

**cf-DNA/cf-RNA Preservative Tubes - Supplementary Protocol for Total RNA
Purification from Leukocytes recovered from cf-DNA/cf-RNA Preservative Tubes
Product # 63950, 63960**

Customer-Supplied Reagents

- 0.2µ filtered 1X PBS - pH 7.4 (RNase-free).
- Leukocyte RNA Purification Kit (Cat. 21200)

Isolation of WBCs/CTCs from cf-DNA/cf-RNA Preservative Tubes

1. Using a blood collection set and a holder, collect blood into Norgen's cf-DNA/cf-RNA Preservative Tube using your institution's recommended procedure for standard venipuncture technique and as indicated in **Section A** in the manual for the cf-DNA/cf-RNA Preservative Tube (Cat. 63950, 63960).
2. To separate plasma, centrifuge the contents of the cf-DNA/cf-RNA Preservative Tube at 425 xg for 20 minutes at room temperature.
3. Carefully transfer the upper plasma layer by pipetting to a fresh tube. Avoid the transfer of any cells during the transfer of the plasma. **DO NOT DISCARD THE BLOOD PELLET FOR WBCs/CTCs ISOLATION.**
4. To the blood pellet, add 1x PBS (pH 7.4) at a volume equivalent to the recovered plasma volume.
5. Mix the blood pellet with the added **1x PBS** by gentle inversion or vortexing to dissolve the majority of the blood pellet.
6. Aliquot up to 5 mL from the 1X PBS resuspended blood pellet into a fresh 50 mL tube.
7. Add 5 volumes of **RBC Lysis Buffer** to the aliquoted 1X PBS resuspended blood pellet. (i.e.: Add 2.5 mL of **RBC Lysis Buffer** to 500 µL of 1X PBS resuspended blood pellet).
8. Incubate at room temperature for 3 to 5 minutes, with brief vortexing during the incubation to mix.

Note: Ensure that the solution changes from a milky, opaque pink to clear red before proceeding to the next step.

9. Centrifuge at **250 x g (~2,000 RPM)** for 3 minutes and decant supernatant.
10. Add 2 additional volumes of **RBC Lysis Buffer** to pelleted white blood cells and mix by gentle vortexing for 10 seconds. (i.e. Add 1 mL of **RBC Lysis Buffer** to every 500 µL of 1X PBS resuspended blood pellet).
11. Centrifuge at 250 x g (~2,000 RPM) for 3 minutes and decant supernatant. A few µL of media may be left behind with the pellet in order to ensure that the pellet is not dislodged.
12. The pellet should be white. If the pellet is still red then repeat Step 10 and Step 11 until the pellet becomes white
13. Add 350 µL of Buffer RL directly to pelleted leukocytes.
14. Lyse cells by gentle vortexing until homogeneity is reached.
15. Add 200 µL of 96 – 100% ethanol (provided by the user) to the mixture and mix by vortexing for 10 seconds.

Note: For input amounts greater than 500 µL of blood or 10⁶ leukocytes, it is recommended that the lysate is passed through a 25 gauge needle attached to a syringe 5-10 times at this point, in order to shear the genomic DNA prior to loading onto the column.

16. Apply the lysate with the ethanol onto the column and centrifuge for 1 minute at 3,500 x g (~6,000 RPM). Discard the flowthrough. Reassemble the spin column with its collection tube.

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at 14,000 x g (~14,000 RPM).

17. Apply 400 μL of Wash Solution A to the column and centrifuge for 1 minute. Discard the flowthrough and reassemble the spin column with its collection tube.

Note: Ensure the entire Wash Solution A has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute

18. Repeat Step 17 twice to wash column for a total of 3 washes.
19. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
20. Add 50 μL of Elution Solution A to the column.
21. Centrifuge for 2 minutes at 200 x g (~2,000 RPM), followed by a 1 minute spin at 14,000 x g (~14,000 RPM). Note the volume eluted from the column. If the entire 50 μL has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.
22. The purified RNA sample may be stored at -20°C for a few days. It is recommended that samples be placed at -70°C for long term storage.

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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