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Enrichment of reticulocyte by 19%Nycodenz-KCl

Materials

Reagents	Cat no.	Lot no.	Company
NycoPrep™ Universal	1106865	10130684	Axis-Shield
High KCl buffer, Ph 7.4	In-house		
RPMI 1640	31800-022	1133370	Gibco
Retic-chex Stain	340235-6	1101037	Streck, USA
PALL filter	RC2VAE	135047	PALL corporation
McCOY	M4892-10X1L	081M8312	Sigma-Aldrich
Human AB serum			Inter-state blood bank

High KCl buffer pH 7.4

20 mM HEPES (Mw 238.3g/mol)	4.7662 g
1 mM $MgCl_2$	0.095 g
1 mM NaH_2PO_4 (NaH_2PO_4*H_2O, 137.99 g/mol)	0.138 g
10 mM glucose (180.16 g/mol)	1.801 g
0.5 mM EGTA (468.3 g/mol)	0.2342 g
115 mM KCl (74.5513 g/mol)	8.5734 g
12 mM NaCl (58.44 g/mol)	0.7013 g

Sterile by filter through 0.22 µm filter. Keep at 4°C.

McCOY'5A incomplete medium 1L

McCOY'5A powder	1 bottle
NaHCO3	2 g
HEPES	5.94 g
D-glucose	1.8 g
Gentamycin	1 ml

Sterile by filter through 0.22 µm filter. Keep at 4°C.

RPMI incomplete medium 1L

RPMI1640 powder	1 pack
NaHCO3	2 g
HEPES	5.94 g

Sterile by filter through 0.22 µm filter. Keep at 4°C.

19% Nycodenz 100 ml

60% Nycoprep Universal	31.7 ml
High KCl buffer pH 7.4	68.3 ml

Method

1. Deplete leukocytes from whole blood by using Pall® filter (gravity flow, no push).
2. Centrifuge the filtrate at 1,000 g for 10 min, 25ºC. Discard supernatant.
3. Wash the packed blood by adding 2 vol of cold KCL buffer and mix well. Centrifuge the cell suspension at 1,000 g for 10 min at 25ºC and discard supernatant. Repeat washing step for 1 more time.
4. Dilute the packed cells to 20% Hct with cold KCL buffer.
5. To prepare purification tubes, transfer 4 ml of 19% Nycodenz to 15 ml tubes (warm to room temperature).
6. For each purification tube, over layer 4 ml of cold 20% Hct blood on to 4 ml of 19% Nycodenz. The total volume of 19% Nycodenz+diluted blood should be between 7-9 ml (the optimal is 8 ml of tube scale).
7. Centrifuge at 3,000 g for 30 min at 25ºC, no break.
8. Collect the reticulocytes at the interface to 50 ml centrifuge tube.
9. Add 5 vol of RPMI incomplete medium. Centrifuge at 1,000 g for 10 min and discard supernatant.
10. Wash the cells 2 more times with 25 ml RPMI incomplete medium.
11. Resuspend the enriched reticulocytes to 50% Hct with McCOY incomplete medium or RPMI.
12. Transfer 5 µl cell suspension to 1.5 ml microcentrifuge tube. Add 5 µl of Retic chex (new methylene blue) and mix well by pipetting. Incubate for 15 min at room temperature. Spot 5 µl of stained cells on the glass slide and make a thin smear.
13. Count the number of reticulin containing cells in the total of 5,000 cells.
14. Keep the enriched retics at 4°C for 2 weeks from blood drawing date. The blood over 2 weeks old should not be used for culture.