# QuantiGene Sample Processing Kit

Fresh or Frozen Tissues

**Catalog Number** QS0104 10 samples, QS0105 25 samples, QS0106 100 samples

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**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 Tissue Homogenates contains reagents and instructions for the preparation of tissue homogenates from fresh or frozen tissues (animal or plant) for use in QuantiGene Singleplex and QuantiGene Plex Assays for RNA targets and QuantiGene Plex DNA assays for DNA targets.

For more information, refer to the appropriate user manual. For quantitating RNA targets, we recommend the use of QuantiGene Sample Assessment Kit to evaluate relative cell number and RNA quality of tissue homogenates. For more information, see the QuantiGene Sample Assessment Kit Package Insert.

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.

## Available procedures for cultured cells

- Preparing tissue homogenates from animal tissues using liquid nitrogen
- Preparing tissue homogenates from animal tissues stored in RNAlater
- Preparing tissue homogenates from plant tissues using Qiagen TissueLyser
- Preparing tissue homogenates from plant tissues using liquid nitrogen

## Contents and storage

Refer to product label for expiration date.

| Cat. No. | Kit size [1] | Component [2] | Quantity | Storage |
|---|---|---|---|---|
| QS0104 | 10 samples | Homogenizing Solution | 10 mL | 15–30°C |
| | | Proteinase K (50 µg/µL) | 36 µL | –20°C |
| QS0105 | 25 samples | Homogenizing Solution | 20 mL | 15–30°C |
| | | Proteinase K (50 µg/µL) | 90 µL | –20°C |
| QS0106 | 100 samples | Homogenizing Solution | 75 mL | 15–30°C |
| | | Proteinase K (50 µg/µL) | 360 µL | –20°C |

[1] A sample is defined as 5 mg animal tissue or 15 mg plant tissue.

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

## Materials required but not supplied

**Table 1 Materials for recommended procedure.**

| Item | Source |
|---|---|
| RNase*Zap*™ (if quantifying RNA) | Cat. No. AM9780 |
| Liquid nitrogen-cooled mortar (mortar, bowl, and housing) | Cat. No. 12-947-1 |
| Mortar (extra, ideal when preparing multiple samples) | Cat. No. 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 |

**Table 2 Materials for alternate procedure.**

| Item | Source |
|---|---|
| RNase*Zap*™ (if quantifying RNA) | Cat. No. AM9780 |
| RNA*later*™[1] or RNA*later*™+ICE[2] (if quantifying RNA) | Cat. No. AM7020 or AM7030 |
| One of the following: | |
| Dounce tissue grinder (for animal tissue only) | Cat. No. 06434 |
| or | |
| TissueLyser | Qiagen (Cat. No. 85300) |
| TissueLyser Adapter Set 2 × 96 | Qiagen (Cat. No. 69984) |
| Collection Microtubes (racked, 96) | Qiagen (Cat. No. 19560) |
| Collection Microtube Caps | Qiagen (Cat. No. 19566) |
| 5-mm Stainless Steel Bead (for animal tissue) | Qiagen (Cat. No. 69989) |
| 3-mm Tungsten Carbide Bead (for plant tissue) | Qiagen (Cat. No. 69997) |

[1] For preparing fresh tissue.

[2] For preparing frozen tissue.

## Preparing tissue homogenates from animal tissues (recommended)

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

- Ensure samples for preparation are on dry ice.
- Pre-chill tubes, spatula, mortar and pestle on dry ice.
- If tissues have been stored in RNAlater, they will become rubbery and difficult to process with liquid nitrogen. Consider using the alternate procedure described in "Preparing tissue homogenates from animal tissues stores in RNA*later*™ (alternate)" on page 2.
- Safety goggles should be worn at all times during this procedure.

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 homogentization" on page 3.

5. Add a small amount of liquid nitrogen (LN2) to a clean mortar while it is sitting on dry ice.
6. Add the preweighed 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. When the tissue becomes a fine powder, allow the LN2 to evaporate, then transfer the powder to an appropriate sized prechilled 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 × *g* for 15 minutes to pellet any remaining cellular debris, then transfer the supernatant to a new tube. Repeat this step once more.
13. Use the homogenate immediately in a QuantiGene Singleplex or QuantiGene Plex assay, or store at –80°C for later use.

## Preparing tissue homogenates from animal tissues stores in RNA*later*™ (alternate)

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 sample types: bone, muscle, pancreas, stomach, and jejunum.

**IMPORTANT!** If quantitating RNA targets, we do not recommend the use of this procedure if samples have not been preserved in RNA*later*™ or RNA*later*™+ICE. Homogenizing fresh or frozen samples at room temperature exposes the sample to significant RNA degradation.

If you are quantitating RNA targets, treat all surfaces with RNase*Zap*™ according to the manufacturer's recommendations.

1. (If quantitating RNA only.) Place tissue in 5 volumes of RNA*later*™ or RNA*later*™+ICE, and incubate according to the manufacturer's recommendations:
   - Fresh tissue in RNA*later*™ at 4°C for 16 hours
   - Frozen tissue in ice-cold RNA*later*™+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 RNA*later*™ by blotting tissue on laboratory wipes.

   **Note:** Carry over of RNA*later*™ or RNA*later*™+ICE may interfere with QuantiGene 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 Cat. No. 19560).
   b. Add one 5-mm Stainless Steel Bead (Qiagen Cat. No. 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 × *g* for 15 minutes to pellet any remaining debris, then transfer the supernatant to a new microcentrifuge tube. Repeat this step once more.
7. Use tissue homogenate immediately in a QuantiGene Singleplex or QuantiGene Plex Assay, or store at –80°C for later use.

## Preparing tissue homogenates from plant tissues (recommended)

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.

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 Cat. No. 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 "Troubleshooting" on page 3.

   **Note:** As an alternative method, transfer samples to 2 mL microcentrifuge tubes and centrifuge the sample at 16,000 × *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 Singleplex or QuantiGene Plex assay, or store at –80°C.

## Preparing tissue homogenates from plant tissues (alternate)

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

- Ensure samples for preparation are on dry ice. Prechill tubes, spatula, mortar, and pestle on dry ice.
- Safety goggles should be worn at all times during this procedure.

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 homogentization" on page 3.
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. **Never grind the tissue without LN2.**
8. When 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 × *g* for 15 minutes to pellet any remaining cellular debris, then transfer the supernatant to a new tube. Repeat this step once more.
12. Use the homogenate immediately in a QuantiGene Singleplex or QuantiGene Plex assay, or store at –80°C.

## Determining complete tissue homogentization

Validate your homogenate by doing the following:

1. Examine the homogenate. It should be clear and non-viscous.
2. Perform a serial dilution of the homogenate and run an appropriate QuantiGene Singleplex 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.

## Troubleshooting

| Observation | Possible cause | Solution |
|---|---|---|
| Filtration issues with QuantiGene Plex assay. | Prepared samples are not cleared of all debris following homogenization. | Pre-filter the sample. |
| When using RNA*later*™, tissue is rubbery and difficult to homogenize. | This is a known phenomenon with preservation of RNA using RNA*later*™ or RNA*later*™+ICE. | Use our alternate method for homogenizing animal tissues. |
| Poor sensitivity in QuantiGene Singleplex or QuantiGene Plex assays. | Samples stored and/or prepared under nonoptimal conditions resulting in significant RNA or DNA degradation. | If quantitating RNA, run Sample Assessment Controls 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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  - Safety Data Sheets (SDSs; also known as MSDSs)

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

## Limited product warranty

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