Panomics

QuantiGene Technical Note

Preparation of Bacterial Homogenates for Use in QuantiGene and QuantiGene Plex Assays

About this Technical Note

Instructions are provided for the preparation of bacteria homogenates for use in QuantiGene 2.0 or QuantiGene Plex 2.0 assays. For more information about these assays, please refer to the appropriate QuantiGene Reagent System User Manual.

Required Materials

Item	Source
Homogenizing Solution	Panomics P/N QS0518, QS0516 or QG0517
Proteinase K (50 µg/µL)	Panomics P/N QS0510, QS0511, QS0512
Ready-Lyse Lysozyme Solution for Nucleic Acid Extraction, 2 x 10^6 U	Epicentre P/N R1802M
TE buffer (10 mM Tris-HCl pH 8.1, 1 mM EDTA pH 8.0)	Ambion P/N 9858
Sodium Chloride Solution, 5M	Sigma P/N S-5150

About this Procedure

This procedure is for the preparation of gram-negative and gram-positive bacterial homogenates for use in QuantiGene 2.0 or QuantiGene Plex 2.0 assays.

Preparing Bacterial Homogenates

To prepare bacterial homogenates:

Step	Action
1	Grow bacteria strain to 10^9 cells.
2	Prepare 4 mL TES buffer (10 mM Tris pH 7.5, 1 mM EDTA, 100 mM NaCl) by adding 3.92 mL TE buffer and 80 µL 5M NaCl solution. Vortex to mix and place on ice.
3	Pellet cells by centrifuging at 4000 rpm for 10 minutes. Discard supernatant, leaving approximately 25 µL of liquid so as not to disturb the pellet.
4	Resuspend cells in 300 µL of cold TES buffer.
5	Dilute Ready-Lyse Lysozyme solution to a concentration of 250 U/µL in TES.
6	Add 3 µL of diluted Ready-Lyse Lysozyme to each tube of 10^9 cells and swirl gently.
7	Incubate at 25 °C for 15 minutes with occasional swirling to remove the cell wall.
8	Add 300 µL of QuantiGene Homogenization Solution and 3 µL of Proteinase K (50 µg/µL). Vortex at maximal speed for 1 minute.
9	Digest at 65 °C for 15 minutes. The final volume is approximately 600 µL. Complete lysing is indicated by solution turning clear with no debris.
10	Use sample immediately in a QuantiGene 2.0 or QuantiGene Plex 2.0 assay, or store at –80 °C for later use.

Determining Complete Sample Homogenization

We strongly recommend that you validate your bacterial homogenization process by doing the following:

- Examine the homogenate. It should be clear of any debris.
- Perform a serial dilution of the homogenate and run a QuantiGene 2.0 or QuantiGene Plex 2.0 assay on the dilution series.
- Verify the expected fold change matches the observed fold change. For example, a 3-fold dilution should generate a 3-fold change (± 20%) in the signal (background subtracted) of the target genes.

Running the QuantiGene 2.0 or QuantiGene Plex 2.0 Assay

Refer to the QuantiGene 2.0 or the QuantiGene Plex 2.0 User Manual as appropriate. Follow the “Capturing Target RNA from Fresh, Frozen, or FFPE Animal Tissues” section for setup of the overnight hybridization reaction and the section “Signal Amplification and Detection of RNA Targets” for the day 2 procedure.

Recommended Sample Input

We recommend starting with three different cell numbers, 10^6/well, 10^5/well, and 10^4/well.

Troubleshooting

Please refer to the QuantiGene 2.0 or QuantiGene Plex 2.0 User Manual for troubleshooting.

Contacting Panomics

For technical support, contact the appropriate resource provided below based on your geographical location. For an updated list of FAQs and product support literature, visit our website at www.panomics.com.

Location	Contact Information
North America	1.877.726.6642 option 3 techsupport@panomics.com
Europe	+39.02.95.360.250 techsupport_europe@panomics.com
Asia	+86.10.59208157 techsupport_asia@panomics.com

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Panomics Inc., 6519 Dumbarton Circle, Fremont, CA 94555, Tel. (877) 726-6642