PreAnalytiX
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# QIAsymphony® SP Sample Recovery Protocol Sheet

## QIAsymphony SP sample recovery procedure for the QIAsymphony PAXgene® Blood RNA protocol V5 for software version 5.0 or higher

This document is the QIAsymphony SP sample recovery procedure for the PAXgene Blood RNA protocol V5 for software version 5.0 or higher. QIAsymphony SP Sample Recovery Protocol Sheet provides information about how to recover samples from the QIAsymphony SP when the PAXgene Blood RNA protocol V5 is interrupted or canceled during sample processing.

**The recovery procedure for the PAXgene Blood RNA protocol is not verified and the performance characteristics have not been established. It is the users' responsibility to validate the performance of the recovery procedure in context of the customer's specific intended use. Not for use in diagnostic procedures.**

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## Principle and procedure

The recovery procedure comprises of 5 steps that should be performed in the following order:

- Protocol installation – upload of required QIAsymphony files for sample removal. This should be done before the first PAXgene Blood RNA protocol run is executed to be prepared.
- Error analysis – identification of the error and the protocol step in which it occurred.
- Sample removal – removal of unprocessed and/or semi-processed samples from the instrument.
- Manual recovery – identification of sample positions in the removed sample prep cartridges and manual processing of removed samples using the PAXgene® Blood miRNA Kit. It could be beneficial to have one of these kits already at site in order to be prepared.
- Cleanup procedure – automatic cleanup of instrument before starting a new run after the recovery procedure for PAXgene Blood RNA was performed

Note: We recommend reading the entire recovery procedure before use to make sure specific instructions are correctly, and completely, understood and followed.

# Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- PAXgene Blood miRNA Kit (50), cat. no. 763134
- Ethanol (96–100%, purity grade p.a.). Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
- Isopropanol (100%, purity grade p.a.)
- Pipettes (20 µL to 5 mL)*
- Sterile, aerosol-barrier, RNase-free pipette tips
- Graduated cylinder
- Microcentrifuge* capable of attaining up to 20,000 x *g*, and equipped with a rotor for 2 mL microcentrifuge tubes
- Shaker-incubator* capable of incubating at 55°C and 65°C and shaking at 1400 rpm equipped with a 1.5 mL microcentrifuge tube adapter (e.g., Eppendorf® Thermomixer Compact* or equivalent)
- Vortex mixer*
- Crushed ice
- Permanent pen for labeling

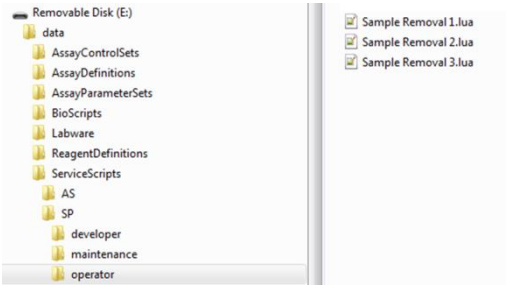
* Ensure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's recommendations.

## Protocol installation

This section describes how to upload the required QIAsymphony files for sample removal.

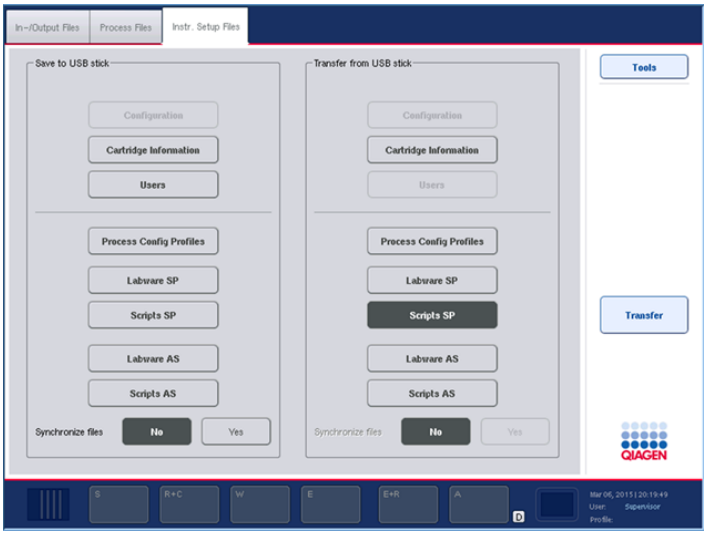
### Procedure

1. Browse the zip file and unzip the Sample Removal package onto the main folder of the USB stick.
2. Check that the **Sample Removal Scripts** are saved under Removable Disk: data/ServiceScripts/SP/Operator as shown below.




3. Remove the USB stick from the computer using the **Safely remove hardware** function.
4. Close all drawers and hoods of the QIAsymphony SP/AS instruments.
5. Power ON the QIAsymphony SP/AS instruments and wait until the **Sample Preparation** screen appears and the initialization procedure has finished.
6. Log in to the QIAsymphony SP/AS instruments as “Supervisor”.
7. Insert the USB stick into one of the USB ports at the front of the QIAsymphony SP.
8. Select **Tools**, and then select **File Transfer**.
9. Select the **Instr. Setup Files** tab.

10. Press **Scripts SP or Scripts** in the **Transfer from USB stick** panel and make sure to set **Synchronize files** to **No**.




11. Press the **Transfer** button in the command bar of the screen to transfer the files from the USB stick to the QIAsymphony SP.
12. After successful data transfer, a message will appear confirming the data transfer: Select **OK**.
13. Remove the QIAsymphony SP USB stick from the USB port.
14. The Sample Removal Scripts are ready to use.

    Note: A restart of the instrument is not necessary, if only the applications files have been uploaded.

## Error analysis

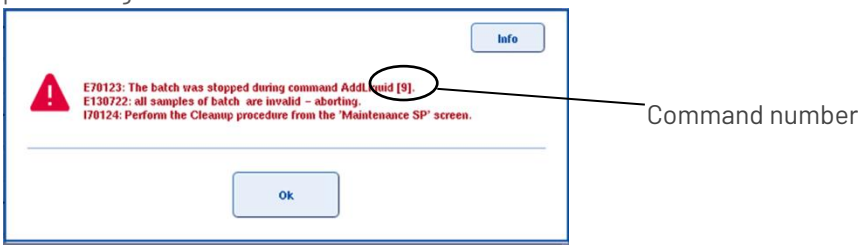
This section describes how to identify the step in which the protocol was interrupted or canceled.

### Procedure

1. Remove the elution rack in order to generate the result file.
2. If the sample button is yellow, press it to obtain the error message box.
3. If the error message box is visible, note the command number in which the batch was stopped (number in brackets [XX]). See the instruction list for sample removal (page 9) and perform the instructions associated with the command number. If no error message is visible, proceed with step 4.

   Note: Do not perform the cleanup procedure on the instrument before the samples are removed!

   Example message box:




4. Download the previously created result file and log file (including the error.log file) using a USB stick or the QIAsymphony Management Console.
5. Open the result file of the erroneous run.
6. The command number can be found in the result file (Figure 1, page 8). Locate the error message under **Messages**. Refer to the instruction list (page 9) and perform the instructions associated with the command number.

   The error message will display a command number or an information message (Figure 1, page 8). If a command number is displayed, note the number in square

brackets ([XX]). If a command number is not displayed, contact QIAGEN Technical Services. Be sure to have the result and error.log files (and, in addition, the trace.log file) to identify the step in which the error occurred.

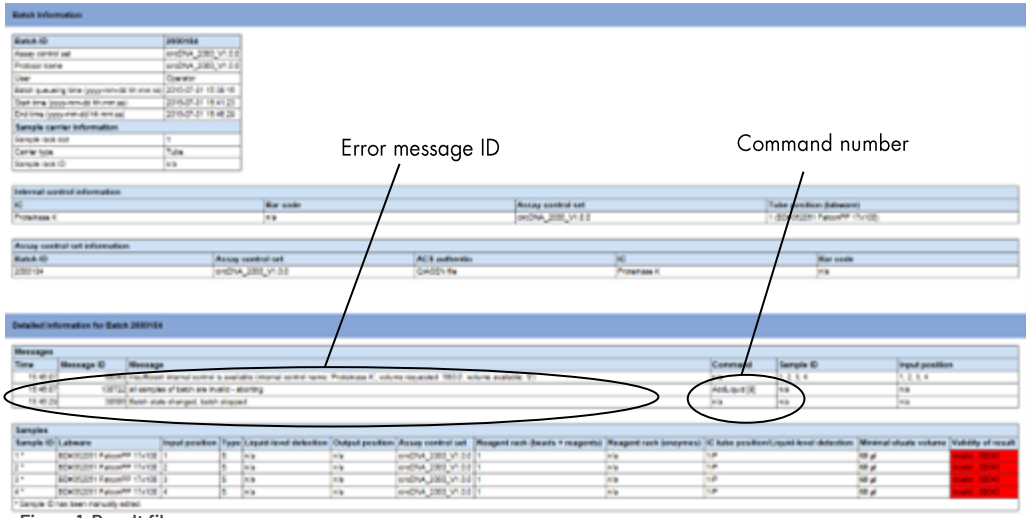



**Figure 1. Result file.**

## Sample removal

This section describes how to remove unprocessed and semi-processed samples from the instrument for the QIAsymphony PAXgene Blood RNA protocol.

General information: Up to command number 131 sample prep cartridges at the right hand side of the extractor belt contain buffer or waste. Sample prep cartridges with samples are located on the left side of the extractor belt, under the magnetic head or at the lysis station.

