February 2020

# QIAGEN GeneRead® QIAact Panel Cleanup Kit Handbook

Σ 48

For cleanup of DNA during construction of targeted, molecularly bar-coded libraries for digital sequencing with next-generation sequencing (NGS) applications that use the QIAGEN GeneReader® instrument

For research use only

Not for use in diagnostic procedures

REF 185446

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Sample to Insight

QIAGEN

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# Kit Contents

The GeneRead™ QIAact Panel Cleanup Kit contains reagents for QIAcube® Connect cleanup of DNA from NGS library preparation for use with the QIAGEN GeneReader instrument.

| **GeneRead QIAact Panel Cleanup Kit**<br>**Catalog number**<br>**Number of reactions** | **(48)**<br>**185446**<br>**48*** |
|---|---|
| **Identity** | **Quantity** |
| Buffer AVE (for elution) | 2 x 20 ml |
| Diluent (for 80% ethanol formulation)† | 3 x 25 ml |
| Buffer SB2 (for size selection) | 2 x 60 ml |
| MinElute® spin columns | 3 x 50 tubes |

* Total reaction number is calculated for 8 automated runs of 6 samples processed on QIAcube Connect. If runs with <6 samples are performed, the total number of processed samples could be less than stated.

† See Appendix ("Preparation of final 80% ethanol, page 23) for instructions.

# Storage

The GeneRead QIAact Panel Cleanup Kit is shipped on cool-packs. Upon receipt, open the kit, and store the MinElute spin columns at 2–8°C in a constant-temperature refrigerator. The remaining kit components can be stored at room temperature (15–25°C).

If stored under these conditions, the kit is stable until the date indicated on the kit label. Check buffers for precipitates before use and re-dissolve at 37°C if necessary. After prolonged storage in light, Buffer SB2 may turn yellow. This does not affect buffer or kit performance. However, light exposure should be avoided, and it is recommended to store Buffer SB2 in the dark.

# Intended Use

The QIAGEN GeneRead QIAact Panel Cleanup Kit is intended for research use only. Not for use in diagnostic procedures.

QIAcube Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the National Institutes of Health (NIH) guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

# Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

**WARNING** **Risk of personal injury**

Do not add bleach or acidic solutions to the sample preparation waste.

All buffers and reagents should be handled using suitable personal protective equipment including disposable gloves, a lab coat and eye protection. Disposal of wastes must be in accordance with all national, state and local health and safety regulations.

Buffer SB2 contains guanidine thiocyanate, which can form highly reactive compounds when combined with bleach. In case liquid containing this buffer is spilt, clean with suitable laboratory detergent and water.

# Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the GeneRead QIAact Panel Cleanup Kit is tested against predetermined specifications to ensure consistent product quality.

# Introduction

Next-generation sequencing (NGS) is a driving force for numerous new and exciting applications, including cancer research, stem cell research, metagenomics, population genetics and medical research. While NGS technology is continuously improving, library preparation remains one of the biggest bottlenecks in the NGS workflow and includes several time-consuming steps that can result in considerable sample loss and potential introduction of handling errors. The GeneRead QIAact Panel Cleanup Kit uses a streamlined, optimized cleanup protocol, saving time and preventing handling errors. Optimized buffer compositions ensure high yields of targeted, molecularly bar-coded sequencing libraries that are ready for use on the GeneReader™ instrument.

GeneRead™ library cleanup protocols are designed to enable straightforward automation on QIAcube Connect.

## Principles of the procedure

The GeneRead QIAact Panel Cleanup Kit provides a fast, automated cleanup procedure for DNA during the process of constructing targeted, molecularly bar-coded libraries.

Genomic DNA samples are first fragmented, end-repaired and A-tailed within a single, controlled multi-enzyme reaction. The prepared DNA fragments are then ligated at their 5′ ends with a specific adapter containing a UMI (Unique Molecular Index) and a sample-specific bar code.

Three cleanup steps follow and these steps can be automated on QIAcube Connect using the GeneRead QIAact Panel Cleanup Kit.

- After ligation, a first cleanup procedure takes place to remove buffer and enzymes from the DNA sample.

