Package Insert

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QuantiGene Sample Processing Kit

Fresh or Frozen 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 Tissue Homogenates contains reagents and instructions for the preparation of tissue homogenates from fresh or frozen tissues (animal or plant) for use in QuantiGene 2.0 and QuantiGene Plex 2.0 assays for RNA targets and QuantiGene Plex DNA assays for DNA targets. For more information, refer to the appropriate user manual.

NOTE: For quantitating RNA targets, we recommend the use of QuantiGene 2.0 Sample Assessment Kit to evaluate relative cell number and RNA quality of 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 Contents and storage conditions for the sample processing kit

Cat. No.	QS0104	QS0105	QS0106	
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 5 mg animal tissue or 15 mg plant tissue.*

† 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:

- *Materials Required but not Supplied*
- *Preparing Tissue Homogenates From Animal Tissues (Recommended)*
- *Preparing Tissue Homogenates From Animal Tissues (Alternate)*
- *Preparing Tissue Homogenates from Plant Tissues (Recommended)*
- *Preparing Tissue Homogenates from Plant Tissues (Alternate)*
- *Determining Complete Tissue Homogenization*
- *Clarifying Homogenates*
- *Troubleshooting*

Materials Required but not Supplied

Materials for Recommended Procedure

Table 2 Required materials for standard homogenization procedure

Item	Source
RNase Zap® (if quantifying RNA)	Ambion P/N AM0780
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 10 mL/ sample)	MLS
Dry ice	MLS
2-mL tubes and/or 15-mL centrifuge tubes (to hold prepared sample)	MLS

Materials for Alternate Procedure

Table 3 Required materials for alternate homogenizing procedure

Item	Source
RNase Zap® (if quantifying RNA)	Ambion P/N AM0780
RNAlater®* or RNAlater®-ICE† (if quantifying RNA)	Ambion P/N AM7020 or AM7030
One of the following: Dounce tissue grinder (for animal tissue only) or TissueLyser TissueLyser Adapter Set 2 x 96 Collection Microtubes (racked, 96) Collection Microtube Caps 5-mm Stainless Steel Bead (for animal tissue) 3-mm Tungsten Carbide Bead (for plant tissue)	 Fisher P/N 06434 Qiagen P/N 85300 Qiagen P/N 69984 Qiagen P/N 19560 Qiagen P/N 19566 Qiagen P/N 69989 Qiagen P/N 69997

** For preparing fresh tissue.*

† For preparing frozen tissue.

Preparing Tissue Homogenates From Animal Tissues (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 goggles should be worn at all times during this procedure.

NOTE: If tissues have been stored in RNAlater, they will become rubbery and difficult to process with liquid nitrogen. Consider using the alternate procedure described on page 4.

Procedure

To prepare tissue homogenates using liquid nitrogen:

1. Place samples on dry ice.
2. Weigh and record the weight of all samples to be prepared. Immediately return samples to dry ice.
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 6.

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 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.

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 1 minute every 10 minutes during this incubation.
12. Centrifuge the sample at 16,000 x g for 15 minutes to pellet any 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 7.

13. Use the homogenate immediately in a QuantiGene or QuantiGene Plex assay, or store at –80 °C for later use.

Preparing Tissue Homogenates From Animal Tissues (Alternate)

About this Alternate Procedure

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

- Bone
- Muscle
- Pancreas
- Stomach
- Jejunum

IMPORTANT: If quantitating RNA targets, 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

If you are quantitating RNA targets, treat all surfaces with RNaseZap according to the manufacturer's recommendations.

Procedure

To prepare tissue homogenates using an alternate procedure:

1. (*If quantitating RNA only.*) 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.
3. (*If quantitating RNA only.*) Completely remove all excess RNAlater by blotting tissue on laboratory wipes.

NOTE: Carry over of RNAlater or RNAlater-ICE may interfere with QuantiGene 2.0 or QuantiGene Plex 2.0 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, Qiagen TissueLyser (high-throughput format):

A. Transfer tissue and Working Homogenizing Solution to collection microtubes (racked, 96 Qiagen P/N 19560).

B. Add one 5-mm Stainless Steel Bead (Qiagen P/N 69989), 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 1 minute 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 microcentrifuge tube. Repeat this step once more.

   NOTE: For an alternative, high-throughput procedure for clarifying samples, see *Clarifying Homogenates* on page 7.

