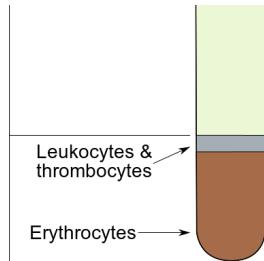


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Preparation of leukocytes by differential lysis of erythrocytes

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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 4°C.

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 centrifuge  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)