Panomics

QuantiGene® Sample Processing Kit

Fresh or Frozen Animal 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 cell lysates, whole blood lysates, animal tissue homogenates, or FFPE tissue homogenates.

About this Kit

This QuantiGene Sample Processing Kit for Tissue Homogenates contains reagents and instructions for the preparation of tissue homogenates from fresh or frozen tissues for use in QuantiGene and QuantiGene Plex assays. For more information, refer to the appropriate *QuantiGene Reagent System User Manual*.

Contents and Storage

Cat. No.	QS0104	QS0105	QS0106	
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 5 mg of tissue.

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 Supplied

Materials for Recommended Procedure

Item	Source
RNase Zap®	Ambion P/N 9780
Liquid nitrogen-cooled mortar (mortar, bowl, and housing)	Fisher P/N 12-947-1
Mortar (extra, ideal when preparing multiple samples)	Fisher P/N 12-947-2
Pestles	Major Laboratory Supplier (MLS)
Spatulas	MLS
Liquid nitrogen (Approximately 100 µL/sample)	MLS
Dry ice	MLS
2-mL tubes and/or 15-mL centrifuge tubes (to hold prepared sample)	MLS

Materials for Alternate Procedure

Item	Source
RNase Zap®	Ambion P/N 9780
RNAlater®[a] or RNAlater®-ICE[b]	Ambion P/N 7020 or 7030

Item	Source
One of the following: Dounce tissue grinder TissueLyser or equivalent	 Fisher P/N 06434 Qiagen P/N 69982

a. For preparing fresh tissue.

b. For preparing frozen tissue.

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 Tissue Homogenates (Recommended)

About this Procedure

Pulverizing tissue with liquid nitrogen is our recommended procedure for preparation of fresh or frozen tissue homogenates.

Before You Start

- Ensure samples for preparation are on dry ice
- Pre-chill tubes, spatula, mortar and pestle on dry ice

! WARNING ! Safety classes should be warn at all times during this procedure.

Preparing Tissue Homogenates

To prepare tissue homogenates:

Step	Action
1	Place samples on dry ice.
2	Weigh and record the weight of all samples to be prepared. Cut sample into cubes, no larger than 2 mm^3.
3	Prepare an appropriate volume of Working Homogenization solution by combining the following per 5 mg tissue: ♦ 300 µL Homogenizing Solution ♦ 3 µL Proteinase K
4	Vortex briefly to mix. **IMPORTANT** If you want to prepare more concentrated samples, for example, 10–15 mg tissue/300 µL Working Homogenization Solution, we strongly recommend you validate the preparation as outlined in “Determining Complete Tissue Homogenization” on page 4.
5	Add a small amount of liquid nitrogen (LN2) to a clean mortar while it is sitting on dry ice.
6	Add the pre-weighed, cut, tissue sample to the mortar containing the LN2.
7	Place one hand over the top of the mortar to prevent tissue from ejecting, and pulverize the tissue with the pestle.
8	Add small amounts of LN2 as it evaporates during the pulverization. **IMPORTANT** Never grind the tissue without LN2.

To prepare tissue homogenates: *(continued)*

Step	Action
9	Once the tissue becomes a fine powder, allow the LN2 to evaporate, then transfer the powder to an appropriate sized pre-chilled tube.
10	Add 300 µL of Working Homogenization Solution for each 5 mg tissue pulverized. Vortex to mix.
11	Incubate the homogenized sample at 65 °C for 30 minutes. Vortex at maximum speed for 15 seconds every 10 minutes during this incubation.
12	Centrifuge the sample at 16,000 x g for 15 minutes to pellet an remaining cellular debris, then transfer the supernatant to a new tube. Repeat this step once more. **Note** For an alternative, high-throughput procedure for clarifying samples, see "Clarifying Homogenates" on page 4.
13	Use the homogenate immediately, or store at –80 °C for later use.

