RBC Lysing Protocol

RBC Lysis Buffer (10X) Cat. No. TNB-4300-L100

Other Materials Required

- Flow Cytometry Staining Buffer (Stain Buffer) (1X PBS with 2% FBS, 0.09% Na-Azide)

Note: This protocol applies to human whole blood preparations. For use with other tissue samples, it is recommended that the investigator optimize conditions to obtain best results. RBC Lysis Buffer (10X) has been tested using blood collected with either heparin or EDTA as the anti-coagulant and found to perform equivalently.

1. Prepare 1X RBC Lysing Buffer by adding 1 part RBC Lysing Buffer (10X) with 9 parts room temperature distilled water.
2. Aliquot a sample of whole blood, typically 50-100 µL, to tube.
3. Add fluorochrome-labeled antibodies for staining directly to sample and mix thoroughly.
4. Incubate for 30 minutes at room temperature and protected from light.
5. Add 2 mL of room temperature 1X RBC Lysing Buffer (prepared in step 1) and pulse vortex (<5 seconds).
6. Incubate for 10-15 minutes at room temperature and protected from light.
7. Centrifuge cells at 500 x *g* for 5 minutes at room temperature.
8. Carefully decant supernatant and wash cells once with 1-2 mL Stain Buffer.
9. Centrifuge cells as in Step 7 and resuspend at the appropriate volume for analysis.

Tonbo Biosciences
4940 Carroll Canyon Road
San Diego, CA 92121-1735

(855) 848-6626 toll-free
(858) 888-7300 direct
(858) 888-7301 fax

info@tonbobio.com
www.tonbobio.com