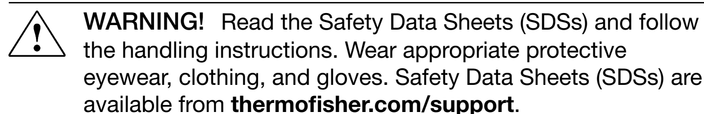
QuantiGene Sample Processing Kit

Blood Samples

Catalog Number QS0110 2 plates, QS0111 10 plates, and QS0112 5 × 10 plates

Doc. Part No. 13058 **Pub. No.** MAN0017265 **Rev.** C.0



WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

About sample processing kits

Sample processing kits are designed for use with QuantiGene Singleplex Assay Kits and Probe Sets or QuantiGene Plex Assay Kits and Plex Sets for quantitation of target-specific RNA directly from a variety of sample types. This QuantiGene Sample Processing Kit for Blood Samples contains reagents and instructions for the preparation of the following blood sample types:

1. Whole blood collected in heparin, citrate, or EDTA tubes
2. Whole blood collected in PAXgene™ blood RNA tubes
3. Whole blood collected in Tempus™ blood RNA tubes
4. Dried blood spots prepared from standard anticoagulant venous blood or finger sticks (referred to as DBS)

Refer to the *QuantiGene Singleplex User Manual* and *QuantiGene Plex Assay User Manual* for more information about running the assays.

Contents and storage

Refer to product label for expiration date.

Cat. No.	Kit size	Component [1,2]	Quantity	Storage
QS0110	2 plates	Proteinase K (50 µg/µL) Lysis Mixture	625 µL 10 mL	–20°C 15–30°C
QS0111	10 plates	Proteinase K (50 µg/µL) Lysis Mixture	3.25 mL 50 mL	–20°C 15–30°C
QS0112	5 × 10 plates	Proteinase K (50 µg/µL) Lysis Mixture	5 × 3.25 mL 5 × 50 mL	–20°C 15–30°C

[1] Place on ice during use. Store at -20 ºC in an enzyme storage box, for example NEB Cool Box (New England Biolabs T0400S). NEVER store at -80 ºC.

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

Preparing whole blood lysates

Use only freshly collected blood. Do not use partially or completely coagulated blood. Follow standard protocols to collect and store whole blood in anticoagulant tubes, then prepare lysates immediately. Storage of non-processed whole blood samples can result in significant RNA degradation. We do not recommend the use of blood that has been previously frozen.

QuantiGene Singleplex and QuantiGene Plex Reagent Systems are compatible with the following anticoagulants:

Anticoagulant	Description	Quantity	Source
Heparin	143 USP units of sodium heparin in a 10 mL collection tube	8–10 mL	Becton Dickinson P/N 366480
Citrate	1.50 mL of ACD-A liquid in a 8.5 mL collection tube	7–8.5 mL	Becton Dickinson P/N 364606
EDTA	10.5 mg K_3EDTA liquid in a 7 mL collection tube	6–7 mL	Covidien P/N 8881311545

1. Pre-warm the Lysis Mixture at 37°C for 30 minutes, followed by gentle swirling.
2. Prepare an appropriate volume of Whole Blood Working Lysis Mixture by combining, in the order listed, the following reagents per assay well and vortex to mix. Scale volumes according to the number of assays to be run.

Reagent	For 1 assay well
Lysis Mixture	32 µL
Water	50 µL
Proteinase K	2 µL
Total volume	84 µL

3. After drawing the blood, mix the tube by inverting 5–10 times and immediately proceed to the next step.
4. For each assay well to be used in the QuantiGene assay, prepare a mixture at a ration of 84 µL of Whole Blood Working Lysis Mixture to 12 µL whole blood. For example, if running technical triplicates, add 252 µL Whole Blood Working Lysis Mixture to 36 µL of whole blood to a 1.5 mL microcentrifuge tube. Vortex immediately for 30-60 seconds.
5. Incubate at 60°C for 1 hour using a VorTemp instrument with shaking at a minimum of 275 rpm.
6. If using immediately for the QuantiGene Singleplex for QuantiGene Plex Assay, keep lysate at room temperature. Do not chill. Alternatively, store at -80 ºC for future use.

