Package Insert

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QuantiGene Sample Processing Kit

Blood Samples

About the Blood Sample Processing Kits

Sample Processing Kits are designed for use with QuantiGene 2.0 Assay Kits and Probe Sets or QuantiGene Plex 2.0 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 and QuantiGene Plex User Manual for more information about running the assays.

Contents and Storage

Kit components have a shelf life of 12 months from the date of receipt.

Cat. No.	**QS0110**	**QS0111**	**QS0112**	
Kit Size	**2-Plate**	**10-Plate**	**5 x 10-Plates**	**Storage**
Component	**Quantity**	**Quantity**	**Quantity**	
Lysis Mixture*	10 mL	50 mL	5 x 50 mL	15-30 °C
Proteinase K† (50 µg/µL)	625 µL	3.25 mL	5 x 3.25 mL	–20 °C

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

† Place on ice during use. We recommend storage at –20 °C in an enzyme storage box, for example NEB Cool Box (New England Biolabs P/N T0400S). NEVER store at –80 °C.

1. 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 been frozen.

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

Anticoagulant	Description	Volume of Collection (mL)	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	Tyco Healthcare (P/N 311545)

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 uL
Water	49 uL
Proteinase K	2 uL
Total Volume	84 uL

3. After drawing the blood, mix the tube by inverting 5–10 and immediately proceed to the next step.
4. For each assay well to be used in the QuantiGene assay, prepare a mixture at a ratio 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 2.0 or QuantiGene Plex 2.0 Assay, keep lysate at room temperature, do not chill. Alternatively, store at –80 °C for future use.

2. 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, pipet 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 QG or QGP assay well will use 65 µLof 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 or frozen, allow them to come completely to room temperature before use (approximately 2 hours), then vortex for 60 seconds to completely resuspend any particulates.
2. Pre-warm 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 uL
Water	61 uL
Proteinase K	2 uL
Total Volume	95 uL

4. For each assay well to be processed in the QuantiGene 2.0 or QuantiGene Plex assays, transfer 65 µL of PAXgene blood to a new 1.5 mL microcentrifuge tube. If running technical triplicates, add 195 µL of PAXgene blood to a new tube.
5. Centrifuge the tubes at 3,000 x *g* for 5 minutes at room temperature to pellet nucleic acids and discard the supernatant. Do not exceed 3,000 x *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 resuspend 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 2.0 or QuantiGene Plex 2.0 Assay, keep lysate at room temperature, do not chill. Alternatively, store at –80 °C for future use.

3. 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 QG 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	Life Technologies P/N 4342792
PBS (Ca2+, Mg2+ free)	Life Technologies P/N 14190-144
100% Ethanol	Major Laboratory Supplier

1. Draw 3 mL (indicated by a black mark on the tube) of blood directly into 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 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 uL
Water	641 uL
Proteinase K	25 uL
Total Volume	1000 uL

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. Pipet 3 mL of 1x PBS (Ca^{2+}, Mg^{2+} 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, 4000 RPM (3200 xg) for 30 minutes.
12. Remove the supernatant by gently pouring it off into a waste container. Note that 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 pipet 5 mL of chilled 70% ethanol into the tube.
14. Cap tube tightly and spin at 4 °C, 4000 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. Pipet 5 mL of chilled 70% ethanol into the tube and cap tube tightly and spin at 4 °C, 4000 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 60 °C.
21. If using immediately for the QuantiGene 2.0 or QuantiGene Plex 2.0 Assay, keep lysate at room temperature, do not chill. Alternatively, store at –80 °C for future use.

4. 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 °C for 30 minutes, followed by gentle swirling.
3. Prepare an appropriate volume of DBS Working Lysis Mixture by combining the following in the order listed and vortexing briefly to mix. Scale volumes according to the number of assays to be run.

Reagent	For 1 DBS Sample
Lysis Mixture	100 uL
RNAase-free water	199 uL
Proteinase K	1 uL
Total Volume	300 uL

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 2000 x 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 2.0 or QuantiGene Plex 2.0 Assay, keep lysate at room temperature, do not chill. Alternatively, store at –80 °C for future use.

Sample Collection and Storage

Inter- and intra-subject variation are important factors to consider for gene expression studies of blood. Inter-subject variations may be related to age, gender, ethnic background, health, nutritional status, metabolism, and medical history. Intra-subject 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 pre-treatment(s), 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. Please refer to our website at www.affymetrix.com 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. We recommend that this product and its components be handled by those trained in laboratory techniques and used according to the principles of good laboratory practice.

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