9 Separation

Separation

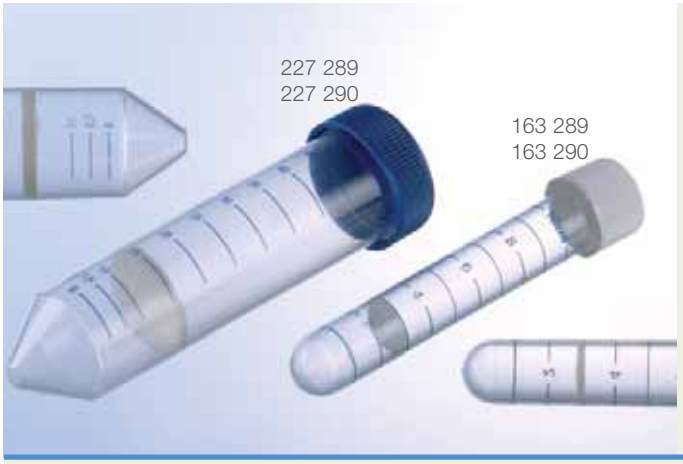
Different separation techniques can be used to enrich certain particles (DNA, RNA, proteins, organelles, vesicles, micelles, cells etc.) specifically from complex biological mixtures such as cell and tissue homogenates, blood, urine and other body fluids, so that they can then be selectively investigated. Separation of these types of particles can be based either on the different sedimentation rates of different particles in a fluid, or on their different densities. **Density gradient centrifugation** (also referred to as band, equilibrium or isopycnic centrifugation), exploits the principle that particles of a certain density migrate into a density gradient until they reach an equilibrium density layer. The first applications of density gradient centrifugation were reported in the early 1950s. Back then, cell organelles were enriched with the aid of buffered saccharose gradients and it is uncontested that the knowledge gained with these enriched materials made a contribution to modern molecular biology.

Soon it was discovered that the enrichment of mammalian cells requires more complex separation media, particularly due to their sensitivity towards osmotic fluctuation. Noble and Boyum described methods for separating mono-nuclear cells from whole blood and bone marrow as early as 1967 and 1968. Based on this pioneering scientific work, numerous applications in today‘s biomedical research and routine diagnostics require highly enriched, viable and functionally intact cell populations as the starting material. The separation of such cells by density gradient centrifu-gation has proven to be the most often used method due to its uncomplicated and robust nature.

With **Leucosep™**, Greiner Bio-One optimised density gradient centrifugation whilst making it user-friendly. Alongside this, **OncoQuick®** was developed to extend the spectrum of applications to deal specifically with oncological targets.

Leucosep™

12 ml and 50 ml Leucosep™ Tubes



Leucosep™

Efficient separation of lymphocytes and mononuclear cells from peripheral blood and bone marrow

Features:

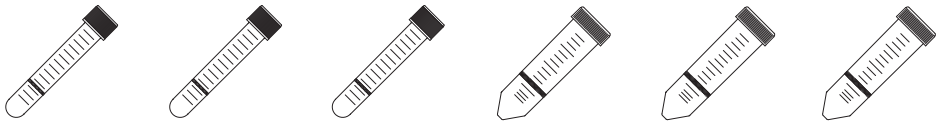
- Enrichment directly from whole blood
- Simplified filling through porous barrier
- Rapid separation in 15 minutes at room temperature
- No additional laboratory equipment required
- Removal of erythrocytes and granulocytes
- No recontamination with erythrocytes
- No blocking of marker molecules
- Pre-filled option with Leucosep™ separation medium
- Available unfilled for usage of different separation media

Leucosep™ was developed for optimal separation of lymphocytes and peripheral mononuclear cells (so-called PBMCs) from human whole blood and bone marrow. The key feature of Leucosep™ is the porous barrier incorporated into the centrifuge tube made of highly translucent polypropylene. This barrier consists of high-grade polyethylene. It shows a precisely controlled pore size and 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 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.

Leucosep™ may be used in combination with all common separation media for PBMC separation. For maximum convenience Leucosep™ tubes are available as pre-filled tubes. The contained Leucosep™ separation medium has a density of 1.077 g/ml and yields excellent separation results.

Typical separation results with Leucosep™ separation medium:	
Vitality	
Viable cells [%]	95 ± 5
Cell yield	
Lymphocytes [% of original number]	60 ± 20
Composition of enriched cell fraction	
Mononuclear cells [%]	95 ± 5
Granulocytes [%]	5 ± 5
Erythrocytes [%]	< 1
Composition of lymphocyte fraction	
T cells [%]	83 ± 3
B cells [%]	6 ± 3
NK cells [%]	11 ± 2

non-cytotoxic | non-pyrogenic



Cat.-No.	163 288	163 289	163 290	227 288	227 289	227 290
Description	Leucosep™ tubes with porous barrier	Leucosep™ tubes with porous barrier	Leucosep™ tubes with porous barrier	Leucosep™ tubes with porous barrier	Leucosep™ tubes with porous barrier	Leucosep™ tubes with porous barrier
Volume [ml]	12	12	12	50	50	50
Separation medium	+ / pre-filled with Leucosep™ separation medium	-	-	+ / pre-filled with Leucosep™ separation medium	-	-
Sterile	as	-	+	as	-	+
Sample volume	3 – 8 ml blood	3 – 8 ml blood	3 – 8 ml blood	15 – 30 ml blood	15 – 30 ml blood	15 – 30 ml blood
Quantity per box/case	50/500	50/500	50/500	25/250	25/300	25/300

as = aseptically produced

Instruction Manual Leucosep™

The Method

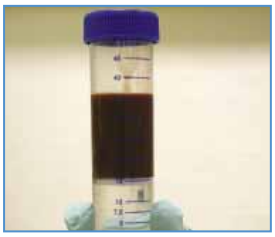
Leucosep™ has been developed for optimal separation of lymphocytes and peripheral mononuclear cells (so-called PBMCs) from human whole blood and bone marrow by means of density gradient centrifugation. The key feature of Leucosep™ is the porous barrier incorporated into the centrifuge tube made of highly translucent 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

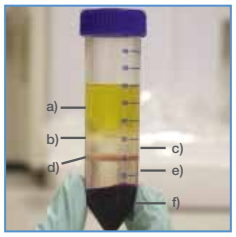
- Warm up separation medium to room temperature (RT) protected from light.
- Fill the Leucosep™ tube with separation medium: 3 ml when using tubes Cat.-No. 163 289 or 163 290; 15 ml when using tubes Cat.-No. 227 289 or 227 290.
- Close the tubes containing the separation medium with the screw cap and 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

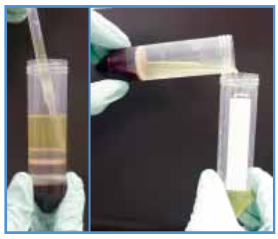
1) Filling with sample material



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 Cat.-No. 163 288, 163 289 or 163 290; 15–30 ml of sample material when using tubes Cat.-No. 227 288, 227 289 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.