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Protocol for the depletion of rbcs from whole blood

Introduction

When drawing venous blood use sodium heparin as an anti-coagulant. Mix thoroughly and process samples within 24 hours. If storage is necessary prior to processing, store the blood at room temperature, shielded from light, and on a rocker. DO NOT refrigerate the cells.

Materials and Equipment

- Human or murine blood sample.
- Sterile tubes (30 ml and 50 ml).
- Serological pipettes of appropriate volumes (sterile)
- Ammonium chloride lysing reagent (0.15 M NH4Cl, 1 mM KHCO3, 0.1 mM EDTA, or a commercial preparation e.g. PharM Lyse™, BD Biosciences #555899)
- Wash buffer (0.1% sodium azide, 0.1% BSA in PBS)
- Benchtop centrifuge (NOT refrigerated) with swing-out rotor and appropriate carriers
- Vortex
- Hemacytometer and microscope for cell counting

Procedure *(may be scaled up or down according to requirements)*

1. **Prepare ammonium chloride lysing reagent.**
2. **Take the fresh blood sample and add the appropriate amount of ammonium chloride lysing reagent** (1 ml per 100 µl blood, or as defined by the manufacturer).
3. **Gently vortex immediately after adding the lysing reagent.**
4. **Incubate for 15 minutes at room temperature, shielded from light.**
5. **Centrifuge at 400 × *g* for 5 minutes.**
6. **Carefully aspirate supernatant without disturbing pellet.**
7. **Add 10 ml wash buffer.**
8. **Centrifuge at 400 × *g* for 5 minutes.**
9. **Carefully aspirate supernatant without disturbing pellet.**
10. **Resuspend cells in wash buffer to a final known volume.**
11. **Count live cells using a hemacytometer and light microscope.** If proceeding straight to staining for flow cytometry, resuspend the cells in any residual wash buffer and distribute them equally between sample tubes.

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