Preparing Tissue Homogenates (alternate)

About this Alternate Procedure

This procedure is for preparing tissue homogenates from 5 mg fresh or frozen animal tissue preserved in RNAlater or RNAlater ICE. This procedure is NOT recommended for preparation of the following samples or sample types:

- Bone
- Muscle
- Pancreas
- Stomach
- Jejunum

IMPORTANT We do not recommend the use of this procedure if samples have not been preserved in RNAlater or RNAlater ICE. Homogenizing fresh or frozen samples at room temperature exposes the sample to significant RNA degradation.

Before you Start

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

Alternate Procedure for Preparing Tissue Homogenates

To prepare tissue homogenates:

Step	Action
1	Place tissue in 5 volumes of RNALater or RNALater-ICE, and incubate according to the manufacturer's recommendations: ♦ Fresh tissue in RNALater at 4 °C for 16 hours ♦ Frozen tissue in ice-cold RNALater-ICE at –20 °C for 16 hours.
2	Prepare an appropriate volume of Working Homogenizing Solution by combining per 5 mg tissue: ♦ 300 µL Homogenizing Solution ♦ 3 µL Proteinase K Vortex briefly to mix.

To prepare tissue homogenates: *(continued)*

Step	Action
3	Completely remove all excess RNAlater by blotting tissue on laboratory wipes. **Note** Carry over of RNAlater or RNAlater-ICE may interfere with QuantiGene or QuantiGene Plex assays.
4	Homogenize the tissue using one of the following methods: Method 1, Dounce tissue grinder: a. Transfer tissue and Working Homogenizing Solution to the Dounce tissue grinder and homogenize **until no visible particles remain**. b. Transfer homogenate to a microfuge tube. Method 2, TissueLyser: a. Transfer tissue and Working Homogenizing Solution to a 2 mL microfuge tube. b. Add 1–2 metal beads, then assemble tubes into TissueLyser according to the manufacturer's recommendations. c. Homogenize tissue at 25 Hz for 1–2 minutes. d. Allow the sample to cool to room temperature, then repeat as necessary **until no visible particles remain**.
5	Incubate the homogenized sample at 65 °C for 30 minutes. Vortex at maximal speed for 15 seconds once every 10 minutes during this incubation. **Note** Some tissues such as connective tissues require longer incubation (up to 18 hours) to reduce viscosity.
6	Centrifuge the sample at 16,000 x g for 15 minutes to pellet any remaining debris, then transfer the supernatant to a new microfuge tube. Repeat this step once more. **Note** For an alternative, high-throughput procedure for clarifying samples, see "Clarifying Homogenates" on page 4.
7	Use tissue homogenate immediately in a QuantiGene or QuantiGene Plex assay, or store at –80 °C for later use.

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

Item	Source
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.

Troubleshooting

Observation	Possible Cause	Recommended Action
Filtration issues with QuantiGene Plex assay	Prepared samples are not cleared of all debris following homogenization.	Pre-filter the sample using the procedure in "Clarifying Homogenates" on page 4.
Tissue is rubbery and difficult to homogenize	This is a known phenomenon with preservation using RNAlater or RNAlater ICE.	Use our recommended method for homogenizing tissues.
Poor sensitivity in QuantiGene or QuantiGene Plex assays	Samples stored and/or prepared under non-optimal conditions resulting in significant RNA degradation.	Run Sample Assessment Controls (Panomics) to assess both sample quantity (18S DNA measurement) and sample quality (28S RNA measurement). If poor sample quality is determined, prepare samples using our recommended liquid nitrogen pulverization method.
	Incomplete sample homogenization. Chunks of tissue remain after homogenization.	Use our recommended liquid nitrogen pulverization method to prepare samples.
Assay signals from QuantiGene or QuantiGene Plex assays are not scaling with sample input	Incomplete sample homogenization. Ratio of tissue sample to Working Homogenizing Solution is too high.	Decrease the amount of tissue sample per recommended volume of Working Homogenization Solution.

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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