

Datasheet

Pancell T

Chemically defined medium for *in vitro* cultivation of T-cells

Product	Description	Catalog-Nr.	Size
Pancell T kit	Kit for <i>in vitro</i> cell culture and expansion of T-cells, including supplement	P04-710414K	500 ml kit
Pancell T medium	Medium for <i>in vitro</i> cell culture and expansion of T-cells	P04-710414	500 ml

Product description

Pancell T is a chemically defined and protein-free (< 0.02%) medium for serum-free cultivation and proliferation of lymphocytes from full blood. The medium can be used for the enrichment and expansion of T-cells *in vitro*.

It allows

- Enrichment of T-cells from PBMC / full blood, after stimulation (e.g., with PHA-L or anti-CD3)
- Stable performance > 4 passages
- Long-term cultivation of immortalized T-cells
- CD3+ > 80%

Composition

Pancell T medium consists of a balanced mixture of salts, amino acids, vitamins, trace elements and hormones for optimized growth. The kit includes a basal medium and a cytokines supplement P04-710414S.

IMPORTANT: Mitogens, such as PHA-L, or antibodies, such as anti-CD3, are necessary for the cell differentiation or the enrichment of T-cells from whole blood / PBMC. These reagents are not included in the Pancell T kit, and can be ordered at <http://www.pan-biotech.com>

Storage conditions and stability

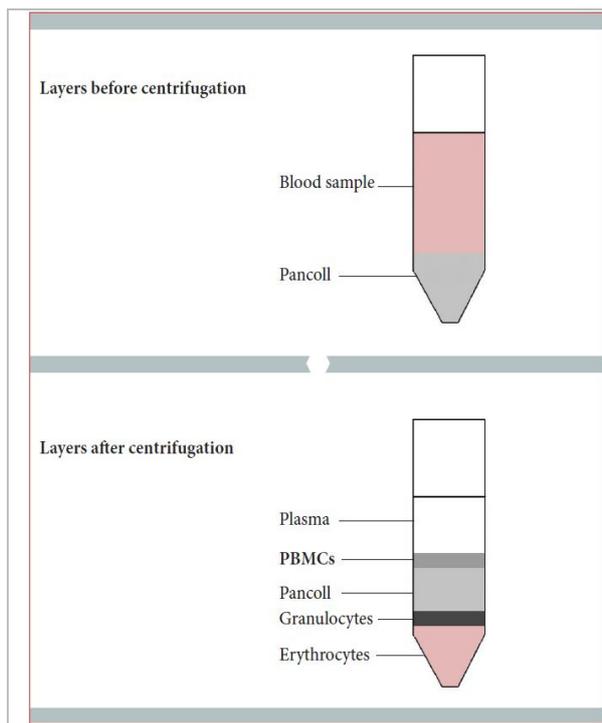
Storage: Pancell T Medium, PHA-L (lyophilized): 2-8°C in the dark
Pancell Growth Supplement: -20°C
Stability: 10 months

Pancell T supplemented with growth factors can be safely stored up to 3 months at 2-8°C in the dark. Repeated warming / cooling cycles and exposure to light should be avoided.

Instructions for Use

Isolation of PBMCs / lymphocytes from full blood by density gradient centrifugation.

Heparinized full blood is diluted 1-3-fold with PBS or RPMI-medium and added to a centrifuge tube filled with lymphocyte separating solution (Pancoll human density 1.077 g/ml, PAN-Biotech Catalog-Nr. P04-60500).



Schematic figure of a density gradient centrifugation

Centrifuge the gradient at 800xg for 15 minutes (or 400xg for 30 minutes) at room temperature (turn off the brake of the centrifuge).

After centrifugation four phases are created:

- top phase contains plasma
- opaque whitely band, Buffy-Coat (lymphocytes)
- separating medium
- pellet with erythrocytes and granulocytes

Carefully remove the plasma with a pipette and transfer the lymphocytes with a new pipette into a new centrifugal tube. Wash the lymphocytes/PBMCs with 10ml of a buffered saline solution e.g. 1x PBS (without Ca and Mg) and subsequently centrifuge for 10 minutes at 500xg. Discard the supernatant and wash the lymphocyte pellet again with 1x PBS (without Ca and Mg). A total of 2-3 washing steps are necessary.

Please note!

- Use Pancoll at room temperature.
- The more diluted the blood sample, the better the purity of mononuclear cells
- The peripheral blood or buffy coat should not be older than 6 hours and should be supplemented with anticoagulants (e.g. heparin)
- Pipette the blood very carefully onto the Pancoll to avoid mixing the phases
- Turn the brake of the centrifuge off
- A too quick brake-process cause the phases to mixing

Stimulation of lymphocytes and resuspension in Pancell T medium

To start and maintain proliferation of primary lymphocytes, the cells need to be stimulated with mitogens. These mitogens are mostly herbal lectins like PHA-L or antibodies like anti-CD3 (PAN-Biotech Catalog-Nr. CB-1103500).

- For stimulation and proliferation of T-cells adjust the cells to a cell density of approx. 1×10^5 /ml, incubate at 37°C, > 90% moisture and 5% CO₂. Freshly prepared medium (containing Supplement 1 and 2) should be stored at 2 - 8 °C.
- The incubation time varies from 48 to 72 hours - according to type and origin of the lymphocytes and the intended use.
- For a further cultivation of lymphocytes: fresh medium and stimulation of the lymphocytes needs to be repeated.

Please note: the proliferation rate of primary lymphocytes from full blood is limited.

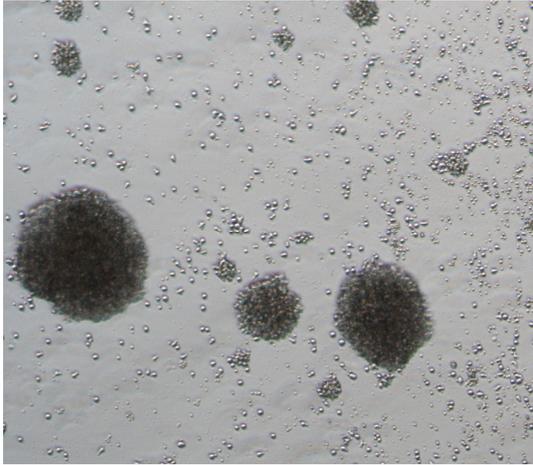


Fig 1: PBMC cultivated in Panell T,
5 days after stimulation

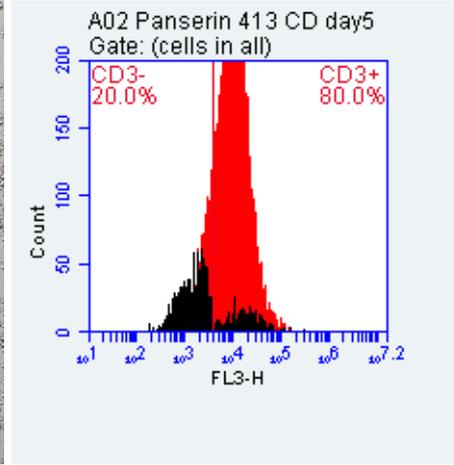


Fig 2: CD3 stained PBMCs Panell T,
5 days after stimulation

Application

This product is intended for research or manufacturing use only.

Technical Support

Additional information will be available on our website: www.pan-biotech.com. 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.

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