Preparation of bacterial homogenates for use in QuantiGene and QuantiGene Plex Assays

Introduction

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

Materials and methods

Table 1. Required materials for preparation of bacterial homogenates.

Item	Source
Invitrogen™ QuantiGene™ Homogenizing Solution	Thermo Fisher Scientific (Cat. No. QS0518, QG0516, or QG0517)
Proteinase K (50 µg/µL)	Thermo Fisher Scientific (Cat. No. QS0510, QS0511, or QS0512)
Ready-Lyse™ Lysozyme Solution for Nucleic Acid Extraction, 2 x 10^6 U	Epicentre (Cat. No. R1802M)
TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)	Thermo Fisher Scientific (Cat. No. AM9858)
Sodium chloride solution, 5 M	Sigma (Cat. No. S-5150)

This procedure is for the preparation of homogenates from gram-negative and gram-positive bacteria, for use in QuantiGene or QuantiGene Plex Assays.

Preparing bacterial homogenates

To prepare a bacterial homogenate:

1. Grow the bacterial strain to enable collection of 10^9 cells.
2. Prepare 4 mL of TES buffer (10 mM Tris, pH 8.0, 1 mM EDTA, 100 mM NaCl) by combining 3.92 mL of TE buffer and 80 µL of 5 M NaCl solution. Vortex to mix, and place on ice.
3. Pellet 10^9 cells by centrifugation at 4,000 rpm for 10 min. Discard supernatant, leaving approximately 25 µL of liquid so as to not 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 Solution to the 10^9 cells, and swirl gently.
7. Incubate at 25°C for 15 min with occasional swirling to remove cell walls.
8. Add 300 µL of QuantiGene Homogenizing Solution and 3 µL of Proteinase K (50 µg/µL). Vortex at maximal speed for 1 min.
9. Digest at 65°C for 15 min. The final volume is approximately 600 µL. Complete lysis is indicated by the sample turning clear with no debris.
10. Use the lysed homogenate immediately in a QuantiGene or QuantiGene Plex Assay, or store at –80°C for later use.

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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.
- Serially dilute the homogenate and run a QuantiGene or QuantiGene Plex Assay on the dilution series.
- Verify that the expected fold change matches the observed fold change. For example, a 3-fold dilution should generate a 3-fold change (±20%) in the background-subtracted signal of the target genes.

Recommended sample input

For the assays, we recommend loading three different quantities of the homogenate, to represent different numbers of cells. We recommend sample inputs equivalent to 10^6 cells/well, 10^5 cells/well, and 10^4 cells/well.

Running the QuantiGene or QuantiGene Plex Assay

Refer to the QuantiGene or QuantiGene Plex 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 “Signal Amplification and Detection of RNA Targets” section for the day 2 procedure.

Troubleshooting

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

Technical help

For an updated list of FAQs and product support literature, visit our website at **thermofisher.com/quantigene**

Find out more at **thermofisher.com/quantigene**

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