Ligated and purified DNA molecules are subject to limited cycles of target enrichment PCR, with one gene-specific primer targeting a region and one universal forward primer complementary to an adapter sequence. This reaction ensures that intended targets and UMIs are enriched sufficiently to be represented in the final library.

- After target enrichment, a second cleanup procedure takes place to remove buffer and enzymes from the DNA sample.

A universal PCR is then carried out to amplify the library and add GeneReader specific sequences which completes the library.

- After the universal PCR, a final cleanup procedure takes place to remove buffer and enzymes from the final library sample.

Once the library is sequenced, results can be analyzed using a dedicated GeneRead™ workflow, which will automatically perform all steps necessary to generate a DNA sequence variant report from your raw NGS data. All detected variants can be further interpreted by QIAGEN Clinical Insight (QCI™) analysis.

For information about multiplexing and clonal amplification input, see the handbook for the respective GeneRead QIAact panel.

## Description of protocols

This handbook contains three protocols for cleanup of DNA during targeted, molecularly bar-coded preparation of libraries that are for use on the QIAGEN GeneReader platform:

- "Protocol: GR QIAact Panel Cleanup 1" (page 9) describes the cleanup after fragmentation, end repair and adapter ligation
- "Protocol: GR QIAact Panel Cleanup 2" (page 13) describes the cleanup after target enrichment PCR
- "Protocol: GR QIAact Panel Cleanup 3" (page 17) describes the cleanup procedure after universal library amplification

# 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.

Make sure that instruments have been checked and calibrated according to the manufacturer's recommendations.

**General laboratory equipment**

- Dedicated pipets (adjustable) (1–10 µl; 10–100 µl; 100–1000 µl)
- Nuclease-free, aerosol-resistant, sterile PCR pipet tips with hydrophobic filters

**Equipment for automated cleanup**

- QIAcube Connect (for information, visit **www.qiagen.com**)
- QIAcube Filter Tips, 1000 µl (cat. no. 990352)
- QIAcube Filter Tips, 200 µl (cat. no. 990332)
- QIAcube Rotor Adapters (containing 1.5 ml Elution Tubes) (cat. no. 990394)
- QIAcube Reagent Bottles (cat. no. 990393)
- Sample Tubes RB (2 ml; cat. no. 990381)

**Additional equipment**

- GeneReader instrument (cat. no. 9002312)

# Protocol: GR QIAact Panel Cleanup 1

This automated protocol describes the cleanup procedure of DNA after fragmentation, end repair and adapter ligation and the generation of adapter-ligated samples that are ready to use for target enrichment PCR.

## Important points before starting

- Prepare 80% ethanol in the Diluent buffer bottle (see "Preparation of final 80% ethanol"; page 23); do not forget to tick the check box on the lid after preparation.
- **IMPORTANT**: Running fewer than 6 samples in a QIAcube Connect run might reduce the total number of sample preparations per kit.

## Cleanup 1: Preparation of Rotor Adapters

For each sample, prepare one Rotor Adapter according to the following steps.

1. Remove the 2 ml collection tube from a MinElute spin column.
2. Cut off the lid of the column as close to the rim as possible.
3. Place the prepared MinElute spin column in position 1 of the adapter (see Figure 1). Position L1 is not used.

   **Note**: Not cutting off the lid might result in the run being aborted and instrument damage.
4. Leave position 2 of the adapter empty.
5. Place one 1.5 ml elution tube (supplied with the Rotor Adapter) into position 3 and put the lid of the tube in slot L3.

   **Note**: Label elution tubes before loading onto QIAcube Connect to avoid mixing up the samples.

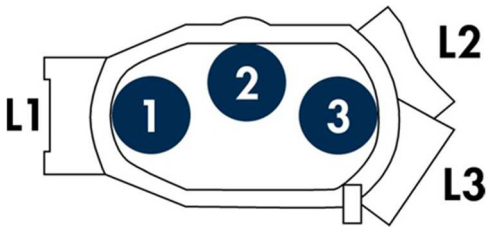


**Figure 1. Rotor Adapters with tube positions (1–3) and lid positions (L1–L3) indicated.** Positions L1, 2 and L2 are not used in this protocol.

## Cleanup 1: Preparation of Reagent Bottle Rack

6. Prepare the bottles according to the volumes in Table 1. Positions 5 and 6 remain empty in the bottle rack and do not require reagent bottles (Figure 2).

