Package Insert

Affymetrix® | Panomics Solutions

QuantiGene Sample Processing Kit

FFPE Tissues

About Sample Processing Kits

Sample Processing Kits are designed for use with both single plex and multiplex QuantiGene assays for quantification of RNA or DNA targets directly from a variety of sample types.

About this Kit

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 2.0 and QuantiGene Plex 2.0 assays for RNA targets and QuantiGene Plex DNA assays for DNA targets. Note that H & E stained slides are compatible with QuantiGene and QuantiGene Plex assays. For quantitating RNA targets, we highly recommend the use of QuantiGene 2.0 Sample Assessment Kit to evaluate relative cell number and RNA quality of FFPE tissue homogenates. For more information, see the QuantiGene 2.0 Sample Assessment Kit Package Insert.

Contents and Storage

Kit components have a shelf life of 12 months from the date of delivery.

Table 1 Kit contents and storage conditions

Cat. No.	**QS0107**	**QS0108**	**QS0109**	
Kit Size	**10 Samples***	**25 Samples***	**100 Samples***	**Storage**
Component	**Quantity**	**Quantity**	**Quantity**	
Homogenizing Solution	10 mL	20 mL	75 mL	15-30 °C
Proteinase K† (50 µg/µL)	36 µL	90 µL	360 µL	–20 °C

** A sample is defined as 25-100 mm² x 50-60 µm total thickness of FFPE tissue sections.*

† Place on ice during use. We recommend storage at –20 °C in an enzyme storage box, for example NEB Cool Box (New England Biolabs P/N T0400S). NEVER store at –80 °C.

Safety Warnings and Precautions

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

What this Package Insert Covers

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

1. Preparing FFPE Tissue Homogenates for QuantiGene Plex Assay- Option 1
2. Preparing FFPE Tissue Homogenates for QuantiGene Plex Assay- Option 2
3. Preparing FFPE Tissue Homogenates for QuantiGene 2.0 Assay
4. Determining Complete Tissue Homogenization

1. 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.

Materials Required but not Supplied

Table 2 Required materials not supplied

Item	Source
RNase Zap® (if quantifying RNA)	Ambion P/N AM0780
Xylene	Sigma P/N 247642-4L
Ethanol 100%	Major Laboratory supplier
Disposable razor blades or scalpels	Major laboratory supplier
Tissue Tek Staining Dish	American Master Tech Scientific

Procedure

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 x W) in square millimeters (mm^2). For best results, ensure adequate sample input by combining the equivalent of 50–60 µm thick x 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):

Table 3 Tissue input for preparing homogenates

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.

2. 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.

Materials Required but not Supplied

Item	Source
RNase Zap® (if quantifying RNA)	Ambion P/N AM0780
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 x W) in square millimeters (mm^2). For best results, ensure adequate sample input by combining the equivalent of 50–60 µm thick x 25-100 mm3 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. Once 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.

3. Preparing FFPE Tissue Homogenates for QuantiGene 2.0 Assay

When preparing FFPE Homogenates for QuantiGene 2.0 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 2.0 assay.

Materials Required but not Supplied

Item	Source
RNase Zap® (if quantifying RNA)	Ambion P/N AM0780
Disposable razor blades or scalpels	Major laboratory supplier

Procedure

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 x W) in square millimeters (mm^2). For best results, ensure adequate sample input by combining the equivalent of 50–60 µm thick x 25-100 mm3 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 2.0 assay or store at –80 °C for future use.

4. Determining Complete Tissue Homogenization

We strongly recommend you validate your homogenate by doing the following:

- Examine the homogenate. It should be clear and non-viscous.
- Perform a serial dilution of the homogenate and run an appropriate QuantiGene or QuantiGene Plex assay with it. Verify 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

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