**Table 1. Instruction list for sample removal**

| Command numbers | Associated sample removal instructions |
|---|---|
| 1-5 | Samples have not been pipetted and are still in the PAXgene Blood RNA tubes or some samples have been transferred to the sample prep cartridge on lysis station.<br><br>Remove the PAXgene Blood RNA tubes or the sample prep cartridge with the samples from the lysis station (Figures 2 & 3, page 29 ) without changing the orientation.<br><br>Perform "Manual Recovery 1" (page 32) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges. |
| 6-18 | All samples are transferred to the sample prep cartridge on lysis station.<br>The lysis step on lysis station is finished or interrupted.<br><br>Remove the sample prep cartridge with the samples from the lysis station or from the left side of the extractor belt (Figures 2 & 3, page 29) without changing the orientation.<br><br>Perform "Manual Recovery 2" (page 36) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

| Command numbers | Associated sample removal instructions |
|---|---|
| 19-20, 23 | Magnetic particles were added for DNA binding step in all samples.<br><br>Remove the cover of the magnetic head.<br><br>Sample prep cartridges with samples are either accessible or under the magnetic head.<br><br>Remove any accessible sample prep cartridges with the samples from the left side of the extractor belt (Figures 2 & 3, page 29) without changing the orientation.<br><br>If any sample prep cartridge is under the magnetic head, perform "Sample Removal 3" on QIAsymphony SP instrument (page 28), to access the cartridges with the samples and then remove them from the left side of the extractor belt without changing the orientation.<br><br>Perform "Manual Recovery 3" (page 40) for all removed samples containing magnetic particles. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

| Command numbers | Associated sample removal instructions |
|---|---|
| 21; 24-25 | Magnetic particles with DNA are bound to the 8-Rod-Cover on the magnetic head.<br><br>Remove the cover of the magnetic head.<br><br>Sample prep cartridges with samples are under the magnetic head.<br><br>Remove any accessible sample prep cartridges with buffer from the right side of the extractor belt (Figures 2 & 3, page 29). Discard the sample prep cartridges with buffer.<br><br>To remove the sample prep cartridges are below the magnetic head, perform "Sample Removal 3" on QIAsymphony SP instrument (page 28), to access the cartridges with the samples, and then remove them from the left side of the extractor belt without changing the orientation.<br><br>Perform "Manual Recovery 3a" (page 43) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). Discard the magnetic particles with DNA. |

| Command numbers | Associated sample removal instructions |
|---|---|
| 22; 26-27 | Magnetic particles with DNA are bound to the 8-Rod-Cover on the magnetic head (Remove DNA).<br><br>Remove the cover of the magnetic head.<br><br>Sample prep cartridges with wash buffer are under the magnetic head or at the right side of the extractor belt.<br><br>Remove any accessible sample prep cartridges with buffer from the right side of the extractor belt. Discard the sample prep cartridges with buffer.<br><br>Remove any accessible sample prep cartridges with the samples from the left side of the extractor belt (Figures 2 & 3, page 29) without changing the orientation. If sample prep cartridges with samples are not accessible on the left side of the extractor belt perform "Sample Removal 3" on QIAsymphony SP instrument (page 28).<br><br>Perform "Manual Recovery 3a" (page 43) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument. (page 65). Discard the magnetic particles with DNA. |
| 30-31 | Sample prep cartridges with samples and without magnetic particles are on the left side of the extractor belt.<br><br>Sample prep cartridges with wash buffer and DNA are under the magnetic head.<br><br>Remove any accessible sample prep cartridges with the samples from the left side of the extractor belt (Figures 2 & 3, page 29) without changing the orientation.<br><br>Case A: Sample volume in sample prep cartridges is less than 1500 µL. Perform "Manual Recovery 3a" (page 43).<br>Case B: Sample volume in sample prep cartridges is more than 1500 µL. Perform "Manual Recovery 4a" (page 48).<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

| Command numbers | Associated sample removal instructions |
|---|---|
| 32-33 | Sample prep cartridges with samples and binding buffer are at the left side of the extractor belt.<br><br>Remove any accessible sample prep cartridges with the samples from the left side of the extractor belt (Figures 2 & 3, page 29) without changing the orientation.<br><br>Perform "Manual Recovery 4a" (page 48) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

| Command numbers | Associated sample removal instructions |
|---|---|
| 28-29, 34-35 | Sample prep cartridges with samples, magnetic particles, and binding buffer at the left side of the extractor belt or under the magnetic head.<br><br>Remove the cover of the magnetic head.<br><br>Case A: Sample prep cartridges with wash buffer are under the magnetic head; Sample prep cartridges with samples, magnetic particles, and binding buffer at the left side of the extractor belt.<br><br>Remove any accessible sample prep cartridges with the samples from the left side of the extractor belt (Figures 2 & 3, page 29) without changing the orientation.<br><br>Perform "Manual Recovery 4" (page 44) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Case B: Sample prep cartridges with wash buffer are at the right side of the extractor belt; sample prep cartridges with samples, magnetic particles, and binding buffer are under the magnetic head. Magnetic particles with RNA are not bound to the 8-Rod-Cover on the magnetic head.<br><br>Remove any accessible sample prep cartridges with buffer from the right side of the extractor belt. Discard the sample prep cartridges with buffer.<br><br>To remove the sample prep cartridges which are under the magnetic head, perform "Sample Removal 3" on QIAsymphony SP instrument (page 28), to access the cartridges with the samples and then remove them from the left side of the extractor belt without changing the orientation.<br><br>Perform "Manual Recovery 4" (page 44) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

| Command numbers | Associated sample removal instructions |
|---|---|
| 36-37, 39, 40-43, 45-46, 49-52 | Magnetic particles with RNA are bound to the 8-Rod-Cover on the magnetic head. Sample prep cartridges with buffer are at the left or right side of the extractor belt or/ and under the magnetic head.<br><br>Remove the cover of the magnetic head.<br><br>Remove any accessible sample prep cartridges with buffer from the right or left side of the extractor belt. Discard the sample prep cartridges with buffer. If any sample prep cartridges are under the magnetic head, perform "Sample Removal 3" on QIAsymphony SP instrument (page 28) to access the cartridges with buffer, and then remove them from the left side of the extractor belt. Discard the sample prep cartridges with buffer.<br><br>Manually prepare new sample prep cartridges for all samples by adding 500 µL Ethanol (96–100%, purity grade p.a.; supplied by user) and 200 µL RNase-Free Water (supplied with the PAXgene Blood miRNA Kit) to every sample prep cartridge well Figure 3 (page 29). Mix these liquids in sample prep cartridge by pipetting.<br><br>Perform "Sample Removal 2" and follow instructions. During the "Sample Removal 2" procedure place the previously prepared sample prep cartridges onto the lysis station. See (Figures 2 & 3, page 29) for how to arrange sample prep cartridges on the lysis station, depending on the number of samples.<br><br>Remove the 8-Rod Cover/sample prep cartridge assembly from the left side of the extractor belt without changing the orientation. To recover samples bound to the magnetic particles, carefully remove the 8-Rod Cover. Be sure that no magnetic particles remain on the removed 8-Rod Cover.<br><br>Discard the removed 8-Rod Cover.<br><br>Perform "Manual Recovery 4" (page 44) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

| Command numbers | Associated sample removal instructions |
| --- | --- |
| 38, 44, 47, 53 | Sample prep cartridges with samples, magnetic particles, and wash buffer are under the magnetic head and sample prep cartridges containing waste buffer are on the right side of the extractor belt.<br><br>Remove the cover of the magnetic head.<br><br>Remove any accessible sample prep cartridges with buffer from the right side of the magnetic head. Discard the sample prep cartridges with buffer.<br><br>To remove the sample prep cartridges under the magnetic head, perform "Sample Removal 3" on QIAsymphony SP instrument (page 28), to access the cartridges with the samples and then remove them from the left side of the magnetic head without changing the orientation.<br><br>Perform "Manual Recovery 4" (page 44) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

| Command numbers | Associated sample removal instructions |
|---|---|
| 48, 54-55, 58-61 | Magnetic particles with RNA are bound to the 8-Rod-Cover on the magnetic head. Sample prep cartridges with buffer are at the left or right side of the extractor belt or/ and under the magnetic head.<br><br>Remove the cover of the magnetic head.<br><br>Remove any accessible sample prep cartridges with buffer from the right or left side of the extractor belt. Discard the sample prep cartridges with buffer. If any sample prep cartridges are under the magnetic head, perform "Sample Removal 3" on QIAsymphony SP instrument (page 28), to access the cartridges with buffer and then remove them from the left side of the extractor belt. Discard the sample prep cartridges with buffer.<br><br>Manually prepare new sample prep cartridges for all samples by adding 200 µL BR5 (supplied at the QIAsymphony PAXgene Blood RNA Kit) to every sample prep cartridge well Figure 3 (page 29).<br><br>Perform "Sample Removal 2" and follow instructions. During the "Sample Removal 2" procedure place the previously prepared sample prep cartridges onto the lysis station. See (Figures 2 & 3, page 29) for how to arrange sample prep cartridges on the lysis station, depending on the number of samples.<br><br>Remove the 8-Rod Cover/sample prep cartridge assembly from the left side of the extractor belt without changing the orientation. To recover samples bound to the magnetic particles, carefully remove the 8-Rod Cover. Be sure that no magnetic particles remain on the removed 8-Rod Cover.<br><br>Discard the removed 8-Rod Cover.<br><br>Perform "Manual Recovery 5" (page 49) for all removed samples. See Figure 4 (page 24) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

