QuantiGene Sample Processing Kit

Cultured Cells

Catalog Number QS0100 2 plates, QS0101 10 plates, QS0102 5 × 10 plates, QS0103 50 plates

Doc. Part No. 13060 Pub. No. MAN0017267 Rev. C.0

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 singleplex and multiplex QuantiGene assays for quantification of RNA or DNA targets directly from a variety of sample types. This QuantiGene Sample Processing Kit for cultured cells contains reagents and instructions for the preparation of cell lysates 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.

Available procedures for cultured cells

- Standard Procedures for Preparing Cell Lysates
- Procedure for Preparing Cell Lysates when Wells have Varying Amounts of Media
- Procedure for Preparing Cell Lysates from Difficult to Lyse Cell Lines
- Procedure for Preparing Cell Lysates from cells grown in 3D Cell Matrix
- Procedure for Preparing Cell Lysates from Fresh or Frozen Cell Pellets
- Procedure for Preparing Cell Lysates from Peripheral Blood Mononuclear Cells (PBMC)
- Alternative Lysis Methods for HTS Users

Contents and storage

Refer to product label for expiration date.

Cat. No.	QS0100	QS0101	QS0102	QS0103	Storage
Kit size	2-plate[1]	10-plate	5 × 10-plate	50-plate	
Component	Quantity	Quantity	Quantity	Quantity	
Lysis mixture[2]	15 mL	75 mL	5 × 75 mL	350 mL	15–30°C
Proteinase K[3]	150 µL	750 µL	5 × 750 µL	3.5 mL	–20°C

[1] A 2-plate kit is sufficient for preparing bulk lysates from up to approximately 1.8×10^7 cells or 2 × 96-well plates containing up to 6×10^4 cells/well.

[2] Before use, redissolve any precipitates by incubating at 37°C, followed by gentle swirling.

[3] Place on ice during use. Store at –20°C in an enzyme storage box, for example NEB Cool Box (New England Biolabs Cat. No. T0400S). NEVER store at –80 °C.

General lysis guidelines

Key factors that influence the complete lysis of the cells include: the Lysis Mixture, Proteinase K, vortexing or pipetting of the sample, storage of the lysates at –80 °C, and incubation at 50°C. The procedures listed in this package insert utilize a combination of these factors to ensure complete lysis of the samples.

The Lysis Mixture provides the proper ionic conditions for the overnight hybridization in the QuantiGene assays and the final concentration of Lysis Mixture should always be 33%. For example, you can lyse cultured cells in their serum-containing media by adding 1/2 volume of undiluted Working Lysis Mixture. Alternatively, you can remove the culture media and lyse in Diluted Working Lysis Mixture (1 volume Working Lysis Mixture plus 2 volumes RNase-free water).

An important consideration when preparing cell lysates for QuantiGene assays is cell density. As cell density increases, so does lysate viscosity. The final cell density should not exceed 400 cells per µL of final lysate when using typical cell lines. The choice of the two lysis methods mentioned above should also take this into consideration. Certain cells, such as PBMCs, can be lysed at higher densities. High viscosity of the lysate can cause poor assay performance. High viscosity of the lysate can also be caused by the presence of un-sheared genomic DNA. Additional pipetting, sonication, or freezing of the sample can aid in shearing the genomic DNA and reducing the viscosity of the sample.

Standard procedure for preparing cell lysates

Use this standard procedure for preparing cell lysates when the volumes of culture media are constant in all wells.

1. Pre-warm the Lysis Mixture at 37°C for 30 minutes, followed by gentle swirling.
2. Prepare Working Lysis Mixture by adding 10 µL of Proteinase K to each mL of Lysis Mixture required. For example, add 65 µL of Proteinase K to 6.5 mL of Lysis Mixture. This volume is sufficient to lyse cells grown in a 96-well plate, using multichannel pipettes and reagent reservoirs.
3. Add 1/2 volume of Working Lysis Mixture to cells in culture media. For example, add 50 µL/well of Working Lysis Mixture to each well of a 96-well cell culture plate containing cells in 100 µL of culture media per well.
4. Thoroughly mix the contents of each well by pipetting up and down 10–15 times. Avoid introducing bubbles. If processing multiple plates, you can use a 96-pipetting station to speed processing. Set pipet tip volume equal to 3/4 of the total lysis volume and pipet speed to maximum.
5. Cover the plate with a lid and incubate at 50–55°C for 30 minutes to lyse the cells.
6. After incubation, mix the contents of each well by pipetting up and down 10–15 times. Examine cells under a microscope to ensure complete cell lysis.
7. Use lysates immediately in a QuantiGene or QuantiGene Plex assays, or store at –80°C for later use.

