Protocol for Isolating RNA from Human Blood Collected into PAXgene™ Blood RNA Tube

The following modified protocol is designed for isolating RNA from human blood samples collected with PAXgene™ RNA tube using E.Z.N.A. Blood RNA Kit (R6814-01/02).

1. Centrifuge the PAXgene™ Blood RNA Tube for 10 minutes at 3000-5000 x g using a swing-bucket rotor.

2. Remove the supernatant by decanting or pipetting. Add 4 ml RNase-free water to the pellet, close the tube with a cap.

3. Vortex until the pellet is completely resuspended. Centrifuge at 3000-5000 x g for 10 minutes using a swing-bucket rotor. Remove and discard the entire supernatant. **Note: Incompletely removal of supernatant will reduce the lysis efficiency and dilute the lysate, and therefore reduce the RNA yield.**

4. Add 300µl TRK Lysis Buffer, vortex the sample until the pellet is completely dissoved.

5. Transfer the sample into a new 1.5 ml microcentrifuge tube, add 590µl of DEPC-water and 10 µl Proteinase K (25mg/ml). Mix throughly by vortexing for 5 seconds. Incubate at 55°C for 10 minutes using a shaker-incubator.

6. Transfer the sample directly into a Homogenizer column placed into a 2 ml collection tube, centrifuge at maximum speed (>13,000 x g) for 3 minutes.

7. Carefully transfer the entire supernatant of the flow-through fraction to a new 1.5 ml tube without disturbing the pellet in the 2ml collection tube.

8. Add 450µl of absolute ethanol (96-100%). Mix the sample throughly by vortexing.

9. Pipet 750µl of sample into the HiBind RNA column placed in a 2 ml collection tube. Centrifuge at 8000-20,000 x g for 1 minute. Discard the flow-through and collection tube.

10. Place the HiBind RNA column into a new 2 ml collection tube. Transfer the remaining sample into the HiBind RNA column with pipettor. Centrifuge at 8000-20,000 x g for 1 minute. Discard the flow-through and re-use the collection tube.

    Note: this is the starting point for optional On-Membrane DNase I digestion procedure. See detail information from standard user manual. If the optional DNase I digestion is desired, using step 11a, otherwise, go to step 11b.

11a. Place the HiBind RNA column into 2 ml collection tube and add 350µ RNA wash Buffer I into the HiBind RNA column. Spin at 8000-20,000 x g for 1 minute. Continue with Optional DNase I digestion protocol. (See standard user manual for details)

11b. Add 500µ RNA wash Buffer I into the HiBind RNA column and spin at 8000-20,000 x g for 1 minute. Discard the flow-through and re-use the collection tube.

12. Place the HiBind RNA column into same collection tube. Add 600µ RNA wash Buffer II into the HiBind RNA column and spin at 8000-20,000 x g for 1 minute. Discard the flow-through and re-use the collection tube.

13. Place the HiBind RNA column into same collection tube. Wash the column again by adding another 600µ RNA wash Buffer II into the HiBind RNA column and spin at 8000-20,000 x g for 1 minute. Discard the flow-through and re-use the collection tube.

14. Place the HiBind RNA column into the same collection tube and centrifuge at maximum speed for 2 minutes.

15. Add 50-70µl DEPC-water or RNAse-Free water directly onto the center of the membrane in the HiBind RNA column. Incubate 1 minute at room temperature. Centrifuge at maximum speed for 2 minutes to elute RNA.

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