   **Note**: Each reagent bottle can hold a total reagent volume of 30 ml.

   **Note**: Calculate the volume of buffer for the corresponding number of samples and add the reserve volume for each bottle to ensure the correct final volume for each buffer.

   For example, the following volume, at least, of Buffer AVE should be loaded onto the instrument for 3 samples: (3 x 150 µl) + 2500 µl = 2950 µl. (Higher volumes can be loaded on the instrument if the maximum volume of 30 ml is not exceeded.)

**Table 1. Reagent bottle preparation (volume per sample plus reserve)**

| Position | Reagent | Volume per sample* | Reserve volume |
|---|---|---|---|
| 1 | Buffer SB2 | 700 µl | 2500 µl |
| 2 | 80% ethanol | 1600 µl | 2500 µl |
| 3 | 80% ethanol | 1600 µl | 2500 µl |
| 4 | Buffer AVE | 150 µl | 2500 µl |
| 5 | – | – | – |
| 6 | – | – | – |

* Not the actual volume pipetted by the instrument.


**Figure 2. Reagent bottle rack.** Positions 5 and 6 are not used in this protocol.

## Cleanup 1: Preparation of samples

7. After the adapter-ligation is completed, spin down the sample tubes or plate, and transfer the complete volume of ligated DNA sample (approximately 50 µl) to the bottom of a Sample Tube RB (2 ml) (cat. no. 990381).

   **Note**: If available sample volume is <50 µl due to pipetting losses, adjustment of sample volume is not required.

## Cleanup 1: Preparation of QIAcube instrument

QIAcube Connect should be handled according to *QIAcube Connect User Manual* available online at **www.qiagen.com**.

8. Place the reagent bottle rack onto the instrument, and remove the screw caps.

   **Note**: Do not discard the screw caps of the buffer bottles. Immediately close the buffer bottles using the screw caps after the protocol is completed to avoid evaporation of buffers.

9. Distribute the Rotor Adapters into the centrifuge buckets according to the number of samples and balance scheme of QIAcube Connect (see *QIAcube Connect User Manual*).

10. Put one tip rack with 1000 µl tips (do not use wide-bore tips) and one rack with 200 µl tips into the tip rack positions on the work deck. See Table 2 for the number of tips required according to the number of samples.

    **Note**: The position of the tips is recognized by the instrument. Make sure to click the tip racks properly into the recess.

**Table 2. Number of tips required (Cleanup 1)**

| Number of samples | Number of 1000 µl tips | Number of 200 µl tips |
|---|---|---|
| 2 | 11 | 1 |
| 3 | 13 | 1 |
| 4 | 15 | 1 |
| 5 | 17 | 1 |
| 6 | 19 | 1 |
| 7 | 21 | 1 |
| 8 | 23 | 1 |
| 9 | 25 | 1 |
| 10 | 27 | 1 |
| 12 | 31 | 1 |

11. Use the shaker adapter marked “2” for 2 ml RB tubes.

12. Place each sample tube into a shaker position according to the number of samples and balance scheme of QIAcube Connect (see *QIAcube ConnectUser Manual*).

    **Note**: The lid of each tube is held in a slot at the edge of the shaker rack to ensure that tubes cannot be displaced during sample processing and to enable loading check of the instrument.

13. To start the protocol, select the **Cleanup** application.

    13a. Select the kit name: **GR QIAact Panel Cleanup Kit**

    13b. Select the material: **Adapter-ligated DNA**

    13c. Select the protocol name: **GR QIAact Panel Cleanup 1**

14. Follow the instructions displayed on the touchscreen and check all steps. Close the door of QIAcube Connect prior to pressing the **Start** button.

    **Note**: For 12 samples, QIAcube Connect run time is approximately 65 minutes.

    **Note**: Elution of the adapter-ligated DNA samples is performed using 24 µl Buffer AVE.

15. Remove the sample tubes from the shaker position and discard.

16. Close the buffer bottles with the corresponding screw caps.

17. Remove the Rotor Adapters following the correct orientation of the samples, and remove the 1.5 ml microcentrifuge tube containing the ligated DNA sample.

