Standard Operating Procedure X:
Blood Collection and Processing for Isolation of RNA

** **NOTE: The following procedure is to be performed wearing laboratory coat, gloves, eye protection, and mask.**

PRINCIPLE

The purpose of this procedure is to obtain total cellular RNA from human whole blood for the subsequent analysis of mRNA populations, either by the polymerase chain reaction (following reverse transcription) or by microarray analyses. This procedure does not contain an initial separation of leukocytes, but rather relies on the denaturing of proteins and precipitation of RNA/DNA from whole blood directly. Following lysis, total cellular RNA is removed from DNA and proteins by precipitation and chromatographic separation. A more detailed description of the principle and protocols can be found in the accompanying documentation from the *PAXgene ™Blood RNA Tube Circular* and the *PAXgene Blood RNA Kit Handbook, version 2.*

It is essential that prior to the use of the PAXgene ™system for collecting blood, the operator has fully read the PAXgene ™Blood RNA Handbook and understands the procedures and their potential risks to the operator and the patient.

PROCEDURE (Adapted from PAXgene Blood RNA Kit Handbook, version 2, Qiagen cat. # 762164)

N.B. All centrifugations are done at room temperature.

1. 2.5 mL arterial or venous whole blood is collected into a room temperature (18-25°C) PAXgene™ Blood RNA Tube. Allow at least 10 seconds for a complete blood draw to take place.

   The collection of blood should be obtained from an existing arterial or venous line, or venipuncture and should be performed by someone experienced in the technique and familiar with infectious precautions. The PAXgene™ Blood RNA tube is held vertically, below the donor's arm, during blood collection. If the PAXgene™ Blood RNA tube is the only tube to be collected, draw into a "discard tube" prior to using the PAXgene™ Blood RNA tube. Otherwise, the PAXgene™ Blood RNA tube should be the last tube drawn.

2. After blood collection, gently invert the PAXgene™ Blood RNA tube 8-10 times. Store the PAXgene™ Blood RNA tube at room temperature. If RNA isolation is to occur at a later time point, the tube may be stored at -70 ˚C.

3. After collection of the blood sample, incubate the PAXgene™ Blood RNA tube for *at least* 2 hours at room temperature to ensure complete lysis of the blood cellular constituents. Incubation overnight may increase yields. If the PAXgene Blood RNA Tubes were stored frozen after blood collection, first equilibrate to room temperature and then store it a room temperature for 2 hours before starting.

   Things to do before starting isolation: 1) Set the temperature of the incubator/water bath to 55˚C; 2) Buffer BR2 may need to be warmed at 37˚C if precipitates have formed; 3) Before using buffer BR4 for the first time, add 4 volumes of absolute ethanol; 4) If using DNase set for the first time, prepare DNase stock solution (see manual).

4. Centrifuge the PAXgene™ Blood RNA tube for 10 minutes at 3,300 x g at room temperature in a swinging-bucket centrifuge. Note: the rotor must contain tube adaptors for round bottom tubes or breakage may occur.

5. Remove the supernatent by decanting or pipetting and discard. Dry the rim of the tube with a clean Kimwipe™ if decanted. Add 4 ml RNase-free water (provided in the PAXgene™ kit) to the pellet and close the tube using a fresh secondary Hemogard closure (provided in the PAXgene™ kit).

6. Vortex thoroughly to dissolve the pellet, then centrifuge for 10 min at 3300 x g in a swinging bucket centrifuge. Decant and discard the entire supernatant.

7. Thoroughly resuspend the pellet in 350 µl Buffer BR1 by vortexing.

8. Using a micropipette**,** transfer the sample (usually 500-1200 µl) into a 1.5 ml microcentrifuge tube. Add 300 µl Buffer BR2 and 40 µl Proteinase K solution.

9. Mix by vortexing for 5 sec, and incubate for 10 minutes at 55°C using a shaker-incubator (at 400-1400 rpm), heating block, or water bath. If using a heating block or water bath, vortex each sample once during the incubation. Do not allow the temperature of the sample to decrease during vortexing. Once finished this step, set the temperature to 65˚C.