| Command numbers | Associated sample removal instructions |
|---|---|
| 56-57, 62-67 | Sample prep cartridges with samples, magnetic particles, and Pre-elution buffer are under the magnetic head possibly waste buffer on the right side of the extractor belt.<br><br>Alternatively sample prep cartridges with sample are on the left side of the extractor belt.<br><br>Remove the cover of the magnetic head.<br><br>Remove any accessible sample prep cartridges with buffer from the right side of the extractor belt. Discard the sample prep cartridges with buffer.<br><br>If any sample prep cartridges are under the magnetic head, perform "Sample Removal 3" on QIAsymphony SP instrument (page 28), to access the cartridges with samples and then remove them from the left side of the magnetic head without changing the orientation.<br><br>If only any sample prep cartridges are on the left side of the extractor belt remove the sample prep cartridges without changing the orientation.<br><br>Perform "Manual Recovery 5" (page 49) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument. (page 65). |

| Command numbers | Associated sample removal instructions |
|---|---|
| 68 | Sample prep cartridges with samples, magnetic particles, and QSX1 buffer on the left side of the extractor belt.<br><br>Remove the cover of the magnetic head.<br><br>Remove any accessible sample prep cartridges with samples from the left side of the extractor belt (Figures 2 & 3, page 29) without changing the orientation.<br><br>Case A: Sample volume in sample prep cartridges is less than 350 µL. Perform "Manual Recovery 5" (page 49).<br><br>Case B: Sample volume in sample prep cartridges is more than 350 µL (approximately 440 µL). Perform "Manual Recovery 5a" (page 52).<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |
| 69 | Sample prep cartridges with samples, magnetic particles, and DNase incubation mix are on the left side of the extractor belt.<br><br>Remove any accessible sample prep cartridges with samples from the left side of the extractor belt (Figures 2 & 3, page 29) without changing the orientation.<br><br>Perform "Manual Recovery 5a" (page 52) for all removed samples See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

| Command numbers | Associated sample removal instructions |
|---|---|
| 70-73 | Sample prep cartridges with samples, magnetic particles, and DNase incubation mix on the left side of the extractor belt or under the magnetic head.<br><br>Remove the cover of the magnetic head.<br><br>Remove any accessible sample prep cartridges with the samples from the left side of the extractor belt (Figures 2 & 3, page 29) without changing the orientation.<br><br>If any sample prep cartridges are under the magnetic head, perform "Sample Removal 3" on QIAsymphony SP instrument (page 28), to access the cartridges with samples and then remove them from the left side of the extractor belt without changing the orientation.<br><br>Perform "Manual Recovery 5b" (page 53) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |
| 74 | Sample prep cartridges with samples, magnetic particles, and proteinase K are on the left side of the extractor belt.<br><br>Remove any accessible sample prep cartridges with samples from the left side of the extractor belt (Figures 2 & 3, page 29) without changing the orientation.<br><br>Case A: Sample volume in sample prep cartridges is less than 550 µL. Perform "Manual Recovery 5c" (page 54) .<br><br>Case B: Sample volume in sample prep cartridges is more than 550 µL (approximately 680 µL). Perform "Manual Recovery 6" (page 55).<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

| Command numbers | Associated sample removal instructions |
|---|---|
| 75-78 | Sample prep cartridges with samples and magnetic particles are on the left side of the extractor belt or on lysis station.<br><br>Remove any accessible sample prep cartridges with the samples from the left side of the extractor belt or from the lysis station (Figures 2 & 3, page 29) without changing the orientation.<br><br>Perform "Manual Recovery 6" (page 55) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |
| 79 | Sample prep cartridges with samples and magnetic particles are on the left side of the extractor belt.<br><br>Remove any accessible sample prep cartridges with samples from the left side of the extractor belt (Figures 2 & 3, page 29) without changing the orientation.<br><br>Case A: Sample volume in sample prep cartridges is less than 1400 µL. Perform "Manual Recovery 6a" (page 57).<br><br>Case B: Sample volume in sample prep cartridges is more than 1400 µL. Perform "Manual Recovery 7" (page 58).<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

| Command numbers | Associated sample removal instructions |
|---|---|
| 80-81, 86 | Sample prep cartridges with sample and magnetic particles are on the left side of the extractor belt or under the magnetic head.<br><br>Remove the cover of the magnetic head.<br><br>Remove any accessible sample prep cartridge with the samples from the left side of the extractor belt (Figures 2 & 3, page 29) without changing the orientation.<br><br>If any sample prep cartridges are under the magnetic head, perform "Sample Removal 3" on QIAsymphony SP instrument (page 9), to access the cartridges with sample and then remove them from the left side of the extractor belt without changing the orientation.<br><br>Perform "Manual Recovery 7" (page 58) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

| Command numbers | Associated sample removal instructions |
|---|---|
| 82-83, 85, 87-89, 91-92, 94-98, 100-101, 103-108, 110-118 | Magnetic particles with RNA are bound to the 8-Rod-Cover on the magnetic head. Sample prep cartridges with buffer are at the left or right side of the extractor belt or/ and under the magnetic head.<br><br>Remove the cover of the magnetic head.<br><br>Remove any accessible sample prep cartridges with buffer from the right or left side of the extractor belt. Discard the sample prep cartridges with buffer. If any sample prep cartridges are under the magnetic head, perform "Sample Removal 3" on QIAsymphony SP instrument (page 9), to access the cartridges with buffer and then remove them from the left side of the extractor belt. Discard the sample prep cartridges with buffer.<br><br>Manually prepare new sample prep cartridges for all samples by adding 200 µL BM3 (supplied at the PAXgene Blood miRNA Kit) to every sample prep cartridge well Figure 3 (page 29).<br><br>Perform "Sample Removal 2" and follow instructions. During the "Sample Removal 2" procedure place the previously prepared sample prep cartridges onto the lysis station. See (Figures 2 & 3, page 29) for how to arrange sample prep cartridges on the lysis station, depending on the number of samples.<br><br>Remove the 8-Rod Cover/sample prep cartridge assembly from the left side of the extractor belt without changing the orientation. To recover samples bound to the magnetic particles, carefully remove the 8-Rod Cover. Be sure that no magnetic particles remain on the removed 8-Rod Cover.<br><br>Discard the removed 8-Rod Cover.<br><br>Perform "Manual Recovery 8" (page 61) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

| Command numbers | Associated sample removal instructions |
|---|---|
| 84, 90, 93, 99, 102, 109 | Sample prep cartridges with samples and magnetic particles are under the magnetic head, sample prep cartridges with waste buffer on the right side of the extractor belt.<br><br>Remove the cover of the magnetic head.<br><br>Remove any accessible sample prep cartridges with buffer from the right side of the extractor belt. Discard the sample prep cartridges with buffer.<br><br>If any sample prep cartridges are under the magnetic head, perform "Sample Removal 3" on QIAsymphony SP instrument (page 9), to access the cartridges with sample and then remove them from the left side of the magnetic head without changing the orientation.<br><br>Perform "Manual Recovery 8" (page 61) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |
| 119, 125 | Sample prep cartridges with samples and magnetic particles are under the magnetic head.<br><br>Remove the cover of the magnetic head.<br><br>To remove sample prep cartridges which are under the magnetic head, perform "Sample Removal 3" on QIAsymphony SP instrument (page 9), to access the cartridges with sample and then remove them from the left side of the extractor belt without changing the orientation.<br><br>Perform "Manual Recovery 8" (page 61) for all removed samples See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

| Command numbers | Associated sample removal instructions |
|---|---|
| 120-122, 126-128 | Magnetic particles with RNA are bound to the 8-Rod-Cover on the magnetic head. Sample prep cartridges with buffer are at the left side of the extractor belt or/ and under the magnetic head.<br><br>Remove the cover of the magnetic head.<br><br>Remove any accessible sample prep cartridges with buffer from the left side of the magnetic head. Discard the sample prep cartridges with buffer. If any sample prep cartridges are under the magnetic head, perform "Sample Removal 3" on QIAsymphony SP instrument (page 9), to access the cartridges with buffer and then remove them from the left side of the extractor belt. Discard the sample prep cartridges with buffer.<br><br>If no sample prep cartridges are present under the magnetic head and magnetic particles are bound to the 8-Rod-Cover on the magnetic head, manually prepare new sample prep cartridges for all samples by adding 80-200 µL BR5 (supplied with the PAXgene Blood miRNA Kit) to every sample prep cartridge well Figure 3 (page 29).<br><br>Perform "Sample Removal 2" and follow instructions. During the "Sample Removal 2" procedure place the previously prepared sample prep cartridges onto the lysis station. See (Figures 2 and 3, page 29) for how to arrange sample prep cartridges on the lysis station, depending on the number of samples.<br><br>Remove the 8-Rod Cover/sample prep cartridge assembly from the left side of the extractor belt without changing the orientation. To recover samples bound to the magnetic particles, carefully remove the 8-Rod Cover. Be sure that no magnetic particles remain on the removed 8-Rod Cover.<br><br>Discard the removed 8-Rod Cover.<br><br>Perform "Manual Recovery 9" (page 63) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

| Command numbers | Associated sample removal instructions |
|---|---|
| 123, 129 | Sample prep cartridges with samples and magnetic particles are under the magnetic head. Sample prep cartridges with buffer are at the right side of the extractor belt.<br><br>Remove the cover of the magnetic head.<br><br>Remove any accessible sample prep cartridges with buffer from the right side of the extractor belt. Discard the sample prep cartridges with buffer.<br><br>To remove sample prep cartridges which are under the magnetic head, perform "Sample Removal 3" on QIAsymphony SP instrument (page 9), to access the cartridges with samples and then remove them from the left side of the extractor belt without changing the orientation.<br><br>Perform "Manual Recovery 9" (page 63) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |
| 124, 130 | Magnetic particles without RNA are bound to the 8-Rod-Cover on the magnetic head. Sample prep cartridges with buffer are on the right side of the extractor belt.<br>Sample prep cartridges with eluted RNA are under the magnetic head.<br><br>Remove the cover of the magnetic head.<br><br>Remove any accessible sample prep cartridges with buffer from the right side of the extractor belt. Discard the sample prep cartridges with buffer.<br><br>To remove sample prep cartridges which are under the magnetic head, perform "Sample Removal 3" on QIAsymphony SP instrument (page 28), to access the cartridges with the eluted RNA and then remove them from the left side of the extractor belt without changing the orientation.<br><br>Perform "Manual Recovery 9" (page 58) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

