Package Insert

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QuantiGene Sample Processing Kit

Blood Samples

About 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.

About this Kit

This QuantiGene Sample Processing Kit for Blood Samples contains reagents and instructions for the preparation of the following blood sample types for use in QuantiGene 2.0 and QuantiGene Plex 2.0 assays:

- EDTA, citrate, or heparin stabilized whole blood (referred to as whole blood)
- Whole blood collected in PAXgene® blood RNA tubes (referred to as PAXgene blood)
- Dried blood spots prepared from standard anticoagulant venous blood or finger sticks (referred to as DBS)

For more information on QuantiGene and QuantiGene Plex assays, refer to the appropriate *QuantiGene User Manual*.

Contents and Storage

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

Table 1 Contents and storage conditions for the sample processing kit

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

** A 2-plate kit is sufficient for preparing bulk lysates from up to approximately 1.8 x 10^7 cells or 2 x 96-well plates containing up to 6 x 10^4 cells/well.*

† 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.

Safety Warnings and Precautions

WARNING: 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.

WARNING: 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.

What this Package Insert Covers

This package insert provides recommendations and step-by-step procedures for the following:

- *Materials Required but not Supplied*
- *Sample Collection and Storage*
- *Preparing Whole Blood Lysates*
- *Preparing PAXgene Blood RNA Lysates*
- *Preparing Dried Blood Spot Lysates*
- *Determining Complete Blood Cell Lysis*
- *Normalizing Gene Expression Data from Blood Samples*

Materials Required but not Supplied

Table 2 Required materials not supplied

Item	Source
RNaseZap®	Ambion P/N AM9780
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
Orbital shaking incubator, 60 °C ± 1 °C, minimum 275 rpm	MLS
96-well plates, 1.2 mL wells†	Qiagen (P/N 19576)
Plate seals†	MLS

* *For dried blood spot procedure only.*
† *For high-throughput whole blood lysis.*

Sample Collection and Storage

General Guidelines

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.

The method of data normalization can also affect the results of blood gene expression studies. For more information, see *Determining Complete Blood Cell Lysis* on page 6.

Whole Blood

Follow standard protocols to collect and store whole blood in anticoagulant tubes, then prepare lysates immediately.

! IMPORTANT: Storage of non-processed whole blood samples can result in significant RNA degradation.

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

Table 3 Assay compatible 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)

PAXgene Blood RNA

Follow the manufacturer’s recommendations for collecting and storing whole blood in PAXgene Blood RNA tubes. Information for ordering is provided below.

Table 4 Ordering information for PAXgene Blood RNA tubes

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

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). Follow the instructions in the PAXgene Blood documentation.

Dried Blood Spots

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 either of the following types of filter paper:

Table 5 Recommended filter papers

Source	Part Number
Schleicher and Schuell	903
Whatman	BFC 180

Preparing Whole Blood Lysates

> **IMPORTANT: For best results, use freshly collected blood. Do not use partially or completely coagulated blood.**

To prepare whole blood lysates:

1. If whole blood samples have been refrigerated or frozen, thaw on ice. If freshly collected, keep at room temperature and use immediately.
   Mix each sample well by inverting 5–10 times before use.
2. Prewarm the Lysis Mixture at 37 °C for 30 minutes, followed by gentle swirling.
3. Prepare an appropriate volume of Whole Blood Working Lysis Mixture by combining in the order listed, the following reagents per assay well:
   - 27 µL Lysis Mixture
   - 41 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. For blood lysis in individual tubes, combine 70 µL Whole Blood Working Lysis Mixture and 10 µL whole blood 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 840 µL (10 assay wells + 20% = 12 assay wells x 70 µL/assay well = 840 µL) Whole Blood Working Lysis Mixture and 120 µL (10 assay wells + 20% = 12 assay wells x 10 µL/assay well = 120 µL) whole blood.
   For blood lysis using a 96-well plate (Qiagen, P/N 19576):
   - Pipet up and down 5 times
   - Cover with a plate seal and vortex for 15–30 seconds at maximal speed

   > **IMPORTANT: Do not exceed 320 µL of blood lysis preparation per well.**

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

Preparing PAXgene Blood RNA Lysates

To prepare PAXgene Blood RNA lysates:

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. Prepare an appropriate volume of PAXgene Blood 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.**

3. Transfer 54 µL of PAXgene blood per assay well to a new tube. Include 20% overage.

For example, for 10 assay wells, transfer 648 µL PAXgene blood (10 assay wells + 20% = 12 assay wells x 54 µL per assay well = 648 µL).

NOTE: 54 µL of PAXgene-stabilized blood is equivalent to approximately 15 µL of whole blood.

4. Centrifuge the tubes at 3000 x g for 5 minutes at room temperature to pellet nucleic acids and discard the supernatant.

IMPORTANT: Do not exceed 3000 x g.

5. Add 80 µL per assay of PAXgene Blood Working Lysis Mixture and vortex for 1 minute on maximal setting to completely resuspend the pellet.
   In the above example, add 960 µL PAXgene Blood Working Lysis Mixture (10 assay wells + 20% = 12 assay wells x 80 µL per assay well = 960 µL).
6. Incubate at 60 °C for 1 hour with shaking at a minimum of 275 rpm.
7. Use lysate immediately in a QuantiGene 2.0 or QuantiGene Plex 2.0 assay, or store at –80 °C for future use.

Preparing Dried Blood Spot Lysates

If dried blood spots have been refrigerated or frozen, allow them to come to room temperature.

To prepare dried blood spot lysates:

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 microcentrifuge tube.
2. Prepare an appropriate volume of DBS Working Lysis Mixture by combining per sample:
   - 100 µL Lysis Mixture
   - 200 µL RNase-free water
   - 1 µL Proteinase K

   Vortex briefly to mix.
3. Add 300 µL of DBS Working Lysis Mixture to each tube and vortex at maximal setting for 1 minute.
4. Incubate the samples at 60 °C for 30 minutes. Vortex for 15 seconds once every 10 minutes during this incubation.
5. Transfer DBS lysates (approximately 200 µL) to a clean microcentrifuge tube.

IMPORTANT: Do not throw away the tubes with the filter paper cutout.

6. Recover the remaining liquid trapped in the filter paper:
   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 5.

   Each prepared sample should have approximately 300 µL of lysate.
7. Use lysate immediately in a QuantiGene 2.0 or QuantiGene Plex 2.0 assay, or store at –80 °C for future use.

Determining Complete Blood Cell Lysis

We strongly recommend that you validate the sample preparation to ensure the collection of the highest quality data. To do this:

After preparing blood lysate 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. 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

Data Normalization

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.

Example

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.

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