

TITLE: Whole blood hemolysis with isotonic ammonium chloride solution

***AUTHORS**

Le P. Ngo¹, Marcus Parrish¹, Jing Ge¹, and Bevin Engelward²

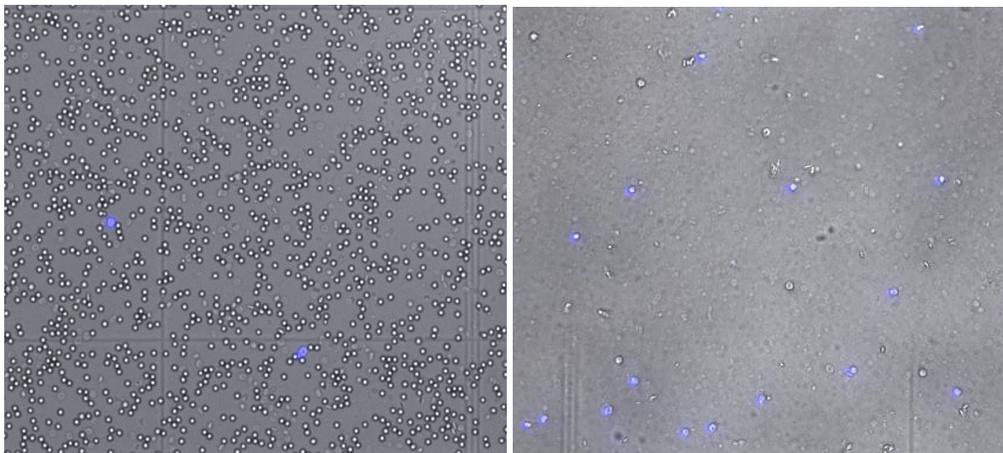
***AUTHOR INFORMATION**

¹Graduate Student, Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA

²Professor, Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA

***ABSTRACT:** This method is applied to freshly collected whole blood samples in order to isolate white blood cells (WBCs) for a number of applications: total WBC DNA extraction, WBC culturing without red cells' interference, and comet assays using Comet Chip. The basic mechanism of hemolysis by isotonic ammonium chloride is as follows. NH_3 diffuses freely through the cell membrane and increases the concentration of intracellular OH^- . OH^- reacts with intracellular CO_2 to form HCO_3^- . In red blood cells (RBCs), the intracellular HCO_3^- is exchanged with the extracellular Cl^- through the $\text{Cl}^-/\text{HCO}_3^-$ trans-membrane anion exchanger of RBCs (a.k.a. Band 3 anion channel). The result is an influx of NH_4Cl inside RBCs, which causes cellular swelling and eventually rupture of the cell membrane. In this protocol, freshly collected whole blood can be effectively hemolyzed after 3-5 minutes of incubation in a homemade ammonium chloride hemolysis buffer at 37°C .

***EXAMPLE DATA**



Before NH_4Cl treatment

After NH_4Cl treatment

Figure. Whole blood samples, before and after hemolysis, are stained with Hoechst 33258. A cell shows blue fluorescence only when it is nucleated. The left image shows the high number of RBCs compared to WBCs in fresh whole blood. The right image shows that ammonium chloride treatment effectively removes most of RBCs.

***PROCEDURES**

Step 1: Prepare isotonic hemolysis buffer stock

Active time: 10 minutes

Total time: 1 hour

Materials (for 1L of 1X RBC lysis buffer):

8.26g crystalline NH_4Cl (0.15M)

1g crystalline KHCO_3 (10mM)

0.037g crystalline EDTA (0.1mM)

Deionized water or distilled water

Parafilm

Aluminum foil

1.1. Mix all materials in a 1-liter autoclavable glass bottle

1.2. Add deionized (or distilled) water to get a final volume of 1L

1.3. Autoclave using liquid setting for 30 minutes to sterilize

1.4. After the buffer has cooled down, wrap the bottle in aluminum foil and seal the lid with parafilm

1.5. Store the autoclaved buffer at 4°C, protected from light

Step 2: Set up prior to experimentation

Active time: 10 minutes

Total time: 40 minutes

Materials:

1X autoclaved RBC lysis buffer stock

1X sterile phosphate buffer saline (PBS - commercial or homemade)

Complete medium for white blood cells (RPMI 1640 + 20% fetal bovine serum)

Falcon tubes (volume depends on the amount of blood used)

Ice

Parafilm

For 1 volume of whole blood:

2.1. Place 10 volumes of 1X RBC lysis buffer stock in a Falcon tube, sealed with parafilm, and incubate for 20 minutes at 37°C, protected from light

2.2. Place 10 volumes of 1X PBS in a Falcon tube and let it cool down for 20 minutes at 4°C

2.3. Place 15 volumes of complete medium in a Falcon tube and incubate for 20 minutes at 37°C

Step 3: Experimentation

Active time: 30 minutes

Total time: 30 minutes

Materials:

Fresh whole blood collected in tubes coated with an anticoagulant

Buffers prepared in steps 1 and 2

3.1. Transfer 1 volume of whole blood to 10 volumes of pre-warmed 1X RBC lysis buffer and gently mix using a 25ml pipette tip or simply invert the tube several times. See **video 1**.

3.2. Incubate at 37°C, 5% CO₂, protected from light for 3-5 minutes or until the cell suspension turns clear. See **video 2** for cell suspension before incubation, **video 3** for after incubation, and **video 4** for a side-by-side comparison.

3.3. Add 10 volumes of cold 1X PBS to the hemolysed blood and gently mix by inverting

3.4. Centrifuge the hemolysed blood at 450xg for 10 minutes, at 4°C

3.5. Aspirate the supernatant and take care not to disrupt the cell pellet

3.6. If the pellet is still red, repeat steps 3.1 – 3.5. This step is not recommended to minimize processing that can damage cells.

3.7. Resuspend the pellet with 10 volumes of pre-warmed complete medium

3.8. Centrifuge the hemolysed blood at 450xg for 5 minutes, at 4°C

3.9. Aspirate the supernatant

3.10. Resuspend the pellet in 1 volume of pre-warmed complete medium

3.11. White blood cells can be processed for analysis immediately or cultured at 1 million cells/ml in complete medium