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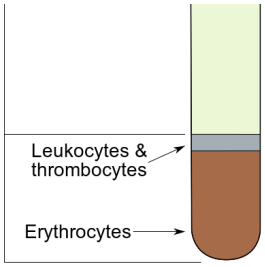
Preparation of leukocytes by differential lysis of erythrocytes



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We use this protocol and it's working

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Abstract

Leukocytes are isolated by centrifugation after specific lysis of erythrocytes

Materials

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)