    **Note**: Always check the position of the MinElute spin columns after QIAcube Connect run. The column should be located on top of the 1.5 ml microcentrifuge tube to ensure DNA elution from the column into the tube.

**Optional stopping point**: Adapter-ligated samples can be stored for up to 3 days at –30°C to –15°C until they are used in the target enrichment PCR step.

# Protocol: GR QIAact Panel Cleanup 2

This automated protocol describes the cleanup procedure of DNA after target enrichment PCR and generates library samples that are ready to use for universal PCR.

## Important points before starting

- Prepare 80% ethanol in the Diluent buffer bottle (see "Preparation of final 80% ethanol"; page 23); do not forget to tick the check box on the lid after preparation.
- **IMPORTANT**: Running fewer than 6 samples in a QIAcube Connect run might reduce the total number of sample preparations per kit.

## Cleanup 2: Preparation of Rotor Adapters

For each sample, prepare one Rotor Adapter according to the following steps.

1. Remove the 2 ml collection tube from a MinElute spin column.
2. Cut off the lid of the column as close to the rim as possible.
3. Place the prepared MinElute spin column in position 1 of the adapter (see Figure 3, below). Position L1 is not used.

   **Note**: Not cutting off the lid might result in the run being aborted and instrument damage.
4. Leave position 2 of the adapter empty.
5. Place one 1.5 ml elution tube (supplied with the Rotor Adapter) into position 3 and put the lid of the tube in slot L3.

   **Note**: Label elution tubes before loading onto QIAcube Connect to avoid mixing up the samples.

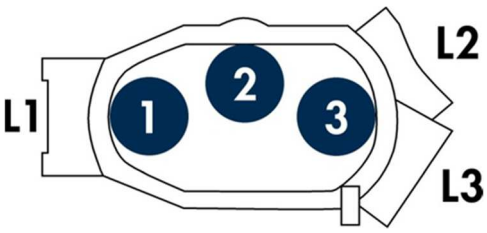


**Figure 3. Rotor Adapters with tube positions (1–3) and lid positions (L1–L3) indicated.** Positions L1, 2 and L2 are not used in this protocol.

## Cleanup 2: Preparation of Reagent Bottle Rack

6. Prepare the bottles according to the volumes in Table 3. Positions 2, 5 and 6 remain empty in the bottle rack and do not require reagent bottles (Figure 4).

   **Note**: Each reagent bottle can hold a total reagent volume of 30 ml.

   **Note**: Calculate the volume of buffer for the corresponding number of samples and add the reserve volume for each bottle to ensure the correct final volume for each buffer.

   For example, the following volume, at least, of Buffer AVE should be loaded onto the instrument for 6 samples: (6 x 30 µl) + 2500 µl = 2680 µl. (Higher volumes can be loaded on the instrument if the maximum volume of 30 ml is not exceeded.)

**Table 3. Reagent bottle preparation (volume per sample plus reserve)**

| Position | Reagent | Volume per sample* | Reserve volume |
|---|---|---|---|
| 1 | Buffer SB2 | 250 µl | 2500 µl |
| 2 | – | – | – |
| 3 | 80% ethanol | 1600 µl | 2500 µl |
| 4 | Buffer AVE | 30 µl | 2500 µl |
| 5 | – | – | – |
| 6 | – | – | – |

* Not the actual volume pipetted by the instrument.


**Figure 4. Reagent bottle rack.** Positions 2, 5 and 6 are not used in this protocol.

## Cleanup 2: Preparation of samples

7. After the target enrichment is completed, spin down the sample tubes or plate, and transfer the complete volume of target-enriched DNA sample (2 PCR reactions from one sample, each 20 µl volume, results in approximately 40 µl) to the bottom of a Sample Tube RB (2 ml; (cat. no. 990381).

   **Note**: If available sample volume is <40 µl due to pipetting losses, adjustment of sample volume is not required.

## Cleanup 2: Preparation of QIAcube instrument

QIAcube Connect should be handled according to *QIAcube Connect User Manual* available online at **www.qiagen.com**.

8. Place the reagent bottle rack onto the instrument, and remove the screw caps.

   **Note**: Do not discard the screw caps of the buffer bottles. Immediately close the buffer bottles using the screw caps after the protocol is completed to avoid evaporation of buffers.

