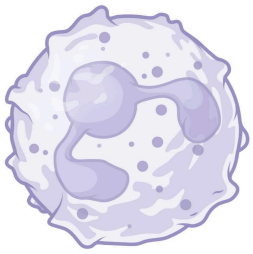
Dec 05, 2024 Version 1

Isolation of human primary neutrophils V.1



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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol was designed for the isolation of human primary neutrophils from full blood or buffy coats. Its thorough optimisation was focused on minimisation of the non-specific activation and population purity.

Image Attribution

neutrophil

Materials

○ RPMI-1640 + HEPES (Sigma-Aldrich, #R5886) supplemented with 0.2% heat-inactivated and filtered FBS (Sigma-Aldrich, #F7524)
○ colour-free RPMI-1640 (Carl Roth, #9104.1) supplemented with 0.2% heat-inactivated and filtered FBS
○ 1x PBS (Sigma-Aldrich, #P2272)
○ 6% Dextran (Sigma-Aldrich, #31392), 150mM NaCl (Sigma-Aldrich, #S9888) in Milli-Q water, filtered
○ 10x ACK - 1.5M NH_4Cl (Sigma-Aldrich, #09718), 100mM $KHCO_3$ (Sigma-Aldrich, #60339), 1mM EDTA disodium salt dihydrate (Sigma-Aldrich, #E5134) in Milli-Q water, pH 7.2, filtered

Protocol materials

- 6% Dextran, 150mM NaCl
- 1X PBS
- 1X ACK buffer
- RPMI+, 0.2% FBS
- Ficoll
- colour-free RPMI, 0.2% FBS

Safety warnings

- work carefully not to activate the neutrophils
- supplement the media with FBS right before use (FBS supplement optional or adjustable)
- prepare 1X ACK right before use

Ethics statement

Prior ethics approval should be obtained before performing these experiments. This protocol requires prior approval by the user's Institutional Review Board (IRB) or equivalent ethics committee(s) for the work with human blood samples.

Before start

- take all reagents out of the refrigerator, temper to room temperature
- supplement the media with FBS
- prepare 1X ACK

Dextran separation

39m

1 transfer blood sample from EDTA vacuette tube into 50ml Falcon tube — 30s

2 add 1 mL of Room temperature 1X PBS per 1 mL of blood sample — 30s

3 add 1 mL of Room temperature 6% Dextran, 150mM NaCl per 1 mL of blood sample — 30s

4 invert the tube 10 times — 30s

5 incubate at Room temperature for 00:30:00 without inverting the tube anymore — 30m

6 transfer the upper phase into new 50ml Falcon tube, avoid transfer of any erythrocytes from the lower phase — 1m

7 spin at 290 rcf, Room temperature, 00:05:00 , swing-up rotor — 5m

Equipment

NAME	TYPE	BRAND	SKU
centrifuge	5702	Eppendorf	022626001

8 using Pasteur pipette and 2 1ml-pipette tips, discard the supernatant 1m

Erythrocyte lysis 11m 30s

9 gently resuspend pellet in 15 mL Room temperature 1X ACK buffer

9.1 first, resuspend in 5 mL , then, add the remaining 10 mL

10 incubate at Room temperature for 00:05:00 starting from the addition of ACK 5m

11 add 35 mL Room temperature 1X PBS to neutralise the lysis 15s

12 invert the tube 10 times 15s

13 spin at 290 rcf, Room temperature, 00:05:00 , swing-up rotor 5m

14 using Pasteur pipette and 2 1ml-pipette tips, discard the supernatant, remove as much red debris as possible 1m

Ficoll separation 37m

15 gently resuspend pellet in 10 mL Room temperature RPMI+, 0.2% FBS 1m

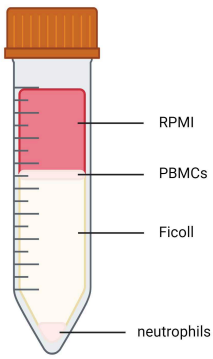
16 carefully load 10 mL Room temperature Ficoll at the bottom of the tube, make sure not to create any bubbles that would destroy the interface, load at the minimum speed 2m

17 spin at

30m

1260 rcf, Room temperature, 00:30:00 , swing-up rotor (S4×750), acceleration and brake ramp: 5

Equipment	
Centrifuge	NAME
5910 R	TYPE
Eppendorf	BRAND
5943000548	SKU



Phase layout after centrifugation

18 with a cut 1ml pipette tip or 5ml pipette tip, transfer PBMCs from the interface into new 15ml Falcon tube

2m

19 using Pasteur pipette and 2 1ml-pipette tips, remove the rest of the supernatant

1m

20 gently resuspend pellet in 5 mL (adjustable) Room temperature colour-free RPMI, 0.2% FBS (percentage of FBS adjustable) 1m

21 count the cells manually or using automated cell counter