Preparing PAXgene™ blood RNA lysates

Follow the manufacturer's recommendations for collecting and storing whole blood in PAXgene™ Blood RNA tubes. Information for ordering is provided below. For processing a small amount of blood sample, pipette the PAXgene™ Blood reagent into a microcentrifuge tube. Add 2.8X volume of PAXgene™ Blood reagent to 1 volume of blood. For example, for 20 µL of blood, add 56 µL of PAXgene™ Blood reagent (the total volume is 76 µL and that each QuantiGene assay well will use 65 µL of this mixture). Follow the instructions in the PAXgene™ Blood documentation.

Item	U.S. supplier	Outside U.S.
PAXgene™ Blood RNA tubes	VWR P/N 77776-026	PreAnalytiX (P/N 762165

1. If PAXgene™ Blood RNA tubes have been refrigerated for frozen, allow them to come completely to room temperature before use (approximately 2 hours), then vortex for 60 seconds to completely re-suspend any particulates.
2. Prewarm the Lysis Mixture at 37°C for 30 minutes, followed by gentle swirling.
3. Prepare an appropriate volume of PAXgene™ Blood Working Lysis Mixture, by combining, in the order listed, the following reagents per assay well and briefly vortexing to mix. Scale volumes according to the number of assays to be run.

Reagent	For 1 assay well
Lysis mixture	32 µL
Water	61 µL
Proteinase K	2 µL
Total volume	95 µL

4. For each assay well to be processed in the QuantiGene Singleplex or QuantiGene Plex assays, transfer 65 µL of PAXgene™ blood to a new 1.5 mL microcentrifuge tube. If running technical replicates, add 195 µL of PAXgene™ blood to a new tube.
5. Centrifuge the tubes at 3,000 × *g* for 5 minutes at room temperature to pellet nucleic acids and discard the supernatant. Do not exceed 3,000 × *g*.
6. Add 95 µL of PAXgene™ Blood Working Lysis Mixture for each assay well. In the previous example, if 195 µL of PAXgene™ blood was originally used, add 285 µL of PAXgene™ Blood Working Lysis Mixture to the tube and vortex for 1 minute on maximal setting to completely re-suspend the pellet.
7. Incubate at 60°C for 1 hour using a VorTemp instrument with shaking at a minimum of 275 rpm.
8. If using immediately for the QuantiGene Singleplex or QuantiGene Plex Assay, keep lysate at room temperature. Do not chill. Alternatively, store at –80°C for future use.

Preparing Tempus™ Blood RNA lysates

Follow the manufacturer's recommendations for collecting and storing whole blood in Tempus™ Blood RNA tubes. Information for ordering is provided below. Tempus™ blood sample processing for QuantiGene platforms involves centrifugation of the diluted lysate to collect RNA precipitate, followed by two washes with 70% chilled ethanol and re-suspending RNA in the Working Lysis Mixture.

Item	U.S. supplier
Tempus™ Blood RNA Tube	Cat. No. 4342792
PBS(Ca2+, Mg2+ free)	Cat. No. 14190-144
100% Ethanol	Major laboratory supplier

1. Draw 3 mL (indicated by a black mark on the tube) of blood directly into a Tempus™ Blood RNA tube under the guidelines of best laboratory practices for drawing blood from individuals. Total volume inside the tube will be 9 mL. Observe appropriate safety practices when drawing blood.
2. Immediately after the blood collection, mix the blood with the solution in the Tempus™ Tube by shaking vigorously or vortexing the tube for 10 seconds. At this point, the tube can be stored at –80°C or used in the next step.
3. Pre-warm the Lysis Mixture at 37°C for 30 minutes, followed by gentle swirling.
4. Make an appropriate volume of 70% ethanol and leave it on ice.
5. Prepare Working Lysis Mixture by combining, in the order listed, the following reagents and keep at room temperature. Scale volumes according to the number of assays to be run.