9. Distribute the Rotor Adapters into the centrifuge buckets according to the number of samples and balance scheme of QIAcube Connect (see *QIAcube Connect User Manual*).

10. Put one tip rack with 1000 µl tips (do not use wide-bore tips) and one rack with 200 µl tips into the tip rack positions on the work deck. See Table 4 for the number of tips required according to the number of samples.

    **Note**: The position of the tips is recognized by the instrument. Make sure to click the tip racks properly into the recess.

**Table 4. Number of tips required (Cleanup 2)**

| Number of samples | Number of 1000 µl tips | Number of 200 µl tips |
|---|---|---|
| 2 | 5 | 1 |
| 3 | 6 | 1 |
| 4 | 7 | 1 |
| 5 | 8 | 1 |
| 6 | 9 | 1 |
| 7 | 10 | 1 |
| 8 | 11 | 1 |
| 9 | 12 | 1 |
| 10 | 13 | 1 |
| 12 | 15 | 1 |

11. Use the shaker adapter marked "2" for 2 ml RB tubes.
12. Place each sample tube into a shaker position according to the number of samples and balance scheme of QIAcube Connect (see *QIAcube Connect User Manual*).

    **Note**: The lid of each tube is held in a slot at the edge of the shaker rack to ensure that tubes cannot be displaced during sample processing and to enable loading check of the instrument.
13. To start the protocol, select the **Cleanup** application.

    13a. Select the kit name: **GR QIAact Panel Cleanup Kit**

    13b. Select the material: **Target-enriched DNA**

    13c. Select the protocol name: **GR QIAact Panel Cleanup 2**
14. Follow the instructions displayed on the touchscreen and check all steps. Close the door of QIAcube Connect prior to pressing the **Start** button.

    **Note**: For 12 samples, QIAcube Connect run time is approximately 30 minutes.

    **Note**: Elution of the target-enriched DNA samples is performed using 17 µl Buffer AVE.
15. Remove the sample tubes from the shaker position and discard.
16. Close the buffer bottles with the corresponding screw caps.
17. Remove the Rotor Adapters following the correct orientation of the samples, and remove the 1.5 ml microcentrifuge tube containing the target-enriched DNA sample.

    **Note**: Always check the position of the MinElute spin columns after QIAcube Connect run. The column should be located on top of the 1.5 ml microcentrifuge tube to ensure DNA elution from the column into the tube.

**Optional stopping point**: Target-enriched samples can be stored for up to 3 days at –30°C to –15°C until they are used in the target enrichment PCR step.

# Protocol: GR QIAact Panel Cleanup 3

This automated protocol describes the cleanup procedure of DNA after universal PCR and generates final library samples that are ready to use for the clonal amplification procedure.

## Important points before starting

- Prepare 80% ethanol in the Diluent buffer bottle (see "Preparation of final 80% ethanol"; page 23); do not forget to tick the check box on the lid after preparation.
- **IMPORTANT**: Running fewer than 6 samples in a QIAcube Connect run might reduce the total number of sample preparations per kit.

## Cleanup 3: Preparation of Rotor Adapters

For each sample, prepare one Rotor Adapter according to the following steps.

1. Remove the 2 ml collection tube from a MinElute spin column.
2. Cut off the lid of the column as close to the rim as possible.
3. Place the prepared MinElute spin column in position 1 of the adapter (see Figure 5, below). Position L1 is not used.

   **Note**: Not cutting off the lid might result in the run being aborted and instrument damage.
4. Leave position 2 of the adapter empty.
5. Place one 1.5 ml elution tube (supplied with the Rotor Adapter) into position 3 and put the lid of the tube in slot L3.

   **Note**: Label elution tubes before loading onto QIAcube Connect to avoid mixing up the samples.

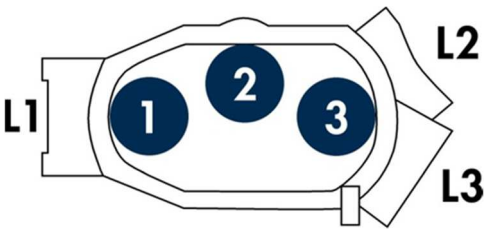


**Figure 5. Rotor Adapters with tube positions (1–3) and lid positions (L1–L3) indicated.** Positions L1, 2, and L2 are not used in this protocol.

