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cf-DNA/cf-RNA Preservative Tubes - Supplementary Protocol for RNA/DNA/Protein 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)
- RBC Lysis Buffer (Cat. 21205)
- RNA/DNA/Protein Purification Plus Micro Kit (Cat. 51600)

Section 1: Recovery of Leukocytes 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 to a fresh tube by pipetting. Avoid the transfer of any cells during the transfer of the plasma. ***DO NOT DISCARD THE BLOOD PELLET FOR LEUKOCYTES ISOLATION***.
4. To the blood pellet add 1x PBS (pH 7.4) with a volume equivalent to the recovered plasma volume. (For example, if the plasma volume recovered from the blood tubes was 6 mL then add 6 mL of 1x PBS (pH 7.4) to the blood pellet).

   Important Note: The addition of 1x PBS to the blood pellet must be done immediately after pipetting out the recovered plasma.

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 a maximum of 2 mL from the resuspended blood pellet for Leukocytes recovery (Section 2).

Section 2: Leukocytes recovery from Norgen's cf-DNA/cf-RNA Preservative Tubes using Norgen's RBC Lysis Buffer (Cat. 21205).

1. Add 5 volumes of RBC Lysis Buffer to the 1X PBS resuspended blood pellet from Section 1 above. (i.e.: Add 2.5 mL of RBC Lysis Buffer to 500 µL of the resuspended blood pellet).
2. 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.

3. Centrifuge at 250 x g (~2,000 RPM) for 3 minutes and decant supernatant.
4. Add 2 additional volumes of RBC Lysis Buffer to the 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 the input resuspended blood pellet volume).
5. Centrifuge at 250 x g (~2,000 RPM) for 3 minutes and decant supernatant. A few µL from the RBC Lysis Buffer may be left behind with the pellet in order to ensure that the pellet is not dislodged.
6. The pellet should be white. If the pellet is still red then repeat Step 4 and Step 5 until the pellet becomes white
7. Proceed immediately to **Section 3** for RNA/DNA/Protein Purification from Leukocytes recovered from Norgen's cf-DNA/cf-RNA Preservative Tubes

Section 3: RNA/DNA/Protein Purification from Leukocytes recovered from Norgen's cf-DNA/cf-RNA Preservative Tubes using Norgen's RNA/DNA/Protein Purification Plus Micro Kit (Cat. 51600).

- Please refer to the detailed protocol for the RNA/DNA/Protein Purification Plus Micro Kit (Cat. 51600) which is available online at www.norgenbiotek.com
- Proceed directly to **Step d in Section 1A (ii)** - Cell Lysate Preparation from Cells Growing in Suspension and Lifted Cells

Technical Support
Contact our Technical Support Team between the hours of 9:00 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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