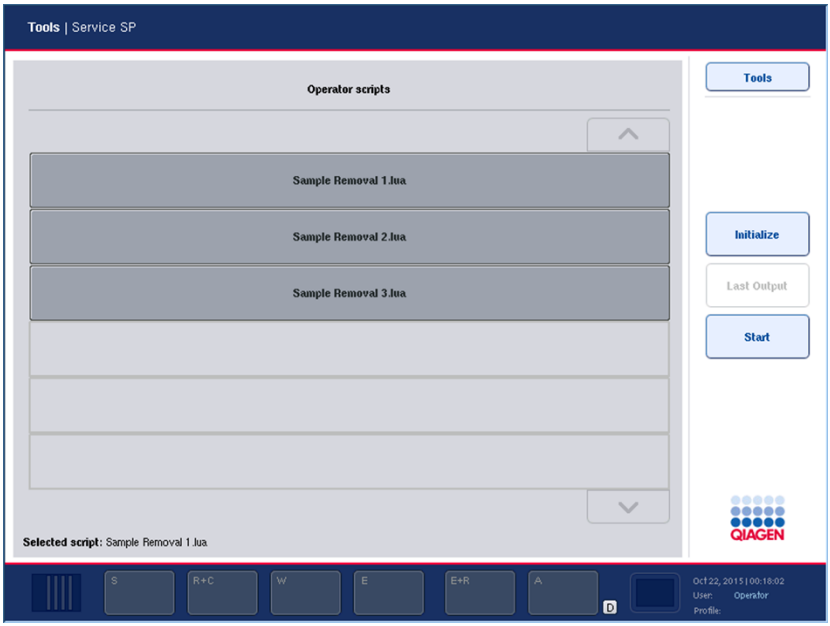
| Command numbers | Associated sample removal instructions |
| --- | --- |
| 131-133 | Sample prep cartridges with eluate on the right side of the extractor belt.<br><br>Remove any accessible sample prep cartridges with eluate from the right side of the extractor belt without changing the orientation.<br><br>Perform "Manual Recovery 9" (page 63) for all removed samples. See Figure 4 (page 30) to identify the position of samples in the sample prep cartridges.<br><br>Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |
| 134-138 | Perform the cleanup procedure on the QIAsymphony SP instrument (page 65). |

## Procedure for sample removal protocol selection

This procedure should be used if the instructions in the “Sample removal” section (page 9) state that a sample removal protocol should be followed.

### Procedure

1. Open the **Tools** menu.
2. Open the **Service SP** menu to view available **Operator scripts**.




3. Select the appropriate sample removal protocol and press **Start**.

   The appropriate protocol is obtained by following the steps in the “Error analysis” section (page 7) and following the instructions associated with the relevant command number in Table 1 (page 9).

   **Note**: Make sure the hood is closed before starting a sample removal protocol (i.e., “Sample Removal 1”, “Sample Removal 2”, or “Sample Removal 3”).

**Note**: Removed sample prep cartridges with intentional samples from the QIAsymphony SP instrument label immediately with numbers 1, 2, and 3 to avoid sample mix-up.

**Important**: The cleanup procedure should be performed before starting a new run. Select the **Tools** tab, open the **Maintenance SP** menu, and select **Cleanup**. For further information, see "Cleanup procedure", page 65.


**Figure 2. Worktable overview.**

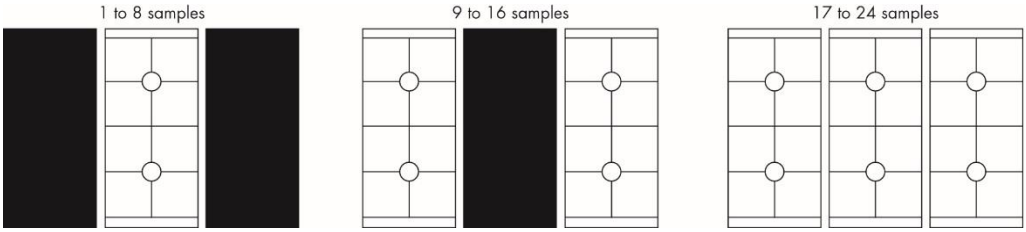



**Figure 3. Position of sample prep cartridges on the lysis station or under / left / right side of the magnetic head, depending on the number of samples.**

## Manual recovery

This section describes how to continue with manual processing of removed samples. Perform 1 of the 9 Manual Recovery protocols for each sample in each removed sample prep cartridge, as instructed in the "Sample removal" section (page 9).

Figure 4 below shows the positions of samples within the 24-tube carrier, sample prep cartridges and in elution racks during sample processing. For the 24-tube carrier, sample positioning is not affected by the number of samples run in a batch and always follows the order shown in Figure 4.

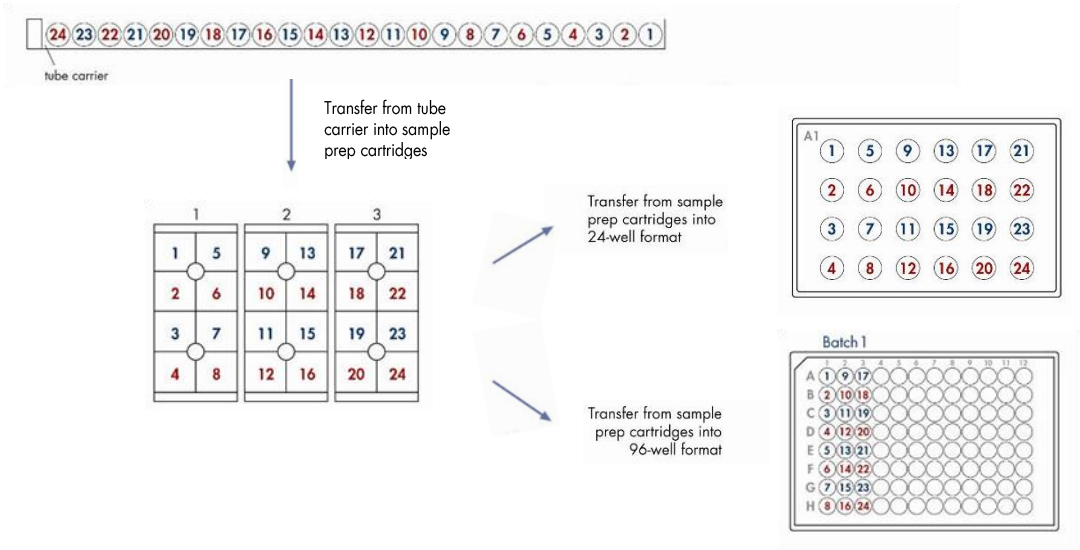



**Figure 4. Sample positioning for batch of 24 samples in tubes.** Sample transfer from tube carrier into sample prep cartridges and into either 24-well or 96-well output format.

## Things to do before starting / general information

For the manual procedure, follow all general instructions in the PAXgene Blood miRNA handbook available under resources section of the product webpage.

- Buffers BM2 and BM3 may form a precipitate upon storage. If necessary, warm to 37°C to dissolve.
- Buffers BM3 and BM4 are supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100%, purity grade p.a.) as indicated on the bottle to obtain a working solution.
- Buffer BM2, BM3, and Flow-through (waste) contain guanidine thiocyanate. Guanidine salts can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.
- If using the RNase-Free DNase Set for the first time, prepare DNase I stock solution. Dissolve the solid DNase I (1500 Kunitz units) in 550 µL of RNase-free water provided with the set. Take care that no DNase I is lost when opening the vial. Do not vortex the reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.

## Manual Recovery 1

This procedure describes how to continue with manual processing of samples recovered from the QIAsymphony SP instrument after an unexpected interruption of a QIAsymphony PAXgene Blood RNA run.

Manual Recovery protocol 1 applies to samples removed from the instrument in front of the lysis step of the procedure or if the QIAsymphony SP instrument cannot start the ordered run.

1. Set the temperature of the shaker incubator to 55°C to prewarm it for later protocol steps.
2. Carefully transfer the entire sample from the removed sample prep cartridge or the PAXgene Blood RNA tube into a 1.5 mL microcentrifuge tube.
3. Freeze the 1.5 mL microcentrifuge tube at –20°C until further processing.
   Note: Even if immediate processing of the samples would be possible, we recommend to freeze the samples for at least 3 h to get the optimal result for the procedure described below. This time could be used to organize all needed equipment and material for the following manual procedure.
4. Thaw the 1.5 mL microcentrifuge tube at ambient temperature (18–25°C) for approximately 30 min.
5. Add 100 µL Buffer BM2 and 20 µL proteinase K. Mix every tube separately by vortexing for 10 s and incubate for 10 min at 55°C in a shaker incubator at 1400 rpm. After incubation, set the temperature of the shaker incubator to 65°C as it will be used in the last step of this protocol.

   Note: Do not mix Buffer BM2 and proteinase K upfront before adding them to the sample.
   Strong impulse vortexing to homogenize the sample is very important.
6. Add 60 µL magnetic particles and vortex until the magnetic particles are visibly dissolved.

Note: magnetic particles are part of the QIAsymphony PAXgene Blood RNA Reagent Cartridge. The magnetic particles are located at magnetic particles trough (MBS). Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the sealed or covered trough containing the magnetic particles vigorously for at least 3 min before first use.

