Instructions for use: Pancoll

Pancoll

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. PAN-Biotech offers a variety of ready-to-use products with a density of 1.063 g/ml up to 1.091 g/ml for a very wide range of cell separation applications.

We offer the following products:

Product	Cat. No. 100 ml	Density g/ml	Osmolality mOsm/kg	pH
Pancoll Human	P04-60100	1.077	280-300	6.5-7.5
Pancoll Animal	P04-63100	1.077	255-275	6.5-7.5
Pancoll Mouse	P04-64100	1.086	270-290	6.5-7.5
Pancoll Rat	P04-65100	1.091	315-335	6.5-7.5
Pancoll Platelets	P04-67100	1.063	310-330	6.5-7.5
Pancoll Monocytes	P04-68100	1.068	325-355	6.5-7.5

The ready-to-use solutions are also available in 500 ml bottles (Cat. No. P04-**500) as well as in prefilled ready-to-use tubes with a separating membrane (for example Pancoll human: 50 ml tubes Cat. No. P04-60125 and 10 ml tubes Cat. No. P04-60225)

Pancoll is stable for at least 3 years at +2°C to +20°C if not opened. Protect from light!

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.

Protocol for Isolating Lymphocytes

1) Transfer Pancoll at ambient temperature under sterile conditions into a suitable sterile centrifuge tube. Alternatively 50 ml tubes, prefilled with 15 ml Pancoll (P04-60125) or 10 ml tubes prefilled with 3 ml Pancoll (P04-60225) can be used. The 50 ml tubes are used with 15-30 ml blood samples and 10 ml tubes are used with 3-8 ml blood samples.
2) Carefully cover the separating solution with a layer of the blood sample which is diluted one to three-fold with physiologic saline solution. **Important: Do not mix the blood sample with Pancoll!**
3) Centrifuge at 800 g at 20°C for 20 minutes. **SWITCH BRAKE OFF!**
4) After centrifugation, carefully remove the upper phase (containing plasma and platelets) using a pipette, without mixing the interphase with the lymphocytes.
5) Using a new pipette, transfer the lymphocyte band above the Pancoll-Layer to a new centrifuge vial. It is important to remove all the lymphocyte band of the interphase with as little volume as possible. If too much Pancoll (lower phase) is picked up, a contamination with granulocytes may occur. If too much supernatant (upper phase) is picked up an increased contamination with platelets with occur.
6) Add at least 3 volumes of a physiological saline solution to the lymphocytes.
7) Suspend the lymphocytes carefully using a pipette.
8) Centrifuge at 300 g at 20°C for 10 minutes.
9) Discard the supernatant.
10) Repeat the washing step two times.

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Typical Results with Pancoll

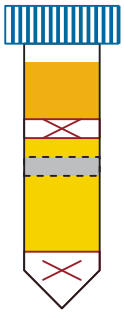
Lymphocytes:	60 ± 20 %	yield of Lymphocytes from original blood samples
	95 ± 5 %	of the Lymphocyte fraction are mononuclear Leukocytes
	> 90 %	live cells (trypan blue-exclusion)
other cells:	3 ± 2 %	Granulocytes
	5 ± 2 %	Erythrocytes
	< 0.5 %	total number of platelets of the original blood sample

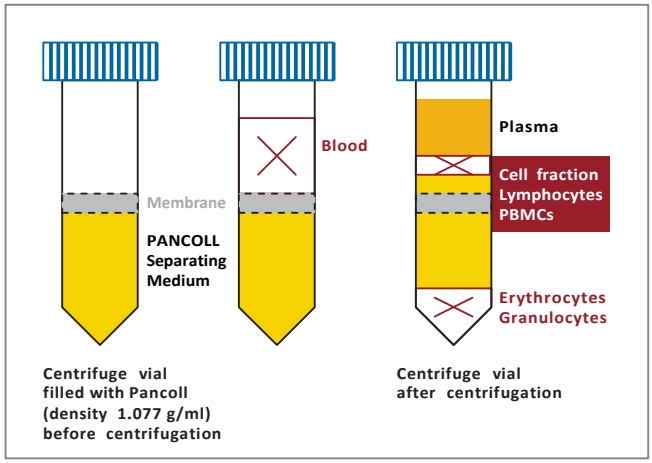
Trouble-shooting

Result:	Possible cause:	Comment:
Contamination of the Lymphocyte fraction with Erythrocytes and Granulocytes	• Temperature too low • Centrifugation speed too low and/or time to short	The density of Pancoll is higher by lower temperatures, the Erythrocytes aggregate less and they can not penetrate (also the Granulocytes) the Pancoll properly **Increase Pancoll temperature to 20°C**
Low yield and viability of Lymphocytes	• Temperature too 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 **Decrease Pancoll temperature to 20°C**
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

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

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