Panomics

QuantiGene® Sample Processing Kit

Cultured Cells

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 Cultured Cells contains reagents and instructions for the preparation of cultured cell lysates for use in QuantiGene, QuantiGene 2.0. QuantiGene Plex, and QuantiGene Plex 2.0 assays. For more information, refer to the appropriate *QuantiGene Reagent System User Manual*.

Contents and Storage

Cat. No.	QS0100	QS0101	QS0102	QS0103	
Kit Size	**2-Plate[a]**	**10-Plate**	**5 x 10-Plates**	**50-Plate**	
Component	**Quantity**	**Quantity**	**Quantity**	**Quantity**	**Storage**
Lysis Mixture[b]	15 mL	75 mL	5 x 75 mL	350 mL	15–30 °C
Proteinase K[c] (50 µg/µL)	150 µL	750 µL	5 x 750 µL	3.5 mL	–20 °C

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

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

c. 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.

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.

Procedure for Preparing Cultured Cell Lysates

About Lysing with Lysis Mixture

In addition to lysing cells, Lysis Mixture provides the proper ionic conditions for the overnight hybridization in QuantiGene assays. Therefore, the final concentration of Lysis Mixture is always 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).

Sample Input Recommendations

Cell lysates should be prepared from a sufficient number of cells to enable quantification of the lowest abundance RNA based on a maximal sample input of 80 µL and the sensitivity of the QuantiGene, QuantiGene 2.0, QuantiGene Plex, or QuantiGene Plex 2.0 assay to be used. Use the table below as a guide.

Target RNA Abundance (copy number/cell)	Recommended Number of Cells/ 80 µL of Lysate				
	QuantiGene		QuantiGene Plex		
	1.0	2.0	1.0	2.0	2.0 Mag
1	N/A	6,000	N/A	30,000	20,000
10	6,000	600	25,000	3,200	2,000
100	600	60	2,500	320	200
≥ 1000	≤ 60	≤ 6	250	32	20

IMPORTANT We do not recommend preparing lysates with greater than 400 cells/µL. If higher density preparations are required, validate complete lysis following the guidelines in “Determining Complete Cell Lysis” on page 3.

Standard Procedure

Use the following procedure when the volumes of culture media are constant in all wells.

Step	Action
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 tissue culture plate containing cells in 100 µL of culture media per well. **IMPORTANT** If volumes of culture media are not constant in all wells, for example because of increased evaporation from edge wells, use the alternative procedure on page 3.
4	Thoroughly mix the contents of each well by pipetting up and down 30 times. Avoid introducing bubbles. **Note** 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 °C for 30 minutes to lyse the cells. **IMPORTANT** Examine cells under a microscope to ensure complete cell lysis.

Step	Action
6	Mix contents of each well by pipetting up and down 30 times. Avoid introducing bubbles. If cell lysates have been prepared from cells grown in a 3D matrix, we recommend an additional procedure to clarify the sample. See "Clarifying Lysates from 3D Matrices Cultures" on page 4 for instructions.
7	Use lysates immediately in a QuantiGene or QuantiGene Plex assay, or store at –80 °C for later use.

Alternate Procedure

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

To prepare lysates using the alternate procedure:

Step	Action
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 30 times. Avoid introducing bubbles. **Note** 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 °C for 30 minutes to lyse the cells. **IMPORTANT** Examine cells under a microscope to ensure complete cell lysis.
7	Mix contents of each well by pipetting up and down 30 times. Avoid introducing bubbles. If cell lysates have been prepared from cells grown in a 3D matrix, we recommend an additional procedure to clarify the sample. See "Clarifying Lysates from 3D Matrices Cultures" on page 4 for instructions.
8	Use lysates immediately in a QuantiGene or QuantiGene Plex assay, or store at –80 °C for later use.

Determining Complete Cell Lysis

The best data is obtained when cells are completely lysed and the lysate is prepared correctly. Proper lysis ensures that all of the RNA has been released and is protected from degradation.

To determine if your cell lysis is complete:

- Examine the lysate for viscosity. It should not be viscous.
- Perform a serial dilution of the lysate 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 Lysates from 3D Matrices Cultures

When using the QuantiGene Plex assay, it is very important that all extracellular debris is removed from the lysate. 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.

Troubleshooting

Observation	Possible Cause	Recommended Action
Lysates too viscous to pipette accurately	Incomplete cell lysis. Cells are lysed at too high a density, or have not been pipetted up and down 30 times as specified in this procedure.	Reduce cell density to less than 400 cells/µL using Diluted Lysis Mixture (1 volume Lysis Mixture and 2 volumes RNase-free water). a. Pipet sample up and down 30 times. b. Incubate for 30 minutes at 50 °C. c. Pipet sample up and down 30 times.
Intact cells remain after 30 minute incubation	Incomplete cell lysis, see above.	See above.
Signals don’t scale with sample input in the QuantiGene or QuantiGene Plex assay	Incomplete cell lysis, see above.	See above.

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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