



EUROPE

WBC Cystine Group

GUIDELINE No. 1

Prepared by :

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MIXED LEUCOCYTE PREPARATION

The preparation method has been in use at Guy's Hospital, London for the last 12 years and was originally established under the guidance of Professor J Schneider, UCSD

Reagents

a. ACD-Dextran solution:

3g dextran (Sigma D-7265, MW $2 \times 10^5 - 3 \times 10^5$)***

2.1g dextrose

0.33g sodium citrate

0.11g anhydrous citric acid

made up to 100ml of 0.9% sodium chloride

b. 0.9% sodium chloride

c. 3.6% sodium chloride

d. Deionised/distilled water

e. 12% 5-sulfosalicylic acid

Preparation of each solution should include filtration through a 0.22 μ m Millipore filter. Reagents should be stored at 4°C between assays and should be routinely inspected for any evidence of bacterial contamination.

*** It appears that this preparation (Sigma D-7265) of dextran is no longer available. I am presently comparing the preparation from Fisher Scientific/Acros Organics (Cat No: 40626-1000, MW c.2.5x10⁵).

Procedure

1. Allow 3ml of ACD-Dextran solution in a disposable 15ml polystyrene test tube to come to room temperature prior to use. Place the 0.9% sodium chloride, 3.6% sodium chloride, and deionised water on ice.
2. Supervised blood collection.
Immediately add 3ml venous blood without anti-coagulant to the ACD-Dextran solution and invert gently until well mixed.
Remote blood collection.
Take 3-5ml venous blood into a lithium heparin sample collection tube, mix, and transfer to the analytical laboratory at ambient temperature. On arrival in the laboratory gently mix the sample by inversion and added 3ml of blood to the ACD-Dextran solution and again invert gently until well mixed.
3. Once in ACD-Dextran place the sample on ice for 45min (tube vertical!) to allow the red cells to precipitate.
4. After 45min transfer the supernatant to a clean 15ml disposable polystyrene test tube. **It is more important to avoid taking red cells rather than to take all the supernatant.** Centrifuge the tube at 450g for 10min in a pre-chilled centrifuge at 5°C.
5. Discard the supernatant using a water vacuum aspirator (or pipette), leaving a red pellet containing the leukocytes.
6. Add 0.8ml of 0.9% sodium chloride to the pellet and gently re-suspend on a vortex mixer. Add 2.4ml of deionised water, start timing immediately, vortex continuously for 90s, and then add 0.8ml of 3.6% sodium chloride, with continued mixing, to restore the suspension to isotonicity. Centrifuge the tube at 450g for 3min at 5°C.
7. Discard the supernatant using a water vacuum aspirator (or pipette) and repeat the hypotonic lysis, step 6. At this stage the pellet is usually free of red cells but if it remains contaminated then a further cycle of hypotonic lysis is necessary.
8. Discard the supernatant using a water vacuum aspirator (or pipette) and add 3ml of 0.9% sodium chloride, vortexing briefly to partially re-suspend the leukocytes. Centrifuge the tube at 450g for 3min at 5°C.
9. Discard the supernatant using a water vacuum aspirator (or pipette) and add, **accuracy is critical**, 150 μ l of deionised water.
10. Lyse the leukocyte suspension using an ultrasonic probe; 4-6 5s bursts.
11. Transfer the suspension to a clean, labelled 1.5ml polypropylene Eppendorf tube to which has been added 50 μ l of 12% 5-sulfosalicylic acid to precipitate the protein. Cap and vortex the tube briefly to ensure mixing.
12. Store the tubes at -70°C until analysis.

It is essential that the sample preparation process is performed as quickly as possible and without delay at any stage.