10. Pipet the lysate directly into the PAXgene Shredder spin column (lilac) placed in a 2 ml processing tube, and centrifuge for 3 minutes at maximum speed (do not exceed 20,000 x g). Carefully transfer the entire supernatant of the flow-through fraction to a fresh 1.5 ml microcentrifuge tube without disturbing the pellet in the processing tube.

11. Add 350 µl of 100% ethanol. Mix by vortexing and centrifuge briefly (1-2 seconds, 1,000 x g) to remove drops from the inside of the tube lid. Do not centrifuge for longer than 1-2 seconds as this may result in pelleting of the nucleic acids and reduced RNA yield.

12. Apply 700 µl of sample to the PAXgene™ RNA spin column (red) sitting in a 2 ml processing tube. Centrifuge for 1 minute at 10,000 x g. Place the PAXgene™ column in a new 2 ml processing tube and discard the old processing tube containing the flow-through.

13. Apply the remaining sample to the PAXgene™ RNA spin column and centrifuge for 1 minute at 10,000 x g. Again, place the PAXgene™ column in a new 2 ml processing tube and discard the old processing tube containing flow-through.

14. Pipet 350 µl of Buffer BR3 to the PAXgene™ RNA spin column and centrifuge for 1 minute at 10,000 x g. Place the PAXgene™ column in a new 2 ml processing tube and discard the old processing tube containing flow-through.

15. Pipet 10 µl DNase I stock solution (prepared before starting) into 70 µl of Buffer RDD in a 1.5 ml microcentrifuge tube. This is enough solution for 1 tube; if doing multiple, make enough for all the tubes. Mix by gently flicking the tube (*do not vortex*) and centrifuge briefly.

16. Pipet DNase I incubation mix (80 µl) directly onto PAXgene™ RNA spin column and place upright at room temperature for 15 minutes.

17. Pipet 350 µl Buffer BR3 to the PAXgene™ RNA spin column and centrifuge for 1 minute at 10,000 x g. Place the PAXgene™ column in a new 2 ml processing tube and discard the old processing tube containing flow-through.

18. Apply 500 µl Buffer BR4 (diluted with ethanol prior to use) to the PAXgene™ RNA spin column and centrifuge for 1 minute at 10,000 x g. Place the PAXgene™ column in a new 2 ml processing tube and discard the old processing tube containing flow-through.

19. Add another 500 µl Buffer BR4 to the PAXgene™ RNA spin column. Centrifuge for 3 minutes at maximum speed to dry the PAXgene™ column membrane (not greater than 20,000 x g).

20. To eliminate residual Buffer BR4, discard the tube containing the flow-through, place the PAXgene™ column in a 2 ml microcentrifuge tube, and centrifuge for 1 minute at full speed (not greater than 20,000 x g).

21. Discard the tube containing the flow-through and transfer the PAXgene™ RNA spin column to a 1.5 ml microcentrifuge tube. Pipet 40 µl Buffer BR5 directly onto the entire surface of the PAXgene™ column membrane (without touching the membrane with the pipet tip) and centrifuge for 1 minute at 10,000 x g.

22. Repeat the elution step (step 21) as described, using 40 µl Buffer BR5.

23. Incubate the eluate for exactly 5 minutes at 65°C in a shaker-incubator without shaking, heating block or water bath. Chill immediately on ice following incubation.

24. If the RNA samples are not to be used immediately, store at -70 ˚C until analysis.

Reagents

PAXgene™ Blood RNA Tube (PreAnalytiX, A Qiagen/BD Company) Cat# 762165
PAXgene™ Blood RNA Kit (Qiagen), Cat# 762164
Absolute ethanol, USP.

Special Note 1.1: CAUTION – do not add bleach or acidic solution directly to the sample-preparation waste.

Special Note 1.2: Un-purified RNA is stable in the PAXgene Blood RNA tubes for up to 50 months at -70 ˚C.

Special Note 1.3: Purification time for the PAXgene kit is approximately 90 minutes.

Special Note 1.4: RNA yield from 2.5 mL whole blood from healthy subjects is ≥3µg. Yields vary depending on the number of leukocytes present in the blood sample.

Special Note 1.5: DNase I may be stored at 4 ˚C for up to 6 weeks without loss of activity. If longer storage is required, aliquot into microcentrifuge tubes and store at -20˚C for up to 9 months. Do not refreeze thawed aliquots.