7. Centrifuge for 1 min at full speed (do not exceed 20,000 x *g*).

   Carefully transfer the entire supernatant, without disturbing the magnetic particle pellet into a PAXgene Shredder spin column (lilac) placed in a 2 mL processing tube. Discard the microcentrifuge tube with the magnetic particles. Centrifuge the PAXgene Shredder spin column for 3 min at full speed (do not exceed 20,000 x *g*).

8. Carefully transfer the entire supernatant of the flow-through from the PAXgene Shredder spin column to a new 1.5 mL microcentrifuge tube without disturbing the pellet in the processing tube.

9. Add 600 µL of isopropanol (100%, purity grade p.a.) and 100 µL BM2, mix every tube separately by vortexing for 3 s.

10. Pipette 650 µL sample into the PAXgene RNA spin column (red) placed in a 2 mL processing tube. Close the lid gently, and centrifuge for 1 min 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

11. Pipette the remaining sample into the PAXgene RNA spin column (red). Close the lid gently, and centrifuge for 1 min at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

12. Add 500 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

    Note: Buffer BM3 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM3 before use.

13. Add 500 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
14. Add 350 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
15. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

    Note: Buffer BM4 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM4 before use.
16. Add 350 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
17. Add 350 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
18. Add 20 µL DNase I stock solution to 90 µL Buffer RDD in a 1.5 mL microcentrifuge tube. Mix by gently flicking the tube and centrifuge briefly to collect residual liquid from the sides of the tube.

    Note: If using the RNase-Free DNase Set for the first time, prepare DNase I stock solution. Dissolve the solid DNase I (1500 Kunitz units) in 550 µL of RNase-free water provided with the set. Take care that no DNase I is lost when opening the vial. Do not vortex the reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.
19. Pipette the DNase I incubation mix (110 µL) directly onto the PAXgene RNA spin column membrane, and incubate on the benchtop (20–30°C) for 15 min.

**Note**: Ensure that DNase I incubation mix is placed directly onto the membrane. DNase digestion will be incomplete if part of the mix is applied to and remains on the walls or O-ring of the spin column.

20. Add 350 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
21. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Discard the flow-through. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
22. Add 400 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 2 min at 20,000 x *g*.

    **Note**: After centrifugation, carefully remove the PAXgene RNA spin column from the processing tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.
23. Discard the processing tube containing flow-through and place the PAXgene RNA spin column in a new 2 mL processing tube. Centrifuge at 20,000 x *g* for 1 min.

    **Note**: It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions.
24. Discard the processing tube containing flow-through. Place the PAXgene RNA spin column in a new 1.5 mL microcentrifuge tube and pipette 40 µL Buffer BR5 directly onto the spin column membrane. Close the lid gently and centrifuge for 1 min at 20,000 x *g* to elute the RNA. Be sure to add Buffer BR5 directly to the spin column membrane. This wets the entire membrane, ensuring maximum elution efficiency.
25. Repeat the elution step as described, using 40 µL Buffer BR5 and the same microcentrifuge tube. Close the lid gently and centrifuge for 1 min at 20,000 x *g* to elute the RNA.

26. Discard the PAXgene RNA spin column.
27. Incubate the eluate for 5 min at 65°C in the shaker–incubator without shaking. After incubation, chill immediately on ice. Do not exceed the incubation time or temperature.
28. Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65).

## Manual Recovery 2

This procedure describes how to continue with manual processing of samples recovered from the QIAsymphony SP instrument after an unexpected interruption of a QIAsymphony PAXgene Blood RNA run.

Manual Recovery protocol 2 applies to samples removed from the instrument after the lysis step in front of magnetic particles pipetting (for DNA Binding).

1. Set the temperature of the shaker incubator to 65°C as it will be used at the end of this protocol.
2. Carefully transfer the entire sample from the removed sample prep cartridge into a 1.5 mL microcentrifuge tube.
3. Add 60 µL magnetic particles

   Note: magnetic particles are part of the QIAsymphony PAXgene Blood RNA Reagent Cartridge. The magnetic particles are located at magnetic particles trough (MBS). Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the sealed or covered trough containing the magnetic particles vigorously for at least 3 min before first use.
4. Mix every tube separately by vortexing for 5 s.

   Note: Strong impulse vortexing to homogenize the sample is very important.
5. Centrifuge for 1 min at full speed (do not exceed 20,000 x *g*).

Carefully transfer the entire supernatant, without disturbing the magnetic particle pellet into a PAXgene Shredder spin column (lilac) placed in a 2 mL processing tube. Discard the microcentrifuge tube with the magnetic particles. Centrifuge the PAXgene Shredder spin column for 3 min at full speed (do not exceed 20,000 x *g*).

6. Carefully transfer the entire supernatant of the flow-through from the PAXgene Shredder spin column to a new 1.5 mL microcentrifuge tube without disturbing the pellet in the processing tube.
7. Add 600 µL of isopropanol (100%, purity grade p.a.) and 100 µL BM2, mix every tube separately by vortexing for 3 s.
8. Pipette 650 µL sample into the PAXgene RNA spin column (red) placed in a 2 mL processing tube. Close the lid gently, and centrifuge for 1 min 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
9. Pipette the remaining sample into the PAXgene RNA spin column (red). Close the lid gently, and centrifuge for 1 min at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
10. Add 500 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

    Note: Buffer BM3 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM3 before use.
11. Add 500 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
12. Add 350 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

13. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

    **Note**: Buffer BM4 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM4 before use.

14. Add 350 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

15. Add 350 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

16. Add 20 µL DNase I stock solution to 90 µL Buffer RDD in a 1.5 mL microcentrifuge tube. Mix by gently flicking the tube and centrifuge briefly to collect residual liquid from the sides of the tube.

    **Note**: If using the RNase-Free DNase Set for the first time, prepare DNase I stock solution. Dissolve the solid DNase I (1500 Kunitz units) in 550 µL of RNase-free water provided with the set. Take care that no DNase I is lost when opening the vial. Do not vortex the reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.

17. Pipette the DNase I incubation mix (110 µL) directly onto the PAXgene RNA spin column membrane, and incubate on the benchtop (20–30°C) for 15 min.

    **Note**: Ensure that DNase I incubation mix is placed directly onto the membrane. DNase digestion will be incomplete if part of the mix is applied to and remains on the walls or O-ring of the spin column.

18. Add 350 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

19. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Discard the flow-through. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
20. Add 400 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 2 min at 20,000 x *g*.

    Note: After centrifugation, carefully remove the PAXgene RNA spin column from the processing tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.
21. Discard the processing tube containing flow-through and place the PAXgene RNA spin column in a new 2 mL processing tube. Centrifuge at 20,000 x *g* for 1 min.

    Note: It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions.
22. Discard the processing tube containing flow-through. Place the PAXgene RNA spin column in a new 1.5 mL microcentrifuge tube and pipette 40 µL Buffer BR5 directly onto the spin column membrane. Close the lid gently and centrifuge for 1 min at 20,000 x *g* to elute the RNA. Be sure to add Buffer BR5 directly to the spin column membrane. This wets the entire membrane, ensuring maximum elution efficiency.
23. Repeat the elution step as described, using 40 µL Buffer BR5 and the same microcentrifuge tube. Close the lid gently and centrifuge for 1 min at 20,000 x *g* to elute the RNA.
24. Discard the PAXgene RNA spin column.
25. Incubate the eluate for 5 min at 65°C in the shaker–incubator without shaking. After incubation, chill immediately on ice. Do not exceed the incubation time or temperature.
26. Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65).

## Manual Recovery 3

This procedure describes how to continue with manual processing of samples recovered from the QIAsymphony SP instrument after an unexpected interruption of a QIAsymphony PAXgene Blood RNA run.

Manual Recovery protocol 3 applies to samples removed from the instrument after magnetic particles were added for DNA binding.

1. Set the temperature of the shaker incubator to 65°C as it will be used at the end of this protocol.
2. Mix the sample by pipetting inside the removed sample prep cartridge. Dissolve clots of magnetic particles as good as possible. Clots that can be pipetted will not interfere in the following steps.
3. Carefully transfer the entire sample with magnetic particles from the removed sample prep cartridge into a 1.5 mL microcentrifuge tube.
4. Mix every tube separately by vortexing for 5 s.

   Note: Strong impulse vortexing to homogenize the sample is very important.
5. Centrifuge for 1 min at full speed (do not exceed 20,000 x *g*).

   Carefully transfer the entire supernatant, without disturbing the magnetic particle pellet into a PAXgene Shredder spin column (lilac) placed in a 2 mL processing tube. Discard the microcentrifuge tube with the magnetic particles. Centrifuge the PAXgene Shredder spin column for 3 min at full speed (do not exceed 20,000 x *g*).
6. Carefully transfer the entire supernatant of the flow-through from the PAXgene Shredder spin column to a new 1.5 mL microcentrifuge tube without disturbing the pellet in the processing tube.
7. Add 600 µL of isopropanol (100%, purity grade p.a.) and 100 µL BM2, mix every tube separately by vortexing for 3 s.

8. Pipette 650 µL sample into the PAXgene RNA spin column (red) placed in a 2 mL processing tube. Close the lid gently, and centrifuge for 1 min 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
9. Pipette the remaining sample into the PAXgene RNA spin column (red). Close the lid gently, and centrifuge for 1 min at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
10. Add 500 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

    Note: Buffer BM3 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM3 before use.
11. Add 500 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
12. Add 350 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
13. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

    Note: Buffer BM4 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM4 before use.
14. Add 350 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

15. Add 350 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
16. Add 20 µL DNase I stock solution to 90 µL Buffer RDD in a 1.5 mL microcentrifuge tube. Mix by gently flicking the tube and centrifuge briefly to collect residual liquid from the sides of the tube.

