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cf-DNA/cf-RNA Preservative Tubes - Supplementary Protocol for Genomic DNA 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).
- Red Blood Cell Lysis Buffer (Cat. 21201)
- Blood DNA Isolation Mini Kit (Cat. 46300)

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 5mL 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. Resuspend the WBC pellet in 200 µL 1X PBS.
14. Add 20 µL of Proteinase K followed by the addition of 300 µL **Lysis Buffer B.** Mix well by vortexing for 10 seconds
15. Incubate at 55ºC for 10 minutes
16. Add 110 µL of 96 – 100% ethanol (provided by the user) to the mixture and mix by vortexing for 10 seconds.
17. Apply the lysate from Step 16 onto the column and centrifuge for 1 minute at 6,000 x g (~8,000 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).

18. Apply 500 µL of **Solution WN** (ensure ethanol was added) to the column and centrifuge for 1 minute at 6,000 x g (~8,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube

Note: Ensure the entire solution WN 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).

19. Apply 500 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

20. Repeat Step 19 one more time to wash column for a total of 2 washes with Wash Solution A.
21. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
22. Add 100 µL of Elution Buffer B to the column.
23. 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 100 µL has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.
24. The purified DNA 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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