QuantiGene Sample Processing Kit

FFPE Tissues

Catalog Number QS0107 10 samples, QS0108 25 samples, QS0109 100 samples

Doc. Part No. 13059 Pub. No. MAN0017266 Rev. C.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

About sample processing kits

Sample Processing Kits are designed for use with both QuantiGene Singleplex and QuantiGene Plex Assays for quantification of RNA or DNA targets directly from a variety of sample types. This QuantiGene Sample Processing Kit for FFPE Tissue Homogenates contains reagents and instructions for the preparation of tissue homogenates from FFPE tissue sections for use in QuantiGene Singleplex and QuantiGene Plex assays for RNA targets and QuantiGene Plex DNA assays for DNA targets. Note that H&E stained slides are compatible with QuantiGene assays. For quantitating RNA targets, use QuantiGene Sample Assessment Kit to evaluate relative cell number and RNA quality of FFPE tissue homogenates. For more information, see the QuantiGene Sample Assessment Kit Package Insert.

Contents and storage

Refer to product label for expiration date.

Cat. No.	Kit size[1]	Component[2]	Quantity	Storage
QS0107	10 samples	Homogenizing Solution Proteinase K (50 µg/µL)	10 mL 36 µL	15–30°C –20°C
QS0108	25 samples	Homogenizing Solution Proteinase K (50 µg/µL)	20 mL 90 µL	15–30°C –20°C
QS0109	100 samples	Homogenizing Solution Proteinase K (50 µg/µL)	75 mL 360 µL	15–30°C –20°C

[1] A sample is defined as 25–100 mm^2 × 50–60 µm total thickness of FFPE tissue sections.

[2] Place on ice during use. We recommend storage at -20 ºC in an enzyme storage box, from example NEB Cool Box (New England Biolands Cat. No. T04005). NEVER store at -80 ºC.

What this package insert covers

This package insert provides recommendations and step-by-step procedures for the following:

- Preparing FFPE Tissue Homogenates for QuantiGene Plex Assay: Option 1
- Preparing FFPE Tissue Homogenates for QuantiGene Plex Assay: Option 2
- Preparing FFPE Tissue Homogenates for QuantiGene Singleplex Assay
- Determining Complete Tissue Homogenization

Preparing FFPE tissue homogenates for QuantiGene Plex Assay: Option 1

The first option for preparing FFPE homogenates utilizes xylene to remove the excess paraffin wax on the mounted slide, followed by lysis of the FFPE tissue with Lysis Buffer and Proteinase K. This method is preferred for QuantiGene Plex because xylene is very efficient at removing the excess paraffin from the slides. If there is excess paraffin in the sample and the sample is incubated with the Luminex beads, the excess wax can cause bead aggregation.

Table 1 Materials required but not supplied

Item	Source
RNase*Zap*™ (if quantifying RNA)	Cat. No. AM9780
Xylene	Sigma (Cat. No. 247642-4L)
Ethanol 100%	Major laboratory supplier
Disposable razor blades or scalpels	Major laboratory supplier
Tissue Tek Staining Dish	American Maser Tech Scientific

1. If you are planning to quantitate RNA targets, treat all surfaces with RNase*Zap*™ according to the manufacturer's instructions.
2. Measure the length (L) and width (W) of the tissue and calculate the cross-sectional area (L × W) in square millimeters (mm^2). For best results, ensure adequate sample input by combining the equivalent of 50–60 µm thick × 25–100 mm^3 area of tissue. For example, if sections are 10 µm in thickness, use 5–6 sections.
3. In a fume hood, place the slides into the first Tissue Tek staining dish containing xylene solution. Incubate for 5 minutes to remove the paraffin wax. Do not agitate slides.
4. In a fume hood, place the slides into second Tissue Tek staining dish containing 100% ethanol. Incubate for 5 minutes to remove any excess xylene solution. Do not agitate the slides.
5. In a fume hood, place the slides into a third Tissue Tek staining dish containing 100% ethanol. Incubate for 5 minutes. Do not agitate slides.
6. Remove the slides from the Tissue Tek staining dish and allow the ethanol to evaporate off the slide. Using a clean razor blade or scalpel, scrape the slide to completely remove the FFPE section and transfer it to a 1.5-mL microfuge tube.
7. Solubilize the tissue using the volumes specified in the tables below, add Homogenizing Solution and Proteinase K to the tissue. For tissue sections (50–60 µm combined total thickness):

Tissue area (mm^2)	Homogenizing solution (µL)	Proteinase K volume (µL)
25–100	300	3
100–225	600	6
>225	900	9

8. Incubate the samples at 65°C for 3–6 hours. For every hour of incubation, vortex samples for 1 minute at maximum speed and inspect the clarity of the lysate. If the lysate is not clear after 3 hours, vortex and incubate for an additional hour for up to 3 additional hours. Do not exceed 6 hours of incubation.
9. Centrifuge the samples in a microfuge at maximal speed for 5 minutes at room temperature to pellet the cellular debris, then transfer the homogenate to a fresh microfuge tube, avoiding any residual paraffin and debris. Repeat if necessary to completely remove debris.
10. Use the homogenate immediately in a QuantiGene Plex assay or store at –80°C for future use.

