# Erythrocyte isolation from whole blood

## Editorial

This protocol is designed to isolate erythrocytes from whole blood. Most platelets will be removed, though some leukocytes may remain. To fully clear leukocytes, use a leukocyte filtration device or simply refrigerate isolated RBCs for 1-2 days until WBCs die naturally.

## Materials

1. 1mL syringe with Luer Lock
2. Luer lock plug
3. 15mL conical
4. Parafilm
5. FACS buffer (PBS + 2% FBS + 1mM EDTA)
6. PBS

## Preparation

1. Remove plunger from syringe then plug smaller opening with Luer lock plug. Save the plunger
   1. May have to trim an 1-1.5 inches off the top of the plunger for it to fit in the centrifuge

## Protocol

1. Add whole blood and fill with FACS buffer or PBS to 1mL
   1. Minimum 1:1 ratio of buffer to whole blood
2. Insert plunger just enough to form a seal, then carefully invert the syringe and remove the plug. Depress plunger slowly to remove excess air and reattach plug
3. Wrap outside of syringe in a kimwipe and insert it into 15mL conical
   1. Kimwipe should surround syringe so that it fits snugly into conical
4. Centrifuge at 800g for 20min at 4C – acceleration at ¾ maximum and brake at ½ maximum
5. Remove syringe from conical without disturbing separated blood. Slowly and carefully unscrew Luer lock plug. Attach 27G needle.
6. Gently depress plunger so that blood comes out dropwise into 15mL conical. Take about ½ the visible volume of RBC layer
7. Resuspend blood in about 3mL FACS
8. Count cells and adjust volume as necessary to obtain desired density
9. Cells can be stored at 4C for 1-2 weeks
   1. Residual WBCs should be dead after 1-2 days