    Note: If using the RNase-Free DNase Set for the first time, prepare DNase I stock solution. Dissolve the solid DNase I (1500 Kunitz units) in 550 µL of RNase-free water provided with the set. Take care that no DNase I is lost when opening the vial. Do not vortex the reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.
17. Pipette the DNase I incubation mix (110 µL) directly onto the PAXgene RNA spin column membrane, and incubate on the benchtop (20–30°C) for 15 min.

    **Note**: Ensure that DNase I incubation mix is placed directly onto the membrane. DNase digestion will be incomplete if part of the mix is applied to and remains on the walls or O-ring of the spin column.
18. Add 350 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
19. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Discard the flow-through. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
20. Add 400 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 2 min at 20,000 x *g*.

    **Note**: After centrifugation, carefully remove the PAXgene RNA spin column from the processing tube so that the column does not come into contact with the flow-through. Otherwise, carryover of ethanol will occur.

21. Discard the processing tube containing flow-through and place the PAXgene RNA spin column in a new 2 mL processing tube. Centrifuge at 20,000 x *g* for 1 min.

    **Note**: It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions.

22. Discard the processing tube containing flow-through. Place the PAXgene RNA spin column in a new 1.5 mL microcentrifuge tube and pipette 40 µL Buffer BR5 directly onto the spin column membrane. Close the lid gently and centrifuge for 1 min at 20,000 x *g* to elute the RNA. Be sure to add Buffer BR5 directly to the spin column membrane. This wets the entire membrane, ensuring maximum elution efficiency.
23. Repeat the elution step as described, using 40 µL Buffer BR5 and the same microcentrifuge tube. Close the lid gently and centrifuge for 1 min at 20,000 x *g* to elute the RNA.
24. Discard the PAXgene RNA spin column.
25. Incubate the eluate for 5 min at 65°C in the shaker–incubator without shaking. After incubation, chill immediately on ice. Do not exceed the incubation time or temperature.
26. Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65).

## Manual Recovery 3a

This procedure describes how to continue with manual processing of samples recovered from the QIAsymphony SP instrument after an unexpected interruption of a QIAsymphony PAXgene Blood RNA run.

Manual Recovery protocol 3a applies to samples removed from the instrument after magnetic particles were added for DNA binding. Magnetic particles with DNA are

abound to the 8-Rod-Cover on the magnetic head or sample prep cartridges with wash buffer and DNA are under the magnetic head.

1. Set the temperature of the shaker incubator to 65°C as it will be used at the end of this protocol.
2. Pipette the sample of the removed sample prep cartridge into a PAXgene Shredder spin column (lilac) placed in a 2 mL processing tube. Centrifuge the PAXgene Shredder spin column for 3 min at full speed (do not exceed 20,000 x *g*).

For next steps see "Manual Recovery protocol 3" step 6.

## Manual Recovery 4

This procedure describes how to continue with manual processing of samples recovered from the QIAsymphony SP instrument after an unexpected interruption of a QIAsymphony PAXgene Blood RNA run.

Manual Recovery protocol 4 applies to samples removed from the instrument after adding magnetic particles for RNA binding and RNA binding buffer.

1. Set the temperature of the shaker incubator to 55°C to prewarm it for later protocol steps.
2. Mix the sample by pipetting inside the removed sample prep cartridge. Dissolve clots of magnetic particles as good as possible. Smaller clots that can be pipetted will not interfere in the following steps.

   **Note**: The best mixing results are achieved by using a 5 mL pipette and ten times of mixing.
3. Carefully transfer of 620 µL sample with magnetic particles from the removed sample prep cartridge into the PAXgene RNA spin column (red). Close the lid

gently, and centrifuge for 1 min at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

4. Repeat the sample transfer step as described, transfer 620 µL sample with magnetic particles from the removed sample prep cartridge into the PAXgene RNA spin column (red). Close the lid gently, and centrifuge for 1 min at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
5. Pipette the remaining sample with magnetic particles into the PAXgene RNA spin column (red). Close the lid gently, and centrifuge for 1 min at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

   **Note**: Magnetic particles are visible on top of the PAXgene RNA spin column membrane.
6. Add 500 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

   **Note**: Buffer BM3 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM3 before use.
7. Add 500 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
8. Add 350 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
9. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

**Note**: Buffer BM4 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM4 before use.

10. Add 350 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
11. Add 350 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
12. Add 20 µL DNase I stock solution to 90 µL Buffer RDD in a 1.5 mL microcentrifuge tube. Mix by gently flicking the tube and centrifuge briefly to collect residual liquid from the sides of the tube.

    **Note**: If using the RNase-Free DNase Set for the first time, prepare DNase I stock solution. Dissolve the solid DNase I (1500 Kunitz units) in 550 µL of RNase-free water provided with the set. Take care that no DNase I is lost when opening the vial. Do not vortex the reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.
13. Pipette the DNase I incubation mix (110 µL) directly onto the PAXgene RNA spin column membrane, and incubate on the benchtop (20–30°C) for 15 min.

    **Note**: Ensure that DNase I incubation mix is placed directly onto the membrane. DNase digestion will be incomplete if part of the mix is applied to and remains on the walls or O-ring of the spin column.
14. Add 350 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
15. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Discard the flow-through. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

16. Add 400 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 2 min at 20,000 x *g*.

    **Note**: After centrifugation, carefully remove the PAXgene RNA spin column from the processing tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

17. Discard the processing tube containing flow-through and place the PAXgene RNA spin column in a new 2 mL processing tube. Centrifuge at 20,000 x *g* for 2 min.

    **Note**: It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions.

18. Discard the processing tube containing flow-through. Place the PAXgene RNA spin column in a new 1.5 mL microcentrifuge tube. Cut off the lid from the 1.5 mL microcentrifuge tube.

19. Transfer the 1.5 mL microcentrifuge tube with the PAXgene RNA spin column to the shaker incubator

    **Note**: During the transfer it is very important to handle the 1.5 mL microcentrifuge tube in combination with the PAXgene RNA spin column with closed lid of PAXgene RNA spin columns.

20. Open the PAXgene RNA spin column lid on shaker incubator. Incubate 10 min at 55°C.

21. After incubation, close the lid of the PAXgene Blood RNA Spin column, and set the temperature of the shaker incubator to 65°C r as it will be used in the last step of this protocol.

22. Discard the 1.5 mL microcentrifuge tube (without lid) and place the PAXgene RNA spin column in a new 1.5 mL microcentrifuge tube.

23. Carefully pipetting of 40 µL Buffer BR5. Do not open the PAXgene Blood RNA spin column lid completely. Take care that no magnetic particles are lost when opening the lid. Close the lid gently and centrifuge for 1 min at 20,000 x *g* to elute the RNA.

24. Repeat the elution step as described, using 40 µL Buffer BR5 and the same microcentrifuge tube. Close the lid gently and centrifuge for 1 min at 20,000 x *g* to elute the RNA.
25. Discard the PAXgene RNA spin column.
26. Incubate the eluate for 5 min at 65°C in the shaker–incubator without shaking. After incubation, chill immediately on ice. Do not exceed the incubation time or temperature.
27. Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65).

## Manual Recovery 4a

This procedure describes how to continue with manual processing of samples recovered from the QIAsymphony SP instrument after an unexpected interruption of a QIAsymphony PAXgene Blood RNA run.

Manual Recovery protocol 4a applies to samples removed from the instrument after adding RNA binding buffer for RNA binding (without magnetic particles).

1. Set the temperature of the shaker incubator to 55°C to prewarm it for later protocol steps.
2. Add 60 µL magnetic particles to the samples inside the removed sample prep cartridge

   Note: magnetic particles are part of the QIAsymphony PAXgene Blood RNA Reagent Cartridge. The magnetic particles are located at magnetic particles trough (MBS). Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the sealed or covered trough containing the magnetic particles vigorously for at least 3 min before first use.

3. Mix the sample by pipetting inside the removed sample prep cartridge. Dissolve clots of magnetic particles as good as possible. Smaller clots that can be pipetted will not interfere in the following steps.

   **Note**: The best mixing results are achieved by using a 5 mL pipette and mixing ten times.

For next steps see "Manual Recovery protocol 4" step 3.

## Manual Recovery 5

This procedure describes how to continue with manual processing of samples recovered from the QIAsymphony SP instrument after an unexpected interruption of a QIAsymphony PAXgene Blood RNA run.

Manual Recovery protocol 5 applies to samples removed from the instrument after pipetting buffer BR5 for Pre-elution step.

1. Set the temperature of the shaker incubator to 55°C to prewarm it for later protocol steps.
2. Carefully transfer the entire sample with magnetic particles from the removed sample prep cartridge into a 1.5 mL microcentrifuge tube.
3. Mix every tube separately by vortexing for 3 s.

   **Note**: Strong impulse vortexing to homogenize the sample is very important.
4. Centrifuge for 1 min at full speed (do not exceed 20,000 x *g*).
5. Carefully transfer the entire supernatant, without disturbing the magnetic particle pellet into a new 1.5 mL microcentrifuge tube. Discard the microcentrifuge tube with the magnetic particle pellet.

6. Add 20 µL DNase I stock solution to 90 µL Buffer RDD in a 1.5 mL microcentrifuge tube. Mix by gently flicking the tube and centrifuge briefly to collect residual liquid from the sides of the tube.

   **Note**: If using the RNase-Free DNase Set for the first time, prepare DNase I stock solution. Dissolve the solid DNase I (1500 Kunitz units) in 550 µL of RNase-free water provided with the set. Take care that no DNase I is lost when opening the vial. Do not vortex the reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.

7. Pipette the DNase I incubation mix (110 µL) directly into the 1.5 mL microcentrifuge tube with the supernatant and mix the DNase supernatant mix by flicking the tube.

