Datasheet

Pancoll

Lymphocyte Separating Medium

Product	Description	Catalogue-No.	Size
Lymphocyte Separating Medium	**Pancoll human, Density: 1.077 g/ml**	**P04-60125M** **P04-60125** **P04-60225M** **P04-60225**	**2 x 50 ml** **25 x 50 ml** **2 x 10 ml** **50 x 10 ml**
Lymphocyte Separating Medium	**Pancoll animal, Density: 1.077 g/ml**	**P04-63225**	**50 x 10 ml**

Product description

Pancoll separating solutions from PAN-Biotech contain a polysaccharide with a molecular weight of 400,000 daltons; this hydrophilic polymer allows for production of aqueous solutions for cell separation with a density of up to 1.2 g/ml.

Storage conditions

Storage: +2 to +20°C, in the dark
Stability: 3 years from date of production
Size: 10 ml (prefilled with 3ml), 50 ml (prefilled with 15ml)

Method of Separation

For lymphocyte separation blood is used which has been defibrinated or treated with anticoagulants (Heparin, EDTA, Citrate), and which is diluted one to three-fold, depending on the haematocrit level of the blood sample, with the required volume of a physiological saline solution. Then the Pancoll solution is carefully covered with a layer of diluted blood in a centrifuge vial, without mixing the phases. After a short centrifugation step (e.g. 800 g for 20 minutes) at room temperature the lymphocytes, together with monocytes and platelets, can be harvested from the white blood cells layer between the plasma sample layer and the Pancoll. The separated cells are then washed twice in physiological saline solution to purify the lymphocytes by removing platelets.
During centrifugation the cells of the blood sample migrate to the Pancoll layer where they get into contact with the polysaccharide contained in Pancoll. The red blood cells are aggregated by this substance at room temperature immediately. Aggregation causes an increase of the sedimentation rate of the red blood cells which aggregate together with the granulocytes as a sediment at the bottom of the centrifuge vial. Lymphocytes, monocytes and platelets are not so dense and cannot enter and pass through the Pancoll layer. These cells are concentrated as white blood cell layer above the Pancoll layer and therefore can be harvested easily by careful pipetting.
In subsequent centrifugation steps the lymphocytes are washed to remove remaining platelets, serum and Pancoll. As a result of this process a highly purified suspension of viable lymphocytes and monocytes (PBMC) is obtained.

Sample preparation

Blood samples should be processed as soon as possible after they have been obtained in order to achieve optimum results and cell viability.
Storing blood samples at room temperature for more than 12 hours will cause a reduced yield of lymphocytes, a change in the surface markers and an impaired response to mitogen stimulation.

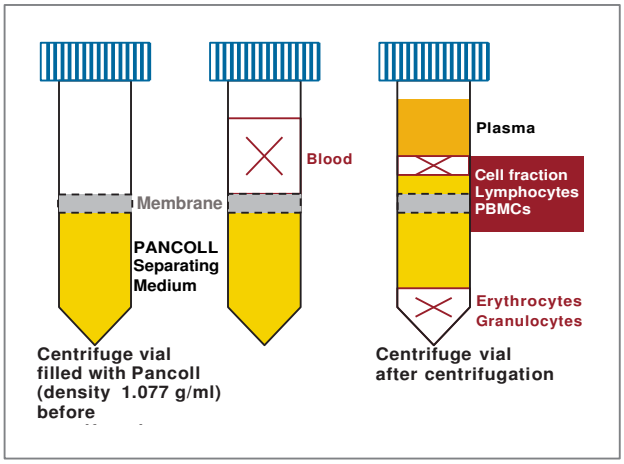
PAN Biotech GmbH – Am Gewerbepark 6 – 94501 Aidenbach – GERMANY
www.pan-biotech.com – phone +49-8543-601630 – fax +49-8543-601649

Typical results with Pancoll

Lymphocytes	60 ± 20 %	yield of Lymphocytes from original blood samples
	95 ± 5 %	of the Lymphocyte fraction are mononuclear Leukocytes
	> 90 %	live cells (trypan blue-exclusion)
Other cells	3 ± 2 %	Granulocytes
	5 ± 2 %	Erythrocytes
	< 0.5 %	total number of platelets of the original blood sample

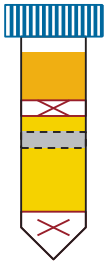
Quick user guide: Lymphocyte separating medium (pre-filled)

- Bring the vial with separating medium (Pancoll) to room temperature
- Dilute blood one to three-fold with buffered saline solution (four-fold for bone marrow) and pour into the vials carefully (15ml - 30ml)
- Centrifuge at 800 g for 20minutes in a centrifuge with a swing-out rotor (or 800 g for 15min)



SWITCH BRAKE OFF!

- After centrifugation the following phases form, from top (see figure)



- Plasma
- Enriched cell fraction (lymphocytes/PBMCs)
- Separating medium with permeable membrane in between
- Pellet (erythrocytes and granulocytes)

- Harvest the cell fraction with a pipette or by pouring off. The membrane prevents contamination with granulocytes and erythrocytes.
- Wash the lymphocytes/PBMCs with 10 ml buffered saline solution (e.g. DPBS) and then centrifuge at 300 g for 10 minutes.
- Repeat the washing step twice – finished

Result:	Possible cause:	Comment:
Contamination of the Lymphocyte fraction with Erythrocytes and Granulocytes	• Temperature to low • Centrifugation speed to low and/or time to short	The density of Pancoll is higher at lower temperatures, the Erythrocytes aggregate less and they cannot penetrate (also the Granulocytes) the Pancoll properly **Increase Pancoll temperature to 20°C**
Low yield and viability of Lymphocytes	• Temperature to high	Adequate times and G-forces have to be kept to assure a complete sedimentation of non-lymphoid cells. Pancoll has a lower density at higher temperatures and Lymphocytes can penetrate to Pancoll easier
Low yield of Lymphocytes with normal viability	• Blood sample not diluted with buffer • Abnormal high haematocrit in blood sample	At very high cell densities Lymphocytes can be included in aggregates of Erythrocytes **Dilute the blood sample**
Low yield of Lymphocytes with contamination of Granulocytes	• Vibrations of the centrifuge rotor can disturb the gradient	Vibrations can result in a broadening of the Lymphocyte band and to a stirring with the cells below **Balance the rotor and switch-off the brake of the centrifuge**
Low yield of Lymphocytes with contamination of other cell types	• Sample contains cells with abnormal densities	Can happen with pathologic blood samples or with samples of non-peripheral blood

Technical support

For technical support, questions or remarks please contact your local PAN-Biotech partner or the technical department of PAN-Biotech via email (info@pan-biotech.com) or phone +49-8543-601630.

PAN Biotech GmbH – Am Gewerbepark 6 – 94501 Aidenbach – GERMANY
www.pan-biotech.com – phone +49-8543-601630 – fax +49-8543-601649