Separating Solutions

Pancoll and Powercoll

Description

Often the isolation of cells or of sub-cellular particles is the first step in gene expression research or in diagnostic examinations. Apart from the bio-specific separation methods physical separation methods are most commonly used. In these methods physical differences such as size and charge of the particles to be separated are utilised. For this purpose so-called separating solutions (= centrifugation media) are used.

These media have to comply with the following criteria:

- Form a density gradient over the desired range
- Desired pH value and desired osmolality easily adjustable
- The solutions should not be too viscous at high density
- Do not cause any functional or morphological changes in biological materials
- Do not penetrate biological membranes

Our separating solutions – Pancoll – are made from a neutral, highly cross-linked, hydrophilic polymer of sucrose with an average molecular weight of 400,000 D.

Powercoll consists of a colloidal suspension of silica particles, loaded with polyvinyl pyrrolidone (PVP).

Storage: 2° C to ambient temperature

When properly stored, separating solutions are stable for at least 36 months. The storage period starts with the manufacturing date.

Pancoll human, density 1.077 g/ml (1)	100 ml 500 ml	P04-60100 P04-60500
Pancoll mouse, density 1.086 g/ml (2)	100 ml 500 ml	P04-64100 P04-64500
Pancoll rat, density 1.091 g/ml (2)	100 ml 500 ml	P04-65100 P04-65500
Pancoll animal, density 1.077 g/ml (2)	100 ml 500 ml	P04-63100 P04-63500
Pancoll monocytes, density 1.068 g/ml (3)	100 ml 500 ml	P04-68100 P04-68500
Pancoll platelets, density 1.063 g/ml (3)	100 ml 500 ml	P04-67100 P04-67500
Powercoll, density 1.077 g/ml	100 ml	P04-61100
Powercoll, density 1.124 g/ml	100 ml	P04-62100

(1) usually on stock, (2) minimum order 10 l, (3) available on request

Lymphocyte Separating Medium

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. 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. The ready-to-use solutions are also available in 500 ml bottles (Cat.no. -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).

Stability

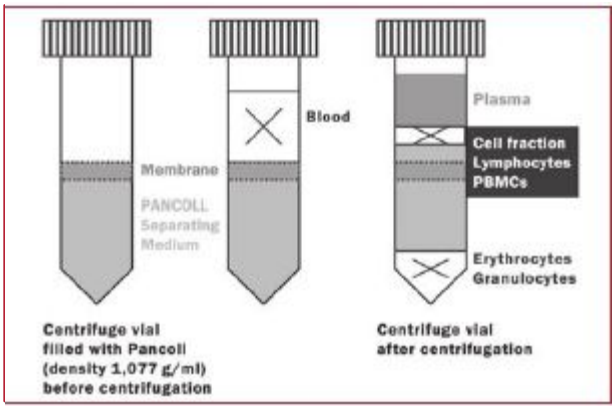
Pancoll is stable for at least 36 months at +2°C to +20°C if not opened. Protect from light!

Typical results with Pancoll

Lymphocytes:	60 ± 20 % 95 ± 5 % > 90 %	yield of lymphocytes from original blood samples of the lymphocyte fraction are mononuclear leukocytes live cells (trypan blue-exclusion)
Other cells:	3±2% 5±2% < 0,5 %	granolocytes erythrocytes total number of platelets of the original blood sample

Method of seperation

For lymphocyte separation blood is used which has been defibrinated or treated with anticoagulants (Heparin, EDTA, Citrate), and which is diluted with the same 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. 800-1000x g for 20-30 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 can not 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.



Pancoll human, density 1.077 g/ml (1)	25 x 50 ml 50 x 10 ml	P04-60125 P04-60225
Pancoll animal, density 1.077 g/ml (3)	50 x 10 ml	P04-63225

(1) usually on stock, (2) minimum order 10 l, (3) available on request