8. Incubate on the benchtop (20–30°C) for 15 min.

   **Note**: Ensure that DNase I incubation mix is placed directly into the supernatant. DNase digestion will be incomplete if part of the mix is applied to and remains on the walls.

9. Add 200 µL Buffer BM2 and 20 µL proteinase K. Mix every tube by vortexing for 3 s and incubate 10 min at 55°C in a shaker incubator at 1400 rpm. After incubation, set the temperature of the shaker incubator to 65°C as it will be used in the last step of this protocol.

   **Note**: Do not mix Buffer BM2 and proteinase K together before adding them to the sample.

10. Add 600 µL of isopropanol (100%, purity grade p.a.), mix every tube separately by vortexing for 3 s.

11. Pipette 650 µL sample into the PAXgene RNA spin column (red) placed in a 2 mL processing tube. Close the lid gently, and centrifuge for 1 min 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

12. Pipette the remaining sample into the PAXgene RNA spin column (red). Close the lid gently, and centrifuge for 1 min at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
13. Add 350 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

    Note: Buffer BM3 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM3 before use.
14. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Discard the flow-through. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

    **Note**: Buffer BM4 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM4 before use.
15. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 2 min at 20,000 x *g*.

    **Note**: After centrifugation, carefully remove the PAXgene RNA spin column from the processing tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.
16. Discard the processing tube containing flow-through and place the PAXgene RNA spin column in a new 2 mL processing tube. Centrifuge at 20,000 x *g* for 1 min.

    **Note**: It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions.
17. Discard the processing tube containing flow-through. Place the PAXgene RNA spin column in a new 1.5 mL microcentrifuge tube and pipette 40 µL Buffer BR5 directly onto the spin column membrane. Close the lid gently and centrifuge for 1 min at 20,000 x *g* to elute the RNA. Be sure to add Buffer BR5 directly to the spin

column membrane. This wets the entire membrane, ensuring maximum elution efficiency.

18. Repeat the elution step as described, using 40 µL Buffer BR5 and the same microcentrifuge tube. Close the lid gently and centrifuge for 1 min at 20,000 x *g* to elute the RNA.
19. Discard the PAXgene RNA spin column.
20. Incubate the eluate for 5 min at 65°C in the shaker–incubator without shaking. After incubation, chill immediately on ice. Do not exceed the incubation time or temperature.
21. Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65).

## Manual Recovery 5a

This procedure describes how to continue with manual processing of samples recovered from the QIAsymphony SP instrument after an unexpected interruption of a QIAsymphony PAXgene Blood RNA run.

Manual Recovery protocol 5a applies to samples removed from the instrument after Pre-elution and pipetting buffer QSX1.

1. Set the temperature of the shaker incubator to 55°C to prewarm it for later protocol steps.
2. Carefully transfer the entire sample with magnetic particles from the removed sample prep cartridge into a 1.5 mL microcentrifuge tube.
3. Mix every tube separately by vortexing for 3 s

   Note: Strong impulse vortexing to homogenize the sample is very important.
4. Centrifuge for 1 min at full speed (do not exceed 20,000 x *g*).

5. Carefully transfer the entire supernatant, without disturbing the magnetic particle pellet into a new 1.5 mL microcentrifuge tube. Discard the microcentrifuge tube with the magnetic particle pellet.
6. Add 20 µL DNase I stock solution directly into the 1.5 mL microcentrifuge tube with the supernatant and mix the DNase supernatant by flicking the tube.

   **Note**: If using the RNase-Free DNase Set for the first time, prepare DNase I stock solution. Dissolve the solid DNase I (1500 Kunitz units) in 550 µL of RNase-free water provided with the set. Take care that no DNase I is lost when opening the vial. Do not vortex the reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.

For next steps see "Manual Recovery protocol 5" step 8.

## Manual Recovery 5b

This procedure describes how to continue with manual processing of samples recovered from the QIAsymphony SP instrument after an unexpected interruption of a QIAsymphony PAXgene Blood RNA run.

Manual Recovery protocol 5b applies to samples removed from the instrument after Pre-elution and DNase incubation step.

1. Set the temperature of the shaker incubator to 55°C to prewarm it for later protocol steps.
2. Carefully transfer the entire sample with magnetic particles from the removed sample prep cartridge into a 1.5 mL microcentrifuge tube.
3. Mix every tube separately by vortexing for 3 s

   **Note**: Strong impulse vortexing to homogenize the sample is very important.
4. Centrifuge for 1 min at full speed (do not exceed 20,000 x *g*).

5. Carefully transfer the entire supernatant, without disturbing the magnetic particle pellet into a new 1.5 mL microcentrifuge tube. Discard the microcentrifuge tube with the magnetic particle pellet.

For next steps see “Manual Recovery protocol 5” step 9.

## Manual Recovery 5c

This procedure describes how to continue with manual processing of samples recovered from the QIAsymphony SP instrument after an unexpected interruption of a QIAsymphony PAXgene Blood RNA run.

Manual Recovery protocol 5c applies to samples removed from the instrument after pre-elution and DNase incubation step.

1. Set the temperature of the shaker incubator to 55°C to prewarm it for later protocol steps.
2. Carefully transfer the entire sample with magnetic particles from the removed sample prep cartridge into a 1.5 mL microcentrifuge tube.
3. Mix every tube separately by vortexing for 3 s

   Note: Strong impulse vortexing to homogenize the sample is very important.
4. Centrifuge for 1 min at full speed (do not exceed 20,000 x *g*).
5. Carefully transfer the entire supernatant, without disturbing the magnetic particle pellet into a new 1.5 mL microcentrifuge tube. Discard the microcentrifuge tube with the magnetic particle pellet.
6. Add 200 µL Buffer BM2. Mix every tube by vortexing for 3 s and incubate for 10 min at 55°C in a shaker incubator at 1400 rpm. After incubation, set the temperature of the shaker incubator to 65°C as it will be used in the last step of this protocol.

For next steps see “Manual Recovery protocol 5” step 10.

## Manual Recovery 6

This procedure describes how to continue with manual processing of samples recovered from the QIAsymphony SP instrument after an unexpected interruption of a QIAsymphony PAXgene Blood RNA run.

Manual Recovery protocol 6 applies to samples removed from the instrument after pipetting proteinase K and BR2 within the DNase digest command section.

1. Set the temperature of the shaker incubator to 55°C as it will be used in the next step of this protocol.
2. Mix the sample mix by pipetting inside the removed sample prep cartridge. Dissolve clots of magnetic particles as good as possible. Smaller clots that can be pipetted will not interfere in the following steps.
3. Carefully transfer the entire sample with magnetic particles from the removed sample prep cartridge into a 1.5 mL microcentrifuge tube.
4. Incubate 10 min at 55°C in a shaker incubator at 1400 rpm. After incubation, set the temperature of the shaker incubator to 65°C as it will be used in the last step of this protocol.
5. Centrifuge for 1 min at full speed (do not exceed 20,000 x g).
6. Carefully transfer the entire supernatant, without disturbing the magnetic particle pellet into a new 1.5 mL microcentrifuge tube. Discard the microcentrifuge tube with the magnetic particle pellet.
7. Add 600 µL of isopropanol (100%, purity grade p.a.) and 100 µL BM2, mix every tube separately by vortexing for 3 s.

8. Pipette 650 µL sample into the PAXgene RNA spin column (red) placed in a 2 mL processing tube. Close the lid gently, and centrifuge for 1 min 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
9. Pipette the remaining sample into the PAXgene RNA spin column (red). Close the lid gently, and centrifuge for 1 min at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
10. Add 350 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

    **Note**: Buffer BM3 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM3 before use.
11. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Discard the flow-through. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

    **Note**: Buffer BM4 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM4 before use.
12. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 2 min at 20,000 x *g*.

    **Note**: After centrifugation, carefully remove the PAXgene RNA spin column from the processing tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.
13. Discard the processing tube containing flow-through and place the PAXgene RNA spin column in a new 2 mL processing tube. Centrifuge at 20,000 x *g* for 1 min.

    **Note**: It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions.

14. Discard the processing tube containing flow-through. Place the PAXgene RNA spin column in a new 1.5 mL microcentrifuge tube and pipette 40 µL Buffer BR5 directly onto the spin column membrane. Close the lid gently and centrifuge for 1 min at 20,000 x *g* to elute the RNA. Be sure to add Buffer BR5 directly to the spin column membrane. This wets the entire membrane, ensuring maximum elution efficiency.
15. Repeat the elution step as described, using 40 µL Buffer BR5 and the same microcentrifuge tube. Close the lid gently and centrifuge for 1 min at 20,000 x *g* to elute the RNA.
16. Discard the PAXgene RNA spin column.
17. Incubate the eluate for 5 min at 65°C in the shaker–incubator without shaking. After incubation, chill immediately on ice. Do not exceed the incubation time or temperature.
18. Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65).

## Manual Recovery 6a

This procedure describes how to continue with manual processing of samples recovered from the QIAsymphony SP instrument after an unexpected interruption of a QIAsymphony PAXgene Blood RNA run.

Manual Recovery protocol 6a applies to samples removed from the instrument after pipetting proteinase K and BR2 within the DNase digest command section.

1. Set the temperature of the shaker incubator to 65°C as it will be used in the next step of this protocol.
2. Mix the sample mix by pipetting inside the removed sample prep cartridge. Dissolve clots of magnetic particles as good as possible. Smaller clots that can be pipetted will not interfere in the following steps.

3. Carefully transfer the entire sample with magnetic particles from the removed sample prep cartridge into a 1.5 mL microcentrifuge tube.
4. Centrifuge for 1 min at full speed (do not exceed 20,000 x *g*).
5. Carefully transfer the entire supernatant, without disturbing the magnetic particle pellet into a new 1.5 mL microcentrifuge tube. Discard the microcentrifuge tube with the magnetic particle pellet.