7. Use tissue homogenate immediately in a QuantiGene or QuantiGene Plex assay, or store at –80 °C for later use.

Preparing Tissue Homogenates from Plant Tissues (Recommended)

About this Recommended Procedure

This recommended procedure is for preparing tissue homogenates from 6 punches or 15 mg of less fibrous fresh or frozen tissue from leaves, seedlings, or fruits such as tomatoes. This procedure is NOT recommended for preparation of more fibrous samples such as tree bark.

Procedure

To prepare tissue homogenates from plant tissues:

1. Prepare an appropriate volume of Working Homogenizing Solution by combining the following per 15 mg tissue:
   - 300 µL Homogenizing Solution
   - 3 µL Proteinase K

   Vortex briefly to mix.
2. Transfer tissue and Working Homogenizing Solution to collection microtubes (racked, 96 Qiagen P/N 19560).
3. Add one 3-mm Tungsten Carbide Bead, then assemble tubes into TissueLyser according to the manufacturer's instructions.
4. Homogenize the tissue at 25 Hz for 15 minutes.
5. Allow sample to cool to room temperature, then repeat as necessary **until no visible particles remain**.

   NOTE: Processing time requires optimization with different tissue types.

6. Incubate the homogenized sample at 65 °C for 30 minutes. Vortex at maximal speed for 1 minute once every 10 minutes during this incubation.
7. To remove cellular debris, refer to *Clarifying Homogenates* on page 7.

   NOTE: As an alternative method, transfer samples to 2-mL microcentrifuge tubes and centrifuge the sample at 16,000 x g for 15 minutes to pellet any remaining debris, then transfer the supernatant to a new microcentrifuge tube. Repeat this step twice more.

8. Use the tissue homogenate immediately in a QuantiGene or QuantiGene Plex assay, or store at –80 °C for later use.

Preparing Tissue Homogenates from Plant Tissues (Alternate)

About this Procedure

Pulverizing tissue with liquid nitrogen is an alternative procedure for preparing more fibrous plant tissues such as tree bark.

Before You Start

- Ensure samples for preparation are on dry ice.

- Prechill tubes, spatula, mortar and pestle on dry ice.

WARNING: **Safety goggles should be worn at all times during this procedure.**

Procedure

To prepare tissue homogenates:

1. Place sample on dry ice.
2. Weigh and record the weight of all samples to be prepared. Immediately return samples to dry ice.
3. Prepare an appropriate volume of Working Homogenization Solution by combining the following per 6 punches or 15 mg tissue:
   - 300 µL Homogenizing Solution
   - 3 µL Proteinase K

   Vortex briefly to mix

   IMPORTANT: **If you want to prepare more concentrated samples, for example, 12 punches or 30 mg tissue/300 µL Working Homogenization Solution, we strongly recommend validating the preparation as outlined in *Determining Complete Tissue Homogenization* on page 6.**
4. Add a small amount of liquid nitrogen (LN2) to a clean mortar while it is sitting on dry ice.
5. Add the preweighed, cut, tissue sample to the mortar containing the LN2.
6. Place one hand over the top of the mortar to prevent tissue from ejecting, and pulverize the tissue with the pestle.
7. Add small amounts of LN2 as it evaporates during the pulverization.

   IMPORTANT: **Never grind the tissue without LN2.**
8. Once the tissue becomes a fine powder, allow the LN2 to evaporate, then transfer the powder to an appropriate sized prechilled tube.
9. Add 300 µL of Working Homogenization Solution for each 15 mg tissue pulverized. Vortex to mix.
10. Incubate the homogenized sample at 65 °C for 30 minutes. Vortex at maximum speed for 1 minute every 10 minutes during this incubation.
11. Centrifuge the sample at 16,000 x g for 15 minutes to pellet any 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 7.**
12. Use the 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 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.

Clarifying Homogenates

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

NOTE: If using magnetic separation, clarification of samples is rarely necessary for animal tissue homogenates. However, if working with plant tissue homogenates, clarifying homogenates is very important to remove remaining fibrous debris.

Required Materials

Table 4 Required materials for clarifying lysates

Item	Source
0.45 µm cellulose nitrate filter plate	Affymetrix, P/N PC5512 or 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

To clarify homogenates:

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

Table 5 Troubleshooting the sample processing assay

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 7.
When using RNAlater, tissue is rubbery and difficult to homogenize	This is a known phenomenon with preservation of RNA using RNAlater or RNAlater ICE.	Use our alternate method for homogenizing animal tissues.
Poor sensitivity in QuantiGene or QuantiGene Plex assays	Samples stored and/or prepared under non-optimal conditions resulting in significant RNA or DNA degradation.	If quantitating RNA, run Sample Assessment Controls (Affymetrix) to assess both sample quantity (18S rDNA measurement) and sample quality (28S rRNA 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.

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