October 2016

QS GeneRead™ DNA FFPE Treatment Kit Handbook

For uracil-N-glycosylase treatment of FFPE tissue specimens using the QIAsymphony® SP Tissue_LC_200_V7_DSP protocol

For Research Use Only

REF 185306

1105007

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

MAT 1105007

Sample to Insight

QIAGEN

Kit Contents

QS GeneRead DNA FFPE Treatment Kit	
Catalog no.	**185306**
Number of samples	**192**
RNase-free Water	50 ml
Lysis Buffer FTB	7 x 0.8 ml
Uracil-N-Glycosylase	3 x 2.5 ml
RNase A (100 mg/ml)	3 x 14 mg
Deparaffinization Solution	50 ml

Reagent Storage and Handling

All components are shipped on dry ice.

Uracil-N-Glycosylase should be stored immediately upon receipt at –20°C in a constant-temperature freezer. When the product is stored under these conditions and handled correctly, performance is guaranteed until the expiration date (see the quality control label on the tube).

The remaining kit components should be stored at room temperature (15–25°C).

Intended Use

For research use only.

This protocol is for uracil-N-glycosylase treatment of formalin-fixed, paraffin-embedded (FFPE) tissues with the QIAsymphony SP and the QS GeneRead DNA FFPE Treatment Kit, using the kit listed in Table 2, page 5, for post-treatment genomic DNA purification. The combined entities cannot be used for the diagnosis, prevention or treatment of a disease.

Quality Control

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

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 convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

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.

- QIAsymphony SP
- ThermoMixer® or shaker–incubator* capable of incubation at 90°C
- Scalpel
- Pipet and pipet tips (to avoid cross-contamination, we recommend pipet tips with aerosol barriers)
- 2 ml Sarstedt® tube (not supplied, Sarstedt cat. no. 72.693 or 72.608)
- Vortexer
- Microcentrifuge with rotor for 2 ml tubes
- Proteinase K (provided in the DNA purification kit listed in Table 2, page 5)
- Plasticware listed in Table 1

Table 1. Plasticware required, according to the number of batches

Plasticware	One batch, 24 samples*	Two batches, 48 samples*	Three batches, 72 samples*	Four batches, 96 samples*
Disposable filter-tips, 200 µl†‡	26	50	74	98
Disposable filter-tips, 1500 µl†‡	72	136	200	264
Sample prep cartridges§	21	42	63	84
8-Rod Covers¶	3	6	9	12

* Using less than 24 samples per batch decreases the number of disposable filter-tips required per run.
† There are 32 filter-tips/filter-tip rack.
‡ Number of required filter-tips includes filter-tips for 1 inventory scan per reagent cartridge.
§ There are 28 sample prep cartridges/unit box.
¶ There are twelve 8-Rod Covers/unit box.

* Make sure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

Important Notes

Table 2. Summary for the Tissue_LC_200_V7_DSP protocols using the QS GeneRead DNA FFPE Treatment Kit

Kits for post-treatment DNA purification	QIAsymphony DSP DNA Mini Kit (cat. no. 937236)
Sample material	FFPE tissue Up to 4 FFPE tissue sections, each with a thickness of up to 10 µm, or 8 sections, with a thickness of up to 5 µm and a surface area of up to 250 mm^2, can be combined in one preparation.
Protocol name	Tissue_LC_200_V7_DSP
Default Assay Control Set	ACS_Tissue_LC_200_V7_DSP
Elution volume	50 µl, 100 µl, 200 µl or 400 µl
Required software version	Version 4.0 or higher

Preparation of sample material

Standard formalin-fixation and paraffin-embedding procedures always result in significant fragmentation of nucleic acids. To limit the extent of DNA fragmentation, be sure to:

- Fix tissue samples in 4–10% formalin as quickly as possible after surgical removal.
- Use a fixation time of 14–24 hours (longer fixation times lead to more severe DNA fragmentation, resulting in poor performance in downstream assays).
- Thoroughly dehydrate samples prior to embedding (residual formalin can inhibit the proteinase K digestion).

Starting material for DNA purification should be a freshly cut section of FFPE tissue. Up to 4 sections, each with a thickness of up to 10 µm, or 8 sections, each with a thickness of up to 5 µm and a surface area of up to 250 mm^2, can be processed in one preparation. If information about the nature of your starting material is not available, we recommend starting with no more than three sections in a single preparation. Depending on DNA yield and purity, it may be possible to use up to eight sections in subsequent preparations.

Loading the "Sample" drawer

Use the information in Table 3 to load the "Sample" drawer.

Table 3. Summary for loading the "Sample" drawer

Sample type	FFPE tissue
Sample input volume	220 µl (required per sample, per protocol)*
Processed sample volume	200 µl
Primary sample tubes	n/a
Secondary sample tubes	2 ml Sarstedt tube (not supplied, Sarstedt cat. no. 72.693 or 72.608)†
Inserts	Depends on type of sample tube used. †
Sample type	FFPE tissue

* The system will not recognize the sample if the volume is less than 220 µl because sample transfer is performed without liquid level detection. Therefore, make sure that the sample input volume is 220 µl.

† See **www.qiagen.com/goto/dsphandbooks** for more information.

n/a: not applicable.

Loading the "Reagents and Consumables" drawer

Use the information in Table 4 to load the "Reagents and Consumables" drawer.

