Standard Operating Procedures Clinical Protocol II:

RNA Isolation from human whole blood using PAXgene blood RNA system

** 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.

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.

METHOD

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

1.Arterial or venous whole blood is collected into a room temperature (18-25o C) PAXgene Blood RNA Tube (PAXgene, PreAnalytiX Inc., Hombrehtikon, Switzerland, distributed by QIAGEN, cat# 762115). 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 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 until the sample is processed.

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.

4. Centrifuge the PAXgene Blood RNA tube for 10 minutes at 3,300 x g in a swinging-bucket centrifuge (in a Beckman Model GPR centrifuge, or equivalent). All centrifugation steps in this protocol are carried out at room temperature, unless otherwise specified.

5. Remove and decant/discard the supernatant. Dry the rim of the tube with a clean Kimwipe. Add 5mL 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 resuspend 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 360µL Buffer BR1 (PAXgene kit) by vortexing.

8. Using a micropipette, transfer the sample (usually 500-1200µL) into a 1.5mL microcentrifuge tube (USA Scientific Cat# 1415-2600). Add 300µL Buffer BR2 (PAXgene) and 40µL Proteinase K solution (PAXgene).

9. Mix by vortexing, and incubate for 20 minutes at 55oC using a shaker-incubator, 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.

10. Centrifuge for 20 minutes at maximum speed in a microcentrifuge (Eppendorf model 5415C, maximum speed 14,000 rpm or 16,000 x g). Transfer the supernatant to a new 1.5mL microcentrifuge 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 column sitting in a 2mL processing tube (all supplied in the PAXgene kit). Centrifuge for 1 minute at $8,000 x g. Place the PAXgene column in a new 2mL processing tube and discard the old processing tube containing the flow-through.

13. Apply the remaining sample to the PAXgene column and centrifuge for 1 minute at $8,000 x g. Again, place the PAXgene column in a new 2mL processing tube and discard the old processing tube containing flow-through.

14. Pipet 350µL Buffer BR3 to the PAXgene column and centrifuge for 1 minute at $8,000 x g. Place the PAXgene column in a new 2mL processing tube and discard the old processing tube containing flow-through.

15. Prepare DNase I stock by dissolving solid DNase I (1500 Kunitz units; Qiagen, cat#79254) in 550µL of RNase free water and mix by inversion (1500 Kuntz Units/0.55mL). Pipet 10µL DNase I stock solution into 70µL of Buffer RDD. Mix by gently flicking the tube (do not vortex) and centrifuge briefly.

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

17. Pipet 350µL Buffer BR3 to the PAXgene column and centrifuge for 1 minute at $8,000 x g. Place the PAXgene column in a new 2mL processing tube and discard the old processing tube containing flow-through.

18. Apply 500µL Buffer BR4 to the PAXgene column and centrifuge for 1 minute at $8,000 x g. Place the PAXgene column in a new 2mL processing tube and discard the old processing tube containing flow-through. Note that Buffer BR4 is supplied as a concentrate. Ensure that the ethanol is added to Buffer BR4 prior to use.

19. Add another 500µL Buffer BR4 to the PAXgene column. Centrifuge for 3 minutes at maximum speed to dry the PAXgene column membrane.

20. To eliminate residual Buffer BR4, discard the tube containing the flow-through, place the PAXgene column in a 2mL processing tube (USA Scientific Cat# 1620-2700), and centrifuge for 1 minute at full speed.

21. Discard the tube containing the flow-through and transfer the PAXgene column to a 1.5mL elution tube (supplied as part of the PAXgene kit). Pipet 40µL Buffer BR5 directly on to the PAXgene column membrane (without touching the membrane with the pipet tip) and centrifuge for 1 minute at 8,000 x g.

22. Repeat the elution step (step 17) as described, using 40µL Buffer BR5.

23. Incubate the eluate for 5 minutes at 65oC in a heating block or water bath. Following incubation, chill immediately on ice.

24. Add 0.1 volumes of 3 M sodium acetate pH 5.2 to the eluate and then add 2.2 volumes of ice-cold absolute ethanol. Vortex and store overnight at -20 C.

25. Centrifuge at 16,000 x g (maximum) at 4 C for 30 minutes. Decant the ethanol, and Speed-Vac on low heat the remaining ethanol to dryness (approximately 5 minutes).

26. Resuspend the RNA pellet in 20µl of RNase-free water. Combine the two samples into a single Eppendorf tube with an Eppendorf pipette.

27. Label and store sample at -80o C until RNA analysis.

Reagents

PAXgene Blood RNA Tube (PreAnalytiX, A Qiagen/BD Company) Cat# 762115

Absolute ethanol, USP grade (AAPER Alcohol and Chemical Corp, Shelbyville, Kentucky).
