# Instruction Manual Leucosep™

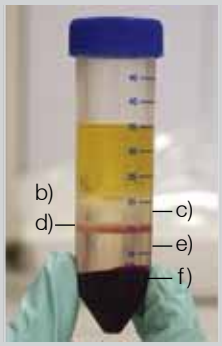
### Preparation

- Warm up separation medium to room temperature (RT) protected from light.
- Fill the Leucosep™ tube with separation medium: 3 ml when using tubes Cat.-No. 163 290; 15 ml when using tubes Cat.-No. 227 290.
- Close the tubes containing the separation medium with the screw cap and centrifugate for 30 sec. 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 (Cat.-No. 163 288 or 227 288) 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.

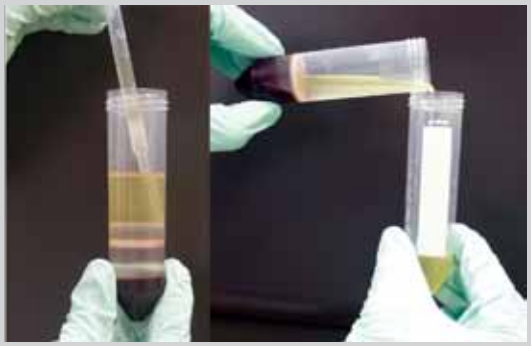
## Procedure

Filling with sample material

Before centrifugation




After centrifugation



Harvest with a Pasteur pipette or by decanting into another centrifuge 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 Cat.-No. 163 288 or 163 290; 15–30 ml of sample material when using tubes Cat.-No. 227 288 or 227 290.

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.



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