## Cleanup 3: Preparation of Reagent Bottle Rack

6. Prepare the bottles according to the volumes in Table 5. Positions 2, 5 and 6 remain empty in the bottle rack and do not require reagent bottles (Figure 6).

   **Note**: Each reagent bottle can hold a total reagent volume of 30 ml.

   **Note**: Calculate the volume of buffer for the corresponding number of samples and add the reserve volume for each bottle to ensure the correct final volume for each buffer.

   For example, the following volume, at least, of Buffer AVE should be loaded onto the instrument for 6 samples: (6 x 40 µl) + 2500 µl = 2740 µl. (Higher volumes can be loaded on the instrument if the maximum volume of 30 ml is not exceeded.)

**Table 5. Reagent bottle preparation (volume per sample plus reserve)**

| Position | Reagent | Volume per sample* | Reserve volume |
|---|---|---|---|
| 1 | Buffer SB2 | 150 µl | 2500 µl |
| 2 | – | – | – |
| 3 | 80% ethanol | 1600 µl | 2500 µl |
| 4 | Buffer AVE | 40 µl | 2500 µl |
| 5 | – | – | – |
| 6 | – | – | – |

* Not the actual volume pipetted by the instrument.


**Figure 6. Reagent bottle rack.** Positions 2, 5 and 6 are not used in this protocol.

## Cleanup 3: Preparation of samples

7. After the universal PCR is completed, spin down the sample tubes or plate, and transfer the complete volume of universally amplified DNA sample (approximately 20 µl) to the bottom of a Sample Tube RB (2 ml) (cat. no. 990381).

   **Note**: If available sample volume is <20 µl due to pipetting losses, adjustment of sample volume is not required.

## Cleanup 3: Preparation of QIAcube instrument

QIAcube Connect should be handled according to *QIAcube Connect User Manual* available online at **www.qiagen.com**.

8. Place the reagent bottle rack onto the instrument, and remove the screw caps.

   **Note**: Do not discard the screw caps of the buffer bottles. Immediately close the buffer bottles using the screw caps after the protocol is completed to avoid evaporation of buffers.

9. Distribute the Rotor Adapters into the centrifuge buckets according to the number of samples and balance scheme of QIAcube Connect (see *QIAcube Connect User Manual*).

10. Put one tip rack with 1000 µl tips (do not use wide-bore tips) and one rack with 200 µl tips into the tip rack positions on the work deck. See Table 6 for the number of tips required according to the number of samples.

    **Note**: The position of the tips is recognized by the instrument. Make sure to click the tip racks properly into the recess.

**Table 6. Number of tips required (Cleanup 3)**

| Number of samples | Number of 1000 µl tips | Number of 200 µl tips |
|---|---|---|
| 2 | 5 | 1 |
| 3 | 6 | 1 |
| 4 | 7 | 1 |
| 5 | 8 | 1 |
| 6 | 9 | 1 |
| 7 | 10 | 1 |
| 8 | 11 | 1 |
| 9 | 12 | 1 |
| 10 | 13 | 1 |
| 12 | 15 | 1 |

11. Use the shaker adapter marked "2" for 2 ml RB tubes.
12. Place each sample tube into a shaker position according to the number of samples and balance scheme of QIAcube Connect (see *QIAcube Connect User Manual*).

    **Note**: The lid of each tube is held in a slot at the edge of the shaker rack to ensure that tubes cannot be displaced during sample processing and to enable loading check of the instrument.
13. To start the protocol, select the **Cleanup** application.

    13a. Select the kit name: **GR QIAact Panel Cleanup Kit**

    13b. Select the material: **Universally amplified DNA**

    13c. Select the protocol name: **GR QIAact Panel Cleanup 3**
14. Follow the instructions displayed on the touchscreen and check all steps. Close the door of QIAcube Connect prior to pressing the **Start** button.

    **Note**: For 12 samples, QIAcube Connect run time is approximately 30 minutes.

    **Note**: Elution of the universally amplified DNA samples is performed using 25 µl Buffer AVE.
15. Remove the sample tubes from the shaker position and discard.
16. Close the buffer bottles with the corresponding screw caps.
17. Remove the Rotor Adapters following the correct orientation of the samples, and remove the 1.5 ml microcentrifuge tube containing the universally amplified DNA sample.

