Technical Note

Preparation of Plasma or Exosome Lysates for Use in QuantiGene 2.0 miRNA Assays

About this Technical Note

Instructions are provided for the preparation of plasma or exosome lysates for use in QuantiGene 2.0 miRNA assays. We would recommend the same procedure for working with serum samples, although we have not tested this procedure with serum samples. For more information on the QuantiGene 2.0 miRNA assay, please refer to the User Manual.

Based on our experience with other sample types, we would expect that all nucleic acids present are released and stabilized with the following procedures. Although we have not tested, we would expect these sample preparations to be compatible with our other QuantiGene and QuantiGene Plex assays, assuming the material present meets the minimum assay requirements for limit of detection.

Required Materials

Table 1 Required materials for plasma preparations

Item	Source
QuantiGene Sample Processing Kit, Blood Samples	Affymetrix P/N QS0110, QS0111 or QS0112
Optional. ExoQuick Exosome Precipitation Solution	System Biosciences P/N EXOQ5A-1 or EXOQ20A-1
Nuclease-free Water	Major Laboratory Supplier (MLS)
Orbital shaking incubator, 60 ± 1 °C, minimum shaking speed of 275 rpm	MLS

Preparing Plasma Lysates

To prepare plasma lysates:

1. If plasma samples have been refrigerated or frozen, thaw on ice. Mix each sample well by inverting 5-10 times before use.
2. Prewarm Lysis Mixture at 37 °C for 30 minutes, followed by gentle swirling.
3. Prepare an appropriate volume of Plasma Working Lysis Mixture by combining, in the order listed, the following reagents per assay well:
   - 27 µL Lysis Mixture
   - 6 µL nuclease-free water
   - 2 µL Proteinase K

   Vortex briefly to mix.

   NOTE: **Scale volumes according to the number of assays to be run. Include 20% overage.**

4. Combine 35 µL Plasma Working Lysis Mixture and 45 µL plasma for each assay well. Vortex immediately for 30-60 seconds.

   Scale volumes according to the number of assays to be run. Include 20% overage. For example, for 10 assay wells, combine 420 µL (10 assay wells + 20% = 12 assay wells * 35 µL/assay well = 420 µL) of Plasma Working Lysis Mixture with 540 µL (10 assay wells + 20% = 12 assay wells * 45 µL/assay well = 540 µL) of plasma.

   IMPORTANT: **Do not exceed 45 µL of plasma/assay well as incomplete lysis and non-linear results may occur.**

5. Incubate at 60 °C for 1 hour with shaking at a minimum of 275 rpm.
6. Use plasma lysates immediately in a QuantiGene 2.0 miRNA assay, or store at –80 °C for future use.

Preparing Plasma Exosome Lysates

To prepare plasma exosome lysates:

1. If plasma samples have been refrigerated or frozen, thaw on ice. Mix each sample well by inverting 5-10 times before use.
2. Combine 400 µL of plasma with 100.8 µL of ExoQuick Exosome Precipitation Solution for each assay well. Vortex to mix well and refrigerate overnight (at least 12 hours).

   Scale volumes according to the number of assays to be run. Include 20% overage. For example, for 10 assay wells, combine 4800 µL (10 assay wells + 20% = 12 assay wells * 400 µL/assay well = 4800 µL) of plasma with 1209.6 µL (10 assay wells + 20% = 12 assay wells * 100.8 µL/assay well = 1209.6 µL) of ExoQuick Exosome Precipitation Solution.
3. After overnight refrigeration, centrifuge ExoQuick/plasma mixture at 1500 x g for 30 minutes. After centrifugation, the exosomes may appear as a beige or white pellet.
4. Aspirate supernatant. Spin down residual ExoQuick solution by centrifugation at 1500 x g for 5 minutes. Remove all traces of solution by careful aspiration and avoid disturbing the precipitated exosome pellet.
5. Pre-warm Lysis Mixture at 37 °C for 30 minutes, followed by gentle swirling.
6. Prepare an appropriate volume of Exosome Working Lysis Mixture by combining, in the order listed, the following reagents per assay well:
   - 27 µL Lysis Mixture
   - 51 µL nuclease-free water
   - 2 µL Proteinase K

   Vortex briefly to mix.

   NOTE: Scale volumes according to the number of assays to be run. Include 20% overage.
7. Use 80 µL of Exosome Working Lysis Mixture to resuspend the exosome pellet for each assay well. Vortex to disturb and resuspend the pellet completely.

   Scale volumes according to the number of assays to be run. Include 20% overage. For example, for 10 assay wells, use 960 µL (10 assay wells + 20% = 12 assay wells * 80 µL/assay well = 960 µL) of Exosome Working Lysis Mixture to resuspend the exosome pellet precipitated from 4800 µL (10 assay wells + 20% = 12 assay wells * 400 µL/assay well = 4800 µL) of plasma.
8. Incubate at 60 °C for 1 hour with shaking at a minimum of 275 rpm.
9. Use exosome lysates immediately in a QuantiGene 2.0 miRNA assay, or store at –80 °C for future use.

Determining Complete Plasma or Plasma Exosome Lysis

We strongly recommend that you validate the sample preparation process by performing a serial dilution of the lysate and running a QuantiGene 2.0 miRNA assay on the dilution series. Dilutions of samples should be prepared using diluted Lysis Mixture (1 volume of Lysis Mixture plus 2 volumes of nuclease-free water, prepared fresh). 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 signal (background subtracted) of the target.

Recommended Sample Input

We recommend starting with the maximal sample input of 80 µL/well. If necessary, dilutions of the sample can be prepared using diluted Lysis Mixture (1 volume of Lysis Mixture plus 2 volumes of nuclease-free water, prepared fresh).

Running the QuantiGene 2.0 miRNA Assay

Refer to the *QuantiGene 2.0 miRNA Assay User Manual* and follow the section titled "Capturing Target miRNA from Cultured Cell or Whole Blood /PAXgene Blood" for setup of the overnight hybridization reaction and the section titled "Signal Amplification and Detection" for the day 2 procedure.

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
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Rev. A 110701