RNA isolation from Tempus blood RNA tubes by Versagene RNA purification kit

The Versagene RNA purification kit can isolate total RNA from 3 mL human whole blood collected in Tempus blood RNA tube. Expected yield is 15 to 25 μg RNA per Tempus tube.

Material

1. Tempus blood RNA tube (ABI, 4342792, ABI)
2. Versagene RNA purification kit (Gentra, VGR-0050BP)
   (5 PRIME perfectPure RNA Blood Purification Kit, Fisher Scientific)
3. 96-100% ethanol

Method

After blood collection, the Tempus blood RNA tubes are mixed vigorously for 10 sec 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 ↓)
- perform all steps, including centrifugation, at room temperature unless specified
- prepare DNase Solution
  add 2.6 mL DNase Buffer to the lyophilized DNase Enzyme, invert gently to mix, aliquot, and store ate -20°C or -80°C

1. after mixing, pour blood from Tempus tube into a clean 50 mL conical tube
2. add 3 mL 95% ethanol and vortex on high for 2 min
3. centrifuge at 3000 g for 30 min at 0°C
4. discard the supernatant
   blot on clean absorbent paper and invert tube on absorbent paper for 2 min
5. add 300 μL Lysis solution and vortex on high speed for 60 sec to thoroughly dissolve the pellet
6. pipet the lysate onto a purification column
   centrifuge at 13000-16000 g for 1 min
   transfer the purification column to a new collection tube
7. add 400 μL Wash 1 Solution and wash the side of the basket during the addition of this solution

centrifuge at 13000-16000 g for 2 min
transfer carefully the purification column to a new collection tube

8. add 50 µL DNase Solution and incubate at room temperature for 15 min
9. add 200 µL DNase Wash Solution
   centrifuge at 13000-16000 g for 1 min
10. add 200 µL DNase Wash Solution
    centrifuge at 13000-16000 g for 1 min
    transfer the purification column to a mew tube
11. add 200 µL Wash 2 Solution
    centrifuge at 13000-16000 g for 1 min
12. add 200 µL Wash 2 Solution
    centrifuge at 13000-16000 g for 2 min
    transfer carefully the purification column to a new tube
    if Wash 2 Solution comes in contact with column, centrifuge at 13000-16000 g for 2 min
13. add 50 µL Elution Solution and incubate at room temperature for 3 min
    centrifuge at 13000-16000 g for 1 min
14. place the purified RNA on ice and then store at -80°C