

## Supplementary Protocol for the Isolation of White Blood Cells (WBCs) / Circulating Tumour Cells (CTCs) 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. 21201)

### 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. Add 5 volumes of **RBC Lysis Buffer** to the 1X PBS resuspended blood pellet. (i.e.: Add 2.5 mL of **RBC Lysis Buffer** to 500 µL of blood)
7. 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.

8. Centrifuge at **250 x g (~2,000 RPM)** for 3 minutes and decant supernatant.
9. 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 input blood volume)
10. 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.
11. The pellet should be white. If the pellet is still red then repeat Step 9 and Step 10 until the pellet becomes white
12. Resuspend the WBCs/CTCs in any buffer of your choice that is suitable for your downstream application.

### 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](http://www.norgenbiotek.com)) or through email at [techsupport@norgenbiotek.com](mailto:techsupport@norgenbiotek.com).

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