Reagent	For 1 tube Lysate
Lysis Mixture	334 µL
Water	641 µL
Proteinase K	25 µL
Total volume	1,000 µL

6. If the Tempus™ Blood Mixture is frozen, thaw completely by leaving it at room temperature (18–25°C).
7. Invert the tubes 10 times to mix.
8. Using a serological pipette, transfer all of the Tempus™ Blood Mixture to a clean 50 mL conical tube. The Tempus™ Blood Mixture must be at 9 mL. If the initial Tempus™ Blood Mixture volume is less than 9 mL, adjust the volume to 9 mL using calcium and magnesium free phosphate buffered saline (PBS). Failure to do so will result in significantly lower RNA yield.
9. Pipette 3 mL of 1x PBS (Ca2+, Mg2+ free) into the tube to bring the volume to 12 mL.
10. Tightly replace cap on the tube, then vortex the tube vigorously at maximal speed for 2 minutes to ensure proper mixing of the content. Sample will become very frothy at this step; this is normal.
11. Centrifuge the tube with a bench-top centrifuge at 4°C, 4,000 rpm (3200 × *g*) for 30 minutes.
12. Remove the supernatant by gently pouring it off into a waste container. Note that the RNA pellet is transparent and invisible. Handle the tube carefully not to dislodge the RNA pellet from the bottom of the tube.
13. Leave the tube inverted on absorbent paper for 2 minutes, then pipette 5 mL of chilled 70% ethanol into the tube.
14. Cap tube tightly and spin at 4°C, 4,000 rpm for 10 minutes.
15. Remove the supernatant by gently pouring it off into a waste container. angle not to disturb the RNA pellet at the bottom of the tube.
16. Pipette 5 mL of chilled 70% ethanol into the tube and cap tube tightly and spin at 4°C, 4,000 rpm for 10 minutes.
17. Remove the supernatant by gently pouring it off into a waste container. Be careful not to disturb the RNA pellet at the bottom of the tube.
18. Leave the tube inverted on absorbent paper for 10 minutes followed by adding 1 mL of Working Lysis Mixture to the tube.

19. Vortex at maximal speed for 30 seconds to mix, and incubate at 60°C for 30 minutes in an oven.
20. Remove the tube from the oven and vortex for 30 seconds to mix. Incubate the tube for an additional 30 minutes at 60°C.
21. If using immediately for the QuantiGene Singleplex or QuantiGene Plex Assay, keep lysate at room temperature. Do not chill. Alternatively, store at –80°C for future use.

Preparing dried blood spot lysates

Dried blood spot samples (DBS) must be collected and stored according to guidelines provided by the National Committee for Clinical Laboratory Standards. Sample collection errors that can result in unsuitable samples include inadequate absorption, non-uniform spots, and exposure of sample to direct sources of heat such as sunlight (CDC, Module 14, Blood Collection and Handling - Dried Blood Spots). A small quantity of blood, typically 50–100 µL, is required to make each dried blood spot. For preparing DBS, we recommend filter papers by Schleicher & Schuell or Whatman as noted below.

Item	Source
Filter paper	Schleicher & Schuell (P/N 903)
Filter paper	Whatman BFC 180
Microfuge tubes, 0.5-2.0 mL capacity	Major Laboratory Supplier (MLS)
Microfuge tubes, 5.0 mL capacity	United Laboratory Plastics (P/N UP-20336FS)
Hole punch, scalpel, or razor blades	MLS

1. Using a clean razor blade, scalpel, or hole puncher, cut the dried blood spots out of the pre-printed filter circles, and transfer each cutout to a 1.5 mL microcentrifuge tube.
2. Pre-warm the Lysis Mixture at 37 ºC for 30 minutes, followed by gentle swirling.
3. Prepare and appropriate volume of DBS Working Lysis Mixture by combining, in the order listed, the following reagents and vortexing briefly to mix. Scale volume according to the number of assays to be run.

