

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

About this Technical Note

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

Required Materials

Table 1 Required materials for preparation of bacterial homogenates

Item	Source
Homogenizing Solution	Affymetrix P/N QS0518, QG0516 or QG0517
Proteinase K (50 µg/µL)	Affymetrix 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:

1. Grow bacteria strain to 10⁹ 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 10⁹ cells and swirl gently.
7. Incubate at 25 ± 1 °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 ± 1 °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 the *QuantiGene Plex 2.0 User Manual* for troubleshooting.

Technical Help

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.affymetrix.com/panomics.

Table 2 Technical Support Contacts

Location	Contact Information
North America	1.877.726.6642 option 1, then option 3; pqbhelp@affymetrix.com
Europe	+44 1628-552550; techsupport_europe@affymetrix.com
Asia	+81 3 6430 430; techsupport_asia@affymetrix.com

US Headquarters

Affymetrix, Inc.
3420 Central Expressway
Santa Clara, CA 95051
Tel: 1-888-362-2447
Direct: 1-408-731-5000
Fax: 1-408-731-5380
Email: info@affymetrix.com
Email: orders_fremont@affymetrix.com
Email: pqbhelp@affymetrix.com

European Headquarters

Panomics Srl
Via Sardegna 1
20060 Vignate-Milano (Italy)
Tel: +39-02-95-360-250
Fax: +39-02-95-360-992
Email: info_europe@affymetrix.com
Email: order_europe@affymetrix.com
Email: techsupport_europe@affymetrix.com
Email: techsupport_asia@affymetrix.com

Asia Pacific Headquarters

Affymetrix Singapore Pte Ltd.
No.7 Gul Circle #2M-01/08
Keppel Logistics Building
Singapore 629563
Tel: +65-6395-7200
Fax: +65-6395-7300
Email: info_asia@affymetrix.com
Email: order_asia@affymetrix.com

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