

QuantiGene® 2.0 Sample Processing Kit

FFPE Tissues

Product Insert

About Sample Processing Kits

Panomics' Sample Processing Kits are designed for use with QuantiGene Assay Kits and Probe Sets or QuantiGene Plex Assay Kits and Plex Sets for quantitation of target-specific RNA directly from cultured cells lysates, whole blood lysates, animal tissue homogenates, or FFPE tissue homogenates.

About this Kit

This QuantiGene 2.0 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 more information, refer to the appropriate *QuantiGene Reagent System User Manual*.

IMPORTANT Because tissues are heterogeneous, and RNA quality in FFPE samples may vary, use the 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 Product Insert*.

Contents and Storage

Cat. No.	QS0107	QS0108	QS0109	
Kit Size	10 Samples ^a	25 Samples ^a	100 Samples ^a	
Component	Quantity	Quantity	Quantity	Storage
Homogenizing Solution	20 mL	50 mL	200 mL	15–30 °C
Proteinase K ^b (50 µg/µL)	36 µL	90 µL	360 µL	–20 °C

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

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

Shelf Life

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

Materials Required but not Supplies

Item	Source
RNase Zap®	Ambion P/N 9780
Disposable razor blades or scalpels	Major laboratory supplier

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 used according to the principles of good laboratory practice.

Intended Use

For research use only. Not for use in diagnosis of disease in humans or animals.

Preparing FFPE Tissue Homogenates

Before You Start Treat all surfaces with RNaseZap according to the manufacturer's recommendations.

Procedure To prepare tissue homogenates from FFPE tissue sections:

Step	Action												
1	<p>Measure and transfer the tissue.</p> <p>For FFPE tissue sections:</p> <ol style="list-style-type: none"> Measure the length (L) and width (W) of the tissue and calculate the cross-sectional area (L x W) in square millimeters (mm²). 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. Avoid transferring excess paraffin. <p>IMPORTANT For best results, ensure adequate sample input by combining the equivalent of 50–60 µm of tissue. For example, if sections are 10 µm in thickness, combine 5–6 sections into a single microfuge tube.</p>												
2	<p>Solubilize the tissue:</p> <ol style="list-style-type: none"> Using the volumes specified in the tables below, add Homogenizing Solution and Proteinase K to the tissue. <p>For tissue sections (50–60 µm combined total thickness):</p> <table border="1"> <thead> <tr> <th>Tissue Area (mm²)</th> <th>Homogenizing Solution (µL)</th> <th>Proteinase K Volume (µL)</th> </tr> </thead> <tbody> <tr> <td>25–100</td> <td>300</td> <td>3</td> </tr> <tr> <td>100–225</td> <td>600</td> <td>6</td> </tr> <tr> <td>> 225</td> <td>900</td> <td>9</td> </tr> </tbody> </table> <ol style="list-style-type: none"> Incubate the samples at 65 °C for 30 minutes to 1 hour. Briefly vortex the samples, then return them to 65 °C overnight (16–20 hours). 	Tissue Area (mm ²)	Homogenizing Solution (µL)	Proteinase K Volume (µL)	25–100	300	3	100–225	600	6	> 225	900	9
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3	<p>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.</p> <p>Note Any residual paraffin will solidify at room temperature during centrifugation, and may appear as a solid residue above the homogenate. It might be necessary to pierce the solid paraffin layer with a pipette tip in order to transfer the homogenate. Discard the pipette tip if it becomes clogged.</p>												
4	<p>Use the homogenate immediately in a QuantiGene 2.0 or QuantiGene Plex 2.0 assay, or store at –80 °C for future use.</p>												

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 a 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.

Clarifying Homogenates

When using the QuantiGene Plex assay, it is very important that all extracellular debris is removed from the homogenate. Failure to remove particulates might result in clogged wells on the Filter Plate following the overnight hybridization step which could lower assay precision.

Required Materials

Item	Source
0.45 µm cellulose nitrate filter plate	Whatman, P/N 7700-3307
96-well polypropylene plate (collection plate)	Fisher P/N 07-201-156 (Corning 3371)
Adhesive plate seal	Major laboratory supplier
Microplate centrifuge	Eppendorf 5804R and rotor A-2 DWP or equivalent

Procedure

Step	Action
1	Determine the number of wells to use on the cellulose nitrate filter plate, based on the number of samples and volume prepared for each sample. Seal the wells that will not be used with an adhesive plate seal. IMPORTANT Do not add more than 300 µL/well.
2	Add the samples to the 0.45 µm cellulose nitrate filter plate.
3	Place cellulose nitrate plate (with samples) on top of the collection plate.
4	Spin the nitrate plate/collection plate assembly in the microplate centrifuge at 1,444 x g for 2–5 minutes at room temperature. If the sample has not filtered through the cellulose plate, spin an additional 2–3 minutes.
5	Use lysates immediately in a QuantiGene or QuantiGene Plex assay, or seal the plate with an adhesive seal and store at –80 °C for later use.

Contacting Panomics

For technical questions, please contact our technical support group by telephone at 1-877-726-6642 option 3, or email at techsupport@panomics.com (US and Canada). In Europe, contact techsupport_europe@panomics.com. In Asia Pacific, contact techsupport_asia@panomics.com. For an updated list of FAQs and product support literature, visit our website at www.panomics.com.

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