Preparing cell lysates when wells have varying amounts of media

Use this procedure when plates have different volumes of culture media as a result of evaporation at the edge wells.

1. Pre-warm the Lysis Mixture at 37°C for 30 minutes, followed by gentle swirling.
2. Prepare Working Lysis Mixture by adding 10 µL of Proteinase K to each mL of Lysis Mixture required. For example, add 65 µL of Proteinase K to 6.5 mL of Lysis Mixture.
3. Prepare Diluted Working Lysis Mixture by adding 2 volumes of RNase-free water to each volume of Working Lysis Mixture. For example, add 13 mL of RNase-free water to 6.5 mL of Working Lysis Mixture.
4. Remove the culture media from the cells and add an appropriate volume of Diluted Working Lysis Mixture to achieve the desired lysate concentration (cells/µL).
5. Thoroughly mix the contents by pipetting up and down 10–15 times. Avoid introducing bubbles. If processing multiple plates, you can use a 96-pipetting station to speed processing. Set pipet tip volume to 3/4 of the total lysis volume and pipet speed to maximum.
6. Cover the plate with a lid and incubate at 50–55°C for 30 minutes to lyse the cells.
7. After incubation, mix the contents of each well by pipetting up and down 10–15 times. Examine cells under a microscope to ensure complete cell lysis.
8. Use lysates immediately in a QuantiGene or QuantiGene Plex assay, or store at –80°C for later use.

Preparing cell lysates from difficult to lyse cell lines

Use this procedure to prepare lysates from difficult to lyse cell lines such as hepatocytes, 3T3L1, and adipocytes.

1. Pre-warm the Lysis Mixture at 37°C for 30 minutes, followed by gentle swirling.
2. Prepare Working Lysis Mixture by adding 10 µL of Proteinase K to each mL of Lysis Mixture required. For example, add 65 µL of Proteinase K to 6.5 mL of Lysis Mixture.
3. Prepare Diluted Working Lysis Mixture by adding 2 volumes of RNase-free water to each volume of Working Lysis Mixture. For example, add 13 mL of RNase-free water to 6.5 mL of Working Lysis Mixture.
4. Remove the culture media from the cells and add an appropriate volume of Diluted Working Lysis Mixture to achieve the desired lysate concentration (cells/µL).
5. Thoroughly mix the contents by pipetting up and down 10–15 times. Avoid introducing bubbles. If processing multiple plates, you can use a 96-pipetting station to speed processing. Set pipet tip volume to 3/4 of the total lysis volume and pipet speed to maximum.
6. Cover the plate with a lid and incubate at 50–55°C for 30 minutes to lyse the cells.
7. After incubation, mix the contents of each well by pipetting up and down 10 times. Examine cells under a microscope to determine Lysis efficiency.
8. Cover the plate with a lid and incubate at 50–55°C for an additional 30 minutes.
9. After incubation, mix the contents of each well by pipetting up and down 10–15 times. Examine cells under a microscope to determine Lysis efficiency. If the cells are still not completely lysed repeat steps 8 and 9.
10. Use lysates immediately in a QuantiGene or QuantiGene Plex assay, or store at –80°C for later use,

Preparing cell lysates from cells grown in 3D cell matrix

Use this procedure to prepare cell lysates from cells grown in a 3-dimensional cell chamber (MatTek 9-mm cell chamber)

1. Pre-warm the Lysis Mixture at 37°C for 30 minutes, followed by gentle swirling.
2. Prepare Working Lysis Mixture by adding 10 µL of Proteinase K per mL of Lysis Mixture required.
3. Prepare diluted Working Lysis Mixture by adding 2 volumes of nuclease-free water to 1 volume of Working Lysis Mixture.
4. Remove the culture media from the cell matrix and add 500 µL of Diluted Working Lysis Mixture to each chamber (9-mm diameter).
5. Cover the well with the lid and incubate at 50–55°C for 30 minutes to lyse the cells.
6. Transfer the contents from the chamber to a 1.5 mL Eppendorf tube.
7. Vortex the tube for 1 minute at the highest speed and store the tube at –80°C.
8. The following day, thaw the frozen lysate on the bench for 30 minutes.
9. Add 2 µL of Proteinase K to each tube.
10. Vortex and incubate at 50–55°C for 30 minutes.
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.
12. Use lysate immediately in a QuantiGene or QuantiGene Plex assay, or store at –80°C for later use.

