

# Technical Notes—How to Deplete Red Blood Cells

This protocol is designed for human peripheral blood, bone marrow and cord blood for red blood cells (RBC) preparations. This protocol outlines the procedure for depletion of RBC from whole blood and from mononuclear cells.

## Materials and Reagents

- Dulbecco's Phosphate Buffered Saline (DPBS) with 2mM EDTA
- WB (washing buffer, 2% Newborn Bovine Serum (NBS), 2mM EDTA in DPBS)
- Ammonium Chloride (StemCell Technologies Cat# 07850)

## Equipment

- Refrigerated centrifuge
- Ice in a bucket
- Pipetman
- Biosafety hood

## Safety Precautions

- Protective gloves should be worn at all times
- Use a safe pipetting device for all pipetting
- NEVER PIPET BY MOUTH

## Procedure For Lysis of RBC From Whole Blood

1. Transfer 25ml of blood into a 50ml conical tube and centrifuge at 1000rpm or 200g in a swing bucket for 10 minutes at room temperature.
2. Discard the supernatant and re-suspend the blood with 25ml of DPBS (containing 5mM EDTA) and centrifuge at 1000rpm for 10 minutes at room temperature.
3. Discard the supernatant and add the Ammonium Chloride Solution at 9:1 ratio. For example, 9ml of Ammonium Chloride Solution to 1ml of blood. You should have 45ml of Ammonium Chloride solution to 5ml of blood.
4. Mix the cell and ammonium solution and place on ice for 10 minutes to allow lysis of the RBC.
5. Centrifuge the tube at 1500rpm for 10 minutes in 4°C.
6. Discard the supernatant and wash the cells twice with WB. The cells are ready for use.

## Procedure For RBC Lysis from Mononuclear Cells

1. Centrifuge the cells after collecting the lymphocyte layer from the separation medium.
2. Discard the supernatant and re-suspend the cells with 25ml of the prepared washing buffer.
3. Centrifuge the tube at 1000rpm for 10 minutes at room temperature.
4. Discard the supernatant and add the Ammonium Chloride Solution at 4:1 ratio. For example, 4ml of Ammonium Chloride to 1ml of blood.
5. Mix the cells and ammonium chloride solution and place on ice for 10 minutes to allow lysis of the RBC.
6. Fill the tube with WB up to 25ml and centrifuge the tube at 200g (1000rpm) for 10 minutes at 4°C.
7. Discard the supernatant and wash the cells twice with the prepared washing buffer. The cells are ready for use.