Reagent	For 1 tube Lysate
Lysis Mixture	100 µL
Water	199 µL
Proteinase K	1 µL
Total volume	300 µL

4. Add 300 µL of DBS Working Lysis Mixture to each tube and vortex at maximal setting for 1 minute.
5. Incubate the samples at 60°C for 30 minutes. Vortex for 15 seconds once every 10 minutes during this incubation.
6. Transfer DBS lysates (approximately 200 µL) to a clean microcentrifuge tube.
7. Do not throw away the tubes with the filter paper cutout. Recover the remaining liquid trapped in the filter paper by performing the following steps:
   a. Using a clean razor blade, make an opening at the bottom of each tube containing a filter paper.
   b. Place each cut tube inside a 5-mL centrifuge tube.
   c. Spin the tubes at 2,000 × *g* for 5 minutes.
   d. Discard the tube with the filter paper, and transfer the liquid at the bottom of the 5-mL centrifuge tube to the microcentrifuge tube from step 6.

   Each prepared sample should have approximately 300 µL of lysate.
8. If using immediately for the QuantiGene Singleplex or QuantiGene Plex Assay, keep lysate at room temperature. Do not chill. Alternatively, store at –80°C for future use.

Sample collection and storage

Intersubject and intrasubject variation are important factors to consider for gene expression studies of blood. Intersubject variations may be related to age, gender, ethnic background, health, nutritional status, metabolism, and medical history. Intrasubject variations arise from biological influences within the body such as hormone variation or diurnal changes. To minimize the impact of these factors on blood gene expression analysis, include randomized samples in studies of sufficient sampling size. In addition, standardize any pretreatments, time of day of blood collection, and post-collection sample handling and storage. Strive to minimize the time between blood collection and preparation of blood lysates.

Determining complete blood cell lysis

We strongly recommend that you validate the sample preparation to ensure the collection of the highest quality data. After preparing blood lysates following one of the procedures above, perform a serial dilution of the prepared lysates and test the samples in a QuantiGene or QuantiGene Plex assay. Dilute the sample using the same Working Lysis Mixture that was used to prepare the samples. Verify that the expected fold change matches the observed fold changes of the target gene. For example, a 3-fold dilution should generate a 3-fold change (±20%) in the signal of the target gene.

Normalizing gene expression data from blood samples

Normalizing data between samples corrects for variations in cell number. Typically, data are normalized to the expression level of one or more invariant housekeeping genes. Blood, however, is one of the most variable tissue types in the body, and the relative proportions of the different blood cell types may vary significantly from time to time and from subject to subject, even though the total number of blood cells does not change significantly. Therefore, it may be necessary to normalize data to common housekeeping genes, blood cell type-specific markers, or both. Refer to our website for a table listing blood cell types and their relative abundance and for a list of available blood cell type-specific marker genes.

Two individuals have similar total white blood cell counts but 5-fold differences in monocyte counts (normal range of monocytes in WBC is 1–5%). If a monocyte-specific target gene, such as a chemokine induced by immune stimulation, is measured, induction levels in the two individuals will appear to differ by 5-fold if normalized to a general cellular housekeeping gene such as GAPDH, but will be equivalent if normalized to a monocyte-specific marker such as CD14.

Safety warnings and precautions

Treat all blood samples as potentially infectious. To avoid the risk of infection when working with blood, wear a lab coat and disposable gloves, and change gloves whenever they become contaminated. Waste can be decontaminated with 10% (v/v) bleach or sodium hypochlorite before being disposed of according to local, state, and federal regulations. If liquid containing potentially infectious agents is spilled, clean the affected area with 10% (v/v) bleach or sodium hypochlorite, then with water. All chemicals should be considered potentially hazardous. This product and its components should be handled by those trained in laboratory techniques and be used according to the principles of good laboratory practice.

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  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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