Thomas Scientific

making a difference

greiner BIO-ONE

Instruction Manual Leucosep

(Thomas Nos. 1182M25, 163289, 1182M26, 1182M27, 227289, 1182M28)

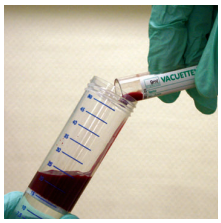
The Method

Leucosep has been developed for optimal separation of lymphocytes and peripheral mononuclear cells (so-called PBMCs) from human whole blood and bone marrow by means of density gradient centrifugation. The key feature of Leucosep is the porous barrier incorporated into the centrifuge tube made of highly transparent polypropylene. This barrier consists of high-grade polyethylene. It does away with the time-consuming and laborious overlaying of the sample material. Anticoagulated blood or bone marrow can simply be poured directly from the blood sampling tube into the Leucosep tube. The porous barrier prevents mixture of the sample material with the separation medium. During centrifugation, lymphocytes and PBMCs are separated from unwanted erythrocytes and granulocytes on the basis of their buoyant density, and enriched in an interphase above the separation medium. When separation is complete, the barrier prevents recontamination of the enriched cell fraction during harvest.

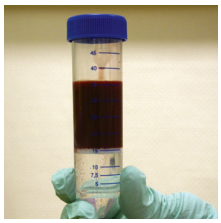
Preparation

- Warm up separation medium to room temperature (RT) protected from light.
- Fill the Leucosep tube with separation medium: 3 ml when using tubes order no. 163289 or 1182M26; 15 ml when using tubes order no. 227289 or 1182M28.
- Close the tubes containing the separation medium with the screw-cap and centrifugate for 30 seconds at 1000 x g and RT. The separation medium is now located below the porous barrier.
- When using tubes that are prefilled with separation medium (order no. 1182M25 or 1182M27) the aforementioned steps can be cancelled. Simply warm up the tubes to RT.
- The tubes are now ready for filling with anticoagulated blood or bone marrow aspirate. Dilution of the sample material with balanced salt solution is not implicitly necessary, but it can help to improve the result of the separation. For blood a dilution ratio of 1:2, for bone marrow a ratio of 1:4 is recommended.

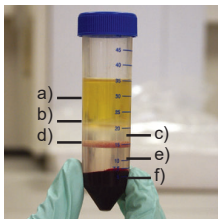
Procedure



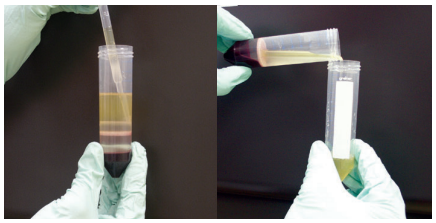
1) Filling with sample material



2) Before centrifugation



3) After centrifugation



4) Harvest by means of a Pasteur pipette or by decanting into another centrifugation tube

1. Pour the anticoagulated sample material (blood or bone marrow aspirate, diluted with balanced salt solution if necessary) directly from the blood sampling tube carefully into the Leucosep tube: 3–8 ml of sample material when using tubes order no.1182M25, 163289 or 1182M26; 15–30 ml of sample material when using tubes order no. 1182M27, 227289 or 1182M28.
2. Centrifugate 10 minutes at 1000 x g and RT or 15 minutes at 800 x g and RT in a swinging bucket rotor. Switch off brakes of the centrifuge.
3. After centrifugation the sequence of layers occurs as follows (seen from top to bottom): a) Plasma – b) enriched cell fraction (interphase consisting of lymphocytes/ PBMCs) – c) separation medium – d) porous barrier – e) separation medium – f) pellet (erythrocytes and granulocytes). Collection and discarding of the plasma layer fraction up to a minimum remnant of 5 to 10 mm above the interphase helps to prevent contamination of the enriched cells with platelets.
4. Harvest the enriched cell fraction (lymphocytes / PBMCs) by means of a Pasteur pipette or by pouring the supernatant above the porous barrier from the Leucosep tube into another centrifugation tube. The porous barrier effectively avoids recontamination with pelleted erythrocytes and granulocytes.
5. Wash the enriched cell fraction (lymphocytes / PBMCs) with 10 ml of phosphate-buffered saline (PBS), subsequently centrifugate for 10 minutes at 250 x g.
6. Repeat washing step twice, resuspend the cell pellet with 5 ml of PBS.

Caution

Handle all biological samples and blood collection lancets, needles, and blood collection sets in accordance with the policies and procedures of your facility.
In case of any exposure or contamination with blood or other biological samples (e.g. accidental puncture injury) initiate appropriate medical treatment as such material has to be considered potentially infective with HBV, HCV (hepatitis), HIV (AIDS), or other infective agents.
For Research Use Only. Not for Diagnostics.