User Information

Pancoll

Pancoll separating solutions from PAN-Biotech contain Ficoll 400, a sugar polymer with a molecular weight of approx. 400,000 daltons. With this hydrophilic polymer aqueous solutions can be made up to a density of approx. 1.2 g/ml.
However, in order to obtain optimum levels of pH and osmolality Ficoll has to be mixed with an acid, preferably diatrizoic acid and sodium hydroxide. We have already made up this optimum mixture for you in our products with a density of 1.063 g/ml to 1.091 g/ml for a very wide range of applications.

We can offer you the following products:

Product	**Cat. No.**	**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 Monocytes	P04-66100	1.068	325-355	6.5-7.5
Pancoll Platelets	P04-67100	1.063	310-330	6.5-7.5

Solutions are available in 100 ml or 500 ml bottles (Cat. No. ---500).

Pancoll will keep for at least 2 years at room temperature if unopened. Protect against light!

On request the various parameters can be specially adjusted to suit your specifications.

Method of Separation

For lymphocyte separation blood is used which has been defibrinated or treated with anticoagulants (Hepari, EDTA, Citrate), and diluted with the same volume of a physiological saline solution beforehand. 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 (normally 400 g for 30 – 40 minutes) at room temperature the lymphocytes, together with monocytes and platelets, can be extracted from the boundary layer between Pancoll and the sample layer.

This material is then washed in physiological saline solution twice in order to clean the lymphocytes by removing the platelets.

The separation process is influenced by various factors.
During centrifugation the cells of the blood sample migrate to the Pancoll boundary layer, where they come into contact with Ficoll 400 (contained in Pancoll). The red blood cells are aggregated by this substance at room temperature immediately. Aggregation causes an increase in the sedimentation of those cells so they sink to the bottom as pellets very quickly, where they are distinctly separated from the lymphocytes. The granulocytes sediment to the bottom of the centrifuge vial in the same way. This process is facilitated by raising their density, which occurs on account of contact with the slightly hypertonic Pancoll solution.
After completing centrifugation the granulocytes and the red blood cells are both found at the bottom of the centrifuge vial, in addition to the Pancoll.

Lymphocytes, monocytes and platelets are not sufficiently dense to enter the Pancoll. These cells therefore collect in a concentrated band as a boundary layer between the blood sample and the Pancoll. This band formation makes it possible to extract lymphocytes with a high yield and low impurities using Pancoll.
In subsequent washing and centrifugation steps the lymphocytes are cleaned to remove platelets, serum and Pancoll. As a result a highly purified suspension of viable lymphocytes and monocytes is obtained which can be used for further studies.

Sample Preparation

Blood samples should be processed as soon as possible after they have been obtained in order to achieve optimum results. The effects of storing blood samples at room temperature for 24 hours are a reduced yield of lymphocytes, a change in the surface markers and an impaired response to mitogen stimulation.

Protocol for Isolating Lymphocytes

1) Under sterile conditions, introduce Pancoll (3 ml) into a suitable sterile centrifuge vial.
2) Carefully cover the separating solution with a layer of the diluted blood sample (4ml). **Important**: **Do not mix** the blood sample and Pancoll!
3) Centrifuge 400 g at 18-20°C for 30 – 40 minutes.
4) After centrifugation, carefully remove the upper phase (containing serum and platelets) using a pipette, without mixing the interphase with the lymphocytes.
5) Using a new pipette, transfer the lymphocyte band to a new centrifuge vial. When doing so it is important to remove all the material of the interphase with as little volume is possible. If too much Pancoll (lower phase) is transferred, this will cause contamination with granulocytes. Excess supernatant (upper phase) leads to increased contamination with platelets.
6) Add at least 3 volumes of a physiological saline solution (6 ml) to the lymphocytes.
7) Suspend the lymphocytes carefully using a pipette.
8) Centrifuge 60-100 g at 18 – 20°C for 10 minutes.
9) Discard the supernatant.
10) Repeat the washing step (items 6-9).

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