Preparing cell lysates from fresh or frozen cell pellets

Use this procedure to prepare cell lysates from cell pellets.

1. Pre-warm the Lysis Mixture at 37°C for 30 minutes, followed by gentle swirling.
2. Prepare Working Lysis Mixture by adding 10 µL of Proteinase K per mL of Lysis Mixture required.
3. Prepare diluted Working Lysis Mixture by adding 2 volumes of nuclease-free water to 1 volume of Working Lysis Mixture. Determine the amount of diluted Working Lysis Mixture needed to lyse the cell pellet at a final concentration of 400 cells/µL.
4. Pellet the fresh cells or remove the cell pellet from the –80°C freezer.
5. Add the predetermined amount of diluted Working Lysis Mixture directly to the cell pellet. Pipette up and down 10–15 times to dissolve the cell pellet, followed by vortexing at maximum speed for 1 minute.
6. Incubate at 50–55°C for 30 minutes.
7. After incubation, vortex at maximum speed for 1 minute and check the viscosity of the lysate using a P1000 pipette.
   - If the viscosity of the lysate is similar to the lysis mixture, proceed to step 8.
   - If the lysate is viscous, pipette up and down 10–15 times and incubate for an additional 30 minutes at 50–55°C. Repeat step if the lysate is still viscous. If not, proceed to step 8.
8. Use lysate immediately in a QuantiGene or QuantiGene Plex assay, or store at –80°C for later use.

Preparing cell lysates from peripheral blood mononuclear cells (PBMC)

Use this procedure to prepare cell lysates from PBMCs.

1. Pre-warm the Lysis Mixture at 37°C for 30 minutes, followed by gentle swirling.
2. Prepare Working Lysis Mixture by adding 20 µL of Proteinase K per mL of Lysis Mixture required.
3. Prepare diluted Working Lysis Mixture by adding 2 volumes of nuclease-free water to 1 volume of Working Lysis Mixture. Determine the amount of diluted Working Lysis Mixture needed to lyse the cell pellet at a final concentration of 1,000 cells/µL.
4. Pellet the fresh cells or remove the cell pellet from the –80°C freezer.
5. Add the predetermined amount of diluted Working Lysis Mixture directly to the cell pellet. Pipette up and down 10–15 times to dissolve the cell pellet, followed by vortexing at maximum speed for 1 minute.
6. Incubate at 50–55°C for 30 minutes.
7. After incubation, vortex at maximum speed for 1 minute and check the viscosity of the lysate using a P1000 pipette. If the viscosity of the lysate is similar to the lysis mixture, proceed to step 8. If the lysate is viscous, pipette up and down 10-15 times and incubate for an additional 30 minutes at 50–55 °C.
8. After incubation, vortex at maximum speed for 1 minute and check viscosity of the lysate.
9. Use lysate immediately in a QuantiGene™ or QuantiGene™ Plex assay, or store at –80 °C for later use.

Alternative lysis methods for HTS users

The following procedures are two different freeze/thaw lysis methods that have been successfully used by high-throughput users.

Method 1

1. Add 1/4 volume of Lysis Mixture (no Proteinase K) to the tissue culture plate.
2. Pipette up and down 2–3 times.
3. Place plate at –20 or –80°C until frozen.
4. Thaw tissue culture plate in refrigerator (0–4°C). Optional. Repeat steps 3 and 4 twice.
5. Add 1/4 volume of Working Lysis Mixture (6.5 mL of Lysis Mixture + 65 µL of Proteinase K).
6. Incubate plate at 37–55°C for 30 minutes.

Method 2

1. Add 1/2 volume of Working Lysis Mixture (6.5 mL of Lysis Mixture + 65 µL of Proteinase K).
2. Pipette up and down 2–3 times.
3. Place plate at –20 or –80°C until frozen.
4. Incubate plate for 30–60 minutes at 37–55°C.

Determining complete cell lysis

Validate your cell lysates by doing the following.

- If possible, view the cells under the microscope to ensure that all of the cells have been lysed.
- Examine the cell lysate. It should be clear and non-viscous. The viscosity of the lysate should be similar to the viscosity of the Lysis Mixture.
- Perform a serial dilution of the cell lysate and run an appropriate QuantiGene Singleplex or QuantiGene Plex assay.
- Verify the expected fold change of assay signal matches the observed fold change for the dilution series. Note that assay signal may also show saturation, depending on the expression level of the target. For example, a 3-fold dilution should generate 3-fold changes (±20%) in the signal (background subtracted) of the targeted genes. The average CV should be less than 15%.

Safety warnings and precautions

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.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.