For next steps see "Manual Recovery protocol 6" step 7.

## Manual Recovery 7

This procedure describes how to continue with manual processing of samples recovered from the QIAsymphony SP instrument after an unexpected interruption of a QIAsymphony PAXgene Blood RNA run.

Manual Recovery protocol 7 applies to samples removed from the instrument after re-binding of RNA to the magnetic particles. Pipetting of binding buffer QSB3 was finished.

1. Set the temperature of the shaker incubator to 55°C as it will be used at the end of this protocol.
2. Mix the samples by pipetting inside the removed sample prep cartridge.

   Note: The best mixing results are achieved by using a 5 mL pipette and ten times of mixing.
3. Carefully transfer 620 µL sample with magnetic particles from the removed sample prep cartridge into the PAXgene RNA spin column (red). Close the lid gently, and centrifuge for 1 min at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

4. Repeat the sample transfer step as described, transfer 620 µL sample with magnetic particles from the removed sample prep cartridge into the PAXgene RNA spin column (red). Close the lid gently, and centrifuge for 1 min at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.
5. Pipette the remaining sample with magnetic particles into the PAXgene RNA spin column (red). Close the lid gently, and centrifuge for 1 min at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

   **Note**: Magnetic particles are visible on top of the PAXgene RNA spin column membrane.
6. Add 350 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

   **Note**: Buffer BM3 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM3 before use.
7. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Discard the flow-through. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

   **Note**: Buffer BM4 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM4 before use.
8. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 2 min at 20,000 x *g*.

   **Note**: After centrifugation, carefully remove the PAXgene RNA spin column from the processing tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

9. Discard the processing tube containing flow-through and place the PAXgene RNA spin column in a new 2 mL processing tube. Centrifuge at 20,000 x *g* for 2 min.

   **Note**: It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions.

10. Discard the processing tube containing flow-through. Place the PAXgene RNA spin column in a new 1.5 mL microcentrifuge tube. Cut off the lid from the 1.5 mL microcentrifuge tube.
11. Transfer the 1.5 mL microcentrifuge tube with the PAXgene RNA spin column to the shaker incubator

    **Note**: During the transfer it is very important to handle the 1.5 mL microcentrifuge tube in combination with the PAXgene RNA spin column with closed lid of PAXgene RNA spin columns.

12. Open the PAXgene RNA spin column lid on shaker incubator. Incubate 10 min at 55°C.
13. After incubation, close the lid of the PAXgene Blood RNA Spin column and set the temperature of the shaker incubator to 65°C as it will be used in the last step of this protocol.
14. Discard the 1.5 mL microcentrifuge tube (without lid) and place the PAXgene RNA spin column in a new 1.5 mL microcentrifuge tube.
15. Carefully pipetting of 40 µL Buffer BR5. Do not open the PAXgene Blood RNA spin column lid completely. Take care that no magnetic particles are lost when opening the lid. Close the lid gently and centrifuge for 1 min at 20,000 x *g* to elute the RNA.
16. Repeat the elution step as described, using 40 µL Buffer BR5 and the same microcentrifuge tube. Close the lid gently and centrifuge for 1 min at 20,000 x *g* to elute the RNA.
17. Discard the PAXgene RNA spin column.

18. Incubate the eluate for 5 min at 65°C in the shaker–incubator without shaking. After incubation, chill immediately on ice. Do not exceed the incubation time or temperature.
19. Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65).

## Manual Recovery 8

This procedure describes how to continue with manual processing of samples recovered from the QIAsymphony SP instrument after an unexpected interruption of a QIAsymphony PAXgene Blood RNA run.

Manual Recovery protocol 8 applies to samples removed from the instrument after DNase digest. Magnetic particles with bound RNA are solved in Buffer QSB1 (wash3).

1. Set the temperature of the shaker incubator to 55°C as it will be used in a later step of this protocol.
2. Mix the samples by pipetting inside the removed sample prep cartridge.
3. Carefully transfer of 620 µL sample with magnetic particles from the removed sample prep cartridge into the PAXgene RNA spin column (red). Close the lid gently, and centrifuge for 1 min at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

   **Note**: If some sample is left in the sample prep cartridge after this step, pipette the remaining sample into the PAXgene RNA spin column (red). Close the lid gently, and centrifuge for 1 min at 20,000 x g. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

   **Note**: Magnetic particles are visible on top of the PAXgene RNA spin column membrane.

4. Add 350 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

   **Note**: Buffer BM3 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM3 before use.

5. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 30 s at 20,000 x *g*. Discard the flow-through. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing flow-through.

   **Note**: Buffer BM4 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM4 before use.

6. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 2 min at 20,000 x *g*.

   **Note**: After centrifugation, carefully remove the PAXgene RNA spin column from the processing tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

7. Discard the processing tube containing flow-through and place the PAXgene RNA spin column in a new 2 mL processing tube. Centrifuge at 20,000 x *g* for 2 min.

   **Note**: It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions.

8. Discard the processing tube containing flow-through. Place the PAXgene RNA spin column in a new 1.5 mL microcentrifuge tube. Cut off the lid from the 1.5 mL microcentrifuge tube.

9. Transfer the 1.5 mL microcentrifuge tube with the PAXgene RNA spin column to the shaker incubator

   **Note**: during the transfer it is very important to handle the 1.5 mL microcentrifuge tube in combination with the PAXgene RNA spin column with closed lid of PAXgene RNA spin columns.

10. Open the PAXgene RNA spin column lid on shaker incubator. Incubate 10 min at 55°C.
11. After incubation, close the lid of the PAXgene Blood RNA Spin column, and set the temperature of the shaker incubator to 65°C as it will be used in the last step of this protocol.
12. Discard the 1.5 mL microcentrifuge tube (without lid) and place the PAXgene RNA spin column in a new 1.5 mL microcentrifuge tube.
13. Carefully pipetting of 40 µL Buffer BR5. Do not open the PAXgene Blood RNA spin column lid completely. Take care that no magnetic particles were lost when opening the lid. Close the lid gently, and centrifuge for 1 min at 20,000 x *g* to elute the RNA.
14. Repeat the elution step as described, using 40 µL Buffer BR5 and the same microcentrifuge tube. Close the lid gently and centrifuge for 1 min at 20,000 x *g* to elute the RNA.
15. Discard the PAXgene RNA spin column.
16. Incubate the eluate for 5 min at 65°C in the shaker–incubator without shaking. After incubation, chill immediately on ice. Do not exceed the incubation time or temperature.
17. Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65).

## Manual Recovery 9

This procedure describes how to continue with manual processing of samples recovered from the QIAsymphony SP instrument after an unexpected interruption of a QIAsymphony PAXgene Blood RNA run.

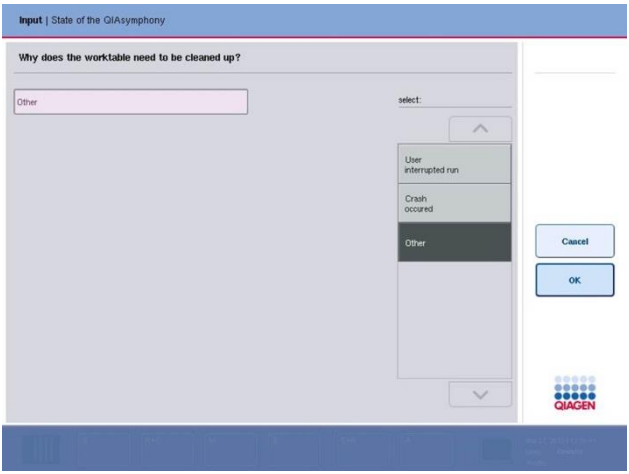
Manual Recovery protocol 9 applies to samples removed from the instrument after adding buffer BR5 for elution.

1. Set the temperature of the shaker incubator to 65°C as it will be used at the end of this protocol.
2. Mix the sample by pipetting inside the removed sample prep cartridge.
3. Carefully transfer the entire sample with magnetic particles from the removed sample prep cartridge into a 1.5 mL microcentrifuge tube.
4. Mix every tube separately by vortexing for 5 s
5. Centrifuge for 1 min at full speed (do not exceed 20,000 x *g*).
6. Carefully transfer the entire supernatant, without disturbing the magnetic particle pellet into a new 1.5 mL microcentrifuge tube. Discard the microcentrifuge tube with the magnetic particles.
7. Repeat the centrifugation step as described, centrifuge for 1 min at full speed (do not exceed 20,000 x *g*).
8. Carefully transfer the entire supernatant, without disturbing the magnetic particle finds into a new 1.5 mL microcentrifuge tube. Discard the microcentrifuge tube with the magnetic particles.
9. Incubate the eluate for 5 min at 65°C in the shaker–incubator without shaking. After incubation, chill immediately on ice. Do not exceed the incubation time or temperature.
10. Finally, perform the cleanup procedure on the QIAsymphony SP instrument (page 65).

## Cleanup procedure

This section describes how to continue after the sample recovery process is complete.

1. Select the Tools tab.
2. Open the Maintenance SP menu, and in the Cleanup section, press the Cleanup button.
3. Follow the instructions on the touchscreen until you receive the message asking if a casework protocol is being used. Press No to start the cleanup procedure.
4. Press the Other button on the following screen.




5. Follow the instructions on the screen until the cleanup procedure is complete.
6. Visually check there are no tips on Tip-Parking station above the liquid waste bottle.

   **Note**: in case of tips still parking; please discard the tips manually.
7. The QIAsymphony SP clean up procedure is finished and the instrument is ready to start a new run.

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