    **Note**: Always check the position of the MinElute spin columns after QIAcube Connect run. The column should be located on top of the 1.5 ml microcentrifuge tube to ensure DNA elution from the column into the tube.

# Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information or protocols in this handbook, or sample and assay technologies. For contact information, see back cover or visit **www.qiagen.com**.

| | | Comments and suggestions |
|---|---|---|
| **Low DNA sample yields** | | |
| a) | Problems with MinElute spin column | Make sure that the MinElute spin column has been transferred to the 1.5 ml microcentrifuge tube of the rotor adapter at the end of QIAcube Connect run. If the spin column is not placed on top of the 1.5 ml tube, no DNA is eluted from the column, and only elution buffer has been transferred into the tube.<br><br>Check if the lid of the spin column was cut off properly (as close to the rim as possible), and repeat the library preparation using the same sample.<br><br>Check if the MinElute spin column has been stored as instructed (upon arrival store MinElute spin columns at 2–8°C). If columns have not been stored as instructed, contact QIAGEN Technical Services. |
| **No DNA sample present after cleanup protocol** | | |
| a) | Presence of inhibitors | Inhibitors, such as ethanol can influence the library preparation reactions. Check if inhibitors are present in the sample. Afterwards, repeat the library construction.<br><br>Check the pH-value of Buffer SB2 and contact QIAGEN Technical Services. |

| | Comments and suggestions |
|---|---|
| b) Loss of DNA | Make sure the final concentration of the 80% ethanol made from the supplied Diluent is correct. The use of lower concentrations of ethanol might lead to loss of DNA. Make sure to use 96–100% non-denatured ethanol to prepare the final 80% ethanol.<br><br>Close the buffer bottles containing 80% ethanol immediately after use to avoid any evaporation. |

For further troubleshooting support, see also the handbook for the respective GeneRead QIAact panel.

# Symbols

The symbols in the following table include symbols used in this handbook.

| | |
|---|---|
|  | Contains reagents sufficient for <N> reactions |
|  | Catalog number |
|  | Material number (i.e., component labeling) |
|  | Manufacturer |

# Appendix

## Preparation of final 80% ethanol

Add 4 volumes (100 ml) ethanol (96–100%, non-denatured) to the bottle containing 25 ml Diluent (for 80% ethanol formulation), and mix well by inverting the bottle 5 times. Tick the check box on the bottle label to indicate that ethanol has been added. Store the 80% ethanol at room temperature (15–25°C).

**Note**: Using lower concentrations of ethanol during cleanup might lead to loss of DNA. Make sure to add the proper 100 ml volume of 96% —100% ethanol (non-denatured) to the Diluent bottle. Always close the bottles immediately after use to avoid evaporation.

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| GeneRead QIAact Panel Cleanup Kit (48) | For 48 reactions: Buffers and reagents for automated cleanup of DNA samples during preparation of targeted, molecularly bar-coded libraries; for use with the QIAGEN QIAcube instruments | 185446 |
| **Related products** | | |
| Filter-Tips, 1000 µl (1024) | Disposable Filter-Tips, racked; (8 x 128); for use with QIAcube instruments | 990352 |
| Filter-Tips, 200 µl (1024) | Disposable Filter-Tips, racked; (8 x 128).; for use with QIAcube instruments | 990332 |
| Rotor Adapters (10 x 24) | Rotor Adapters for use with the centrifuge buckets in QIAcube instruments | 990394 |
| Reagent Bottles, 30 ml (6) | Reagent Bottles for use with buffers of preparation kits for QIAcube instruments | 990393 |
| Sample Tubes RB (2 ml) | Sample Tubes round-bottom for use with sample material on QIAcube instruments | 990381 |
| **QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits** | | |
| QIAcube Connect* | Instrument, connectivity package, 1-year warranty on parts and labor | Inquire |
| Starter Pack, QIAcube | Filter-tips, 200 µl (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder | 990395 |

* All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training and preventive subscription. Contact your local sales representative to learn about your options.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

# Document Revision History

| Date | Changes |
|---|---|
| February 2020 | Updated text, ordering information and intended use for QIAcube Connect. |

## Notes

## Notes