Table 4. Summary for loading the "Reagents and Consumables" drawer

Position A1 and/or A2	Reagent cartridge
Position B1	n/a
Tip rack holder 1–17	Disposable filter-tips, 200 µl or 1500 µl
Unit box holder 1–4	Unit boxes containing sample prep cartridges or 8-Rod Covers

n/a: not applicable.

Loading the "Waste" drawer

Use the information in Table 5 to load the "Waste" drawer.

Table 5. Summary for loading the "Waste" drawer

Unit box holder 1–4	Empty unit boxes
Waste bag holder	Waste bag
Liquid waste bottle holder	Empty liquid waste bottle

Loading the "Eluate" drawer

We recommend loading the Elution rack into slot1, cooling position.*

Note: Depending on the settings, the numbers of filter-tips listed in Table 1, page 4, may differ from the numbers displayed in the touchscreen. We recommend loading the maximum possible number of tips.

Elution volume

Elution volume (50 µl, 100 µl, 200 µl or 400 µl) can be selected in the QIAsymphony touchscreen (Table 2, page 5). Depending on the sample type and DNA content, the final eluate volume may vary by up to 15 µl less than the selected volume. Due to potential variation of the eluate volume, we recommend checking the actual eluate volume when using an automated Assay Set-Up System, which does not verify the eluate volume prior to transfer. Elution with lower volumes increases the final DNA concentration but slightly reduces the yield. We recommend using an elution volume appropriate for the intended downstream application.

* See **www.qiagen.com/goto/dsphandbooks** for more information.

Protocol: Pretreatment for FFPE Tissue

Important point before starting

- QIAsymphony magnetic particles copurify RNA and DNA if both are present in the sample. To minimize RNA content in the sample, an RNA digestion step using RNase A is included in the pretreatment protocol.

Things to do before starting

- Check Lysis Buffer FTB for precipitates. If necessary, incubate at 30°C with occasional shaking to dissolve precipitate.
- Deparaffinization Solution solidifies at temperatures below 18°C. Incubate at 30°C to liquefy.
- Set a ThermoMixer or shaker–incubator to the temperature required for the pretreatment.*

Deparaffinization using Deparaffinization Solution

1. Using a scalpel, trim excess paraffin from the sample block.
2. Cut up to four sections with a thickness of 10 µm or up to eight sections with a thickness of 5 µm.

   Note: If the sample surface has been exposed to air, discard the first 2–3 sections.
3. Immediately place the sections in a 2 ml Sarstedt tube (not supplied) that is compatible with the sample carrier of the QIAsymphony SP.
4. Add 160 µl or 320 µl Deparaffinization Solution (see table below), vortex vigorously for 10 s, and centrifuge briefly to bring the sample to the bottom of the tube.

Thickness of sections	Number of sections	Volume of Deparaffinization Solution
5 µm	1–4	160 µl
	5–8	320 µl
10 µm	1–2	160 µl
	3–4	320 µl

5. Place the tube in a ThermoMixer or shaker–incubator and incubate at 56°C for 3 min, and then allow to cool at room temperature (15–25°C).

   Note: If insufficient Deparaffinization Solution is used or if too much paraffin is carried over with the sample, the Deparaffinization Solution may become waxy or solid after cooling. If this occurs, add additional Deparaffinization Solution, and repeat the 56°C incubation.

* Make sure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

6. Add 55 µl RNase-free Water, 25 µl Lysis Buffer FTB and 20 µl proteinase K.

   Note: A master mix comprising RNase-free Water, Lysis Buffer FTB and proteinase K may be prepared in advance.

   Note: Use proteinase K from the enzyme rack of the DNA purification kit listed in Table 2, page 5.

7. Vortex and briefly centrifuge the samples.

   Note: Upon the addition of proteinase K, the Deparaffinization Solution will form a layer above Lysis Buffer FTB.

8. Incubate at 56°C for 1 h.

   Note: If using only one ThermoMixer or heating block, leave the sample at room temperature after the 56°C incubation in step 8, until the ThermoMixer or heating block has reached 90°C for step 9.

9. Incubate at 90°C for 1 h.

   Incubation at 90°C in Lysis Buffer FTB partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented DNA.

   Note: If using only one ThermoMixer or heating block, leave the sample at room temperature after the 90°C incubation in step 9, until the ThermoMixer or heating block has reached 50°C for step 11.

   After incubation, pulse centrifuge to collect the liquid at the bottom of the tube.

10. Add 115 µl RNase-free Water to the lower phase and mix. Pulse centrifuge to collect the liquid at the bottom of the tube.

11. Add 35 µl Uracil-N-Glycosylase to the lower phase, vortex and incubate at 50°C for 1 h in a ThermoMixer or heating block.

12. Add 2 µl RNase A (100 mg/ml) to the lower phase, mix and incubate for 2 min at room temperature.

13. Centrifuge at full speed for 1 min at room temperature.

14. Carefully transfer tubes (containing both phases) to the sample carrier of the QIAsymphony SP, and select the protocol listed in Table 2, page 5.

Ordering Information

Product	Contents	Cat. no.
QS GeneRead DNA FFPE Treatment Kit (192)	For uracil-N-glycosylase treatment of FFPE tissue specimens using the QIAsymphony SP Tissue_LC_200_V7_DSP protocol	185306

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.

Limited License Agreement for GeneRead DNA FFPE Treatment Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.qiagen.com.