RNA isolation from PAXgene blood RNA tubes by PAXgene blood RNA kit

The PAXgene blood RNA kit can isolate total RNA from 2.5 mL human whole blood collected in PAXgene blood RNA tube. Typical yields of RNA isolation using the PAXgene blood RNA system are between 4 and 10 μg.

Material

1. PAXgene blood RNA tube (BD/QIAGEN, 762165, BD)
2. PAXgene blood RNA kit (BD/QIAGEN, 762174, BD)
3. RNase-free DNase set (QIAGEN, 79254, 諾貝爾)
4. 96-100% ethanol

Method

After blood collection, the PAXgene blood RNA tubes are gently inverted 8- 10 times and are stored at -20°C. If tubes are to be kept at temperatures below -20°C, they are first frozen at -20°C for 24 hr and then are transferred to -80°C.

- equilibrate at room temperature for at least 2 hr (22°C ↓)
- incubate at room temperature for an additional 2 hr if tubes are immediately frozen after blood collection
- invert the tubes 10 times after thawing
- perform all centrifugation steps of the procedure at room temperature
- add 4 volumes of 96-100% ethanol to BR4 to obtain a working solution at the first time

1. centrifuge the PAXgene blood RNA tube at 3200 g for 10 min
2. remove the supernatant
   add 5 mL RNase-free water to the pellet and close by using a fresh secondary Hemogard closure
3. thoroughly resuspend the pellet by vortexing
   centrifuge at 3200 g for 10 min
   discard the supernatant
4. add 360 μL Buffer BR1 and vortex
5. transfer to a microcentrifuge tube
   add 300 μL Buffer BR2 and 40 μL proteinase K and vortex
   incubate at 55°C for 10 min and vortex sample at maximum speed for 10-20 sec

6. vortex for 30 sec and then centrifuge at maximum speed (10000 g ↑) for 10 min
   transfer supernatant to a new microcentrifuge tube
7. add 350 µL 96-100% ethanol, mix by vortexing, and centrifuge briefly
8. add 700 µL sample to the PAXgene spin column
   centrifuge at 8000 g ↑ (10000 rpm ↑) for 1 min
9. add the remaining sample to the PAXgene spin column
   centrifuge at 8000 g ↑ for 1 min
10. add 350 µL buffer BR3 to the PAXgene spin column
    centrifuge at 8000 g ↑ for 1 min
    add 10 µL DNase I stock solution to 70 µL Buffer RDD, mix by gently flicking the tube, and centrifuge briefly
    add DNase I incubation mix directly onto the PAXgene spin column membrane
    incubate at room temperature for 15 min
    add 350 µL Buffer BR3 to the PAXgene spin column
    centrifuge at 8000 g ↑ for 1 min
11. add 500 µL Buffer BR4
    centrifuge at 8000 g ↑ for 1 min
12. add another 500 µL Buffer BR4
    centrifuge at maximum speed for 3 min to dry the PAXgene spin column membrane
    centrifuge at maximum speed for 1 min
13. transfer to a 1.5 mL elution tube and add 40 µL Buffer BR5
    centrifuge at 8000 g ↑ for 1 min to elute RNA
14. add another 40 µL Buffer BR5 or re-apply the eluate
    centrifuge at 8000 g ↑ for 1 min to elute RNA
15. incubate eluate at 65°C for 5 min in a heating block and then chill immediately on ice