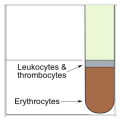

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Preparation of leukocytes by differential lysis of erythrocytes

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1 Works for me dx.doi.org/10.17504/protocols.io.bcv2iw8e

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ABSTRACT

Leukocytes are isolated by centrifugation after specific lysis of erythrocytes

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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MATERIALS TEXT

REAGENTS

1. Lysis Buffer (155 mmol/L ammonium chloride; 10 mmol/L sodium bicarbonate; 0.1 mmol/L EDTA).

Dissolve 8.3 g ammonium chloride, 0.84 g sodium bicarbonate and 29.3 mg EDTA in about 900 mL reagent-grade water. Titrate to pH 7.4 with HCl, then make the volume to 1 litre. Store at 4oC.

2. Isotonic Saline (0.9%, w/v)

Dissolve 9 g NaCl in 1 litre reagent-grade water. Store at 4 degrees Celsius.

1 Centrifuge **10 ml** EDTA blood to pellet all cells **1500 rpm, 4°C 00:10:00** 10m

2 Remove plasma into a clean container and freeze.

3 Restore original blood volume with 0.9% saline and transfer the blood suspension into a **50 ml** conical centrifuge tube.

4 Add **40 ml** cold lysis buffer

5 Stand **On ice** , mixing occasionally, until erythrocytes are lysed (the red cell suspension remains red in colour, but becomes transparent). This should take only about **00:05:00** to **00:10:00** 10m

6 Centrifuge **1500 rpm, 4°C 00:05:00** 5m

7 Discard supernatant and resuspend leucocyte pellet in **5 ml** cold lysis buffer.

8 Stand **On ice** for **00:10:00** 10m

9 Dilute cell suspension to **50 ml** with cold 0.9% saline. Mix and centifuge **1500 rpm, 4°C 00:05:00** 5m

10 Discard supernatant, then resuspend leucocyte pellet in **10 ml** 0.9% saline. Care should be taken to obtain an even cell suspension without being too vigorous and causing cell disruption.

11 Divide the cell suspension into two equal aliquots, into two **10 ml** conical centrifuge tubes.

12 Centrifuge **1500 rpm, 4°C 00:05:00** 5m

13 Remove all supernatant and dry walls of centrifuge tube with a tissue.

14 Store leucocyte pellets and plasma at **-20 °C** (or **-80 °C**)