Preparing FFPE tissue homogenates for QuantiGene Plex Assay: Option 2

This procedure does not use the xylene to remove the excess paraffin wax but instead uses a razor blade to remove the excess wax. This procedure can work with the QuantiGene Plex assay but if there is high bead aggregation, we recommend using Option 1.

Table 2 Materials required but not supplied.

Item	Source
RNase*Zap*™ (if quantifying RNA)	Cat. No. AM9780
Disposable razor blades or scalpels	Major laboratory supplier

1. If you are planning to quantitate RNA targets, treat all surfaces with RNase*Zap*™ according to the manufacturer's instructions.
2. Measure the length (L) and width (W) of the tissue and calculate the cross-sectional area (L × W) in square millimeters (mm^2). For best results, ensure adequate sample input by combining the equivalent of 50–60 µm thick × 25–100 mm^3 area of tissue. For example, if sections are 10 µm in thickness, use 5–6 sections.
3. Using a clean razor blade or scalpel, carefully remove the excess wax around the tissue area to be processed. When the excess wax and tissue has been removed, scrape the desired tissue using the razor blade and transfer to a 1.5 mL microfuge tube. If multiple sections of the same sample are used, combine the sections into the same tube.
4. Solubilize the tissue using the volumes specified in the tables below, add homogenizing solution and Proteinase K to the tissue. For tissue sections (50–60 µm combined total thickness):

Tissue area (mm^2)	Homogenizing solution (µL)	Proteinase K volume (µL)
25–100	300	3
100–225	600	6
>225	900	9

5. Incubate the samples at 65°C for 3–6 hours. For every hour of incubation, vortex samples for 1 minute at maximum speed and inspect the clarity of the lysate. If the lysate is not clear after 3 hours, vortex and incubate for another hour for up to 3 additional hours. Do not exceed 6 hours of incubation.
6. Centrifuge the samples in a microfuge at maximal speed for 5 minutes at room temperature to pellet the cellular debris, then transfer the homogenate to a fresh microfuge tube, avoiding any residual paraffin and debris. Repeat if necessary to completely remove debris.
7. Use the homogenate immediately in a QuantiGene Plex assay or store at –80°C for future use.

Preparing FFPE tissue homogenates for QuantiGene Singleplex Assay

When preparing FFPE Homogenates for QuantiGene Singleplex Assay, carefully remove the excess paraffin wax on the mounted slide using a razor blade. The xylene method can be used, but the excess wax will not impact the performance in the QuantiGene Singleplex Assay.

Table 3 Materials required but not supplied.

Item	Source
RNase*Zap*™ (if quantifying RNA)	Cat. No. AM9780
Disposable razor blades or scalpels	Major laboratory supplier

1. If you are planning to quantitate RNA targets, treat all surfaces with RNase*Zap*™ according to the manufacturer's instructions.
2. Measure the length (L) and width (W) of the tissue and calculate the cross-sectional area (L × W) in square millimeters (mm^2). For best results, ensure adequate sample input by combining the equivalent of 50–60 µm thick × 25–100 mm^3 area of tissue. For example, if sections are 10 µm in thickness, use 5–6 sections.
3. Using a clean razor blade or scalpel, remove the excess wax around the area of interest.
4. Scrape the slide to completely remove the FFPE section and transfer it to a 1.5 mL microfuge tube.
5. Solubilize the tissue using the volumes specified in the tables below, add homogenizing solution and Proteinase K to the tissue. For tissue sections (50–60 µm combined total thickness):

Tissue area (mm^2)	Homogenizing solution (µL)	Proteinase K volume (µL)
25–100	300	3
100–225	600	6
>225	900	9

6. Incubate the samples at 65°C for 3–6 hours. For every hour of incubation, vortex samples for 1 minute at maximum speed and inspect the clarity of the lysate. If the lysate is not clear after 3 hours, vortex and incubate for another hour up to 6 hours total. Do not exceed 6 hours of incubation.
7. Centrifuge the samples in a microfuge at maximal speed for 5 minutes at room temperature to pellet the cellular debris, then transfer the homogenate to a fresh microfuge tube, avoiding any residual paraffin and debris. Repeat if necessary to completely remove debris.
8. Use the homogenate immediately in a QuantiGene Singleplex Assay or store at –80°C for future use.

Determining complete tissue homogenization

Validate your homogenate by doing the following:

- Examine the homogenate. It should be clear and nonviscous.
- Perform a serial dilution of the homogenate and run an appropriate QuantiGene Singleplex or QuantiGene Plex assay with it. Verify that the expected fold change matches the observed fold change. For example, a 3-fold dilution should generate 3-fold changes (±20%) in the signal (background subtracted) of the targeted genes.

Safety warnings and precautions

All chemicals should be considered potentially hazardous. This product and its components should be handled by those trained in laboratory techniques and be used according to the principles of good laboratory practice.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.