Datasheet

Panserin 413

Serum-free Medium for T- and CIK-Cells

Product	Description	Catalogue-No.	Size
Panserin 413	**Serum-free medium for culture and expansion of T-and CIK-Cells**	**P04-71413M** **P04-71413** **P04-71413S**	**100 ml** **500 ml** **1000 ml**

Product description
Panserin 413 is a ready-to-use medium for the serum-free cultivation and proliferation of lymphocytes from whole blood. The medium can be used for the enrichment of T-cells, CIK, LAK and NK cells.
It allows

- Expansion of T-cells from PBMC / whole blood after stimulation (e.g., with PHA-L or anti-CD3).
- Expansion of NK-, LAK- and CIK-cells after stimulation.
- Long-term cultivation of immortalized T-cells.

Storage conditions and stability
Storage: 2-8°C in the dark
Stability: 10 months from date of production
Size: 100 ml, 500 ml, 1000 ml, other sizes on request
Panserin 413 with supplements / 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.

Composition
Based on RPMI 1640 and DMEM-F12 the medium was optimized with additional salts, trace elements, albumin, cholesterol, lipids and vitamins and thereby allows an optimal growth of T-cells.
Growth factors for the cell differentiation or the enrichment of T-cells from whole blood / PBMC are not included in Panserin 413 and must be added if necessary.
We offer suitable supplements for Panserin 413 with the Cat. No. P04-413S.
On our homepage you will find numerous human cytokines and growth factors.
http://www.pan-biotech.com/en/biologicals/human-cytokines-and-growth-factors

Suitability
FOR RESEARCH USE ONLY!
Not approved for human or animal diagnostic or therapeutic procedures.

Instructions for Use

Isolation of PBMCs / lymphocytes from whole blood by density gradient centrifugation
Heparinized whole blood is diluted 1-3-fold with DPBS or RPMI medium and added to a centrifuge tube filled with lymphocyte separating solution (Pancoll human density 1.077 g/ml, PAN-Biotech Cat. No. P04-60100 / P04-60500). Pipette the blood carefully on the Pancoll to avoid a mixing in different phases.
Centrifuge the gradient at 800 g for 20 mintues at room temperature (turn off the brake of the centrifuge!)
Carefully remove the plasma with a pipette and transfer the lymphocytes with a new pipette into a new centrifugal tube. Wash the lymphocytes/PMBCs with 10ml of a buffered saline solution e.g. 1x DPBS (without Ca^{2+}/Mg^{2+}) and subsequently centrifuge for 10 minutes at 300 g. Discard the supernatant and wash the lymphocyte pellet again with 1x DPBS (without Ca^{2+}/Mg^{2+}). A total of 2-3 washing steps are necessary.

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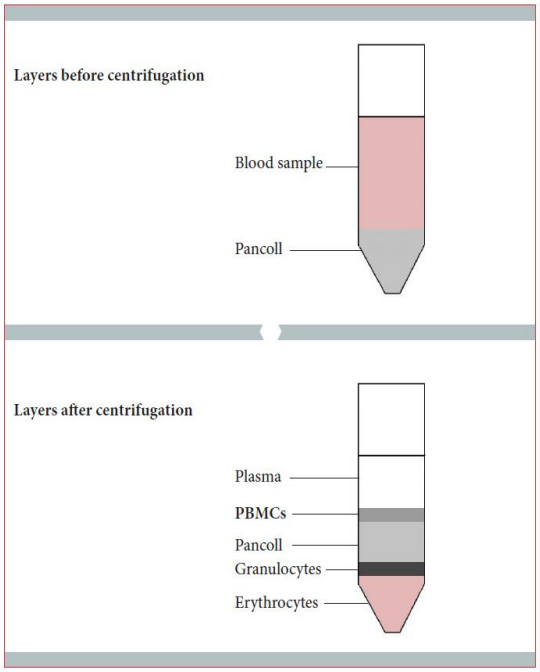
Please note

- 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
- Braking-rapidly causes a mixture of the phases



Schematic figure of a density gradient centrifugation

Centrifuge the gradient at 800 g for 20 minutes at room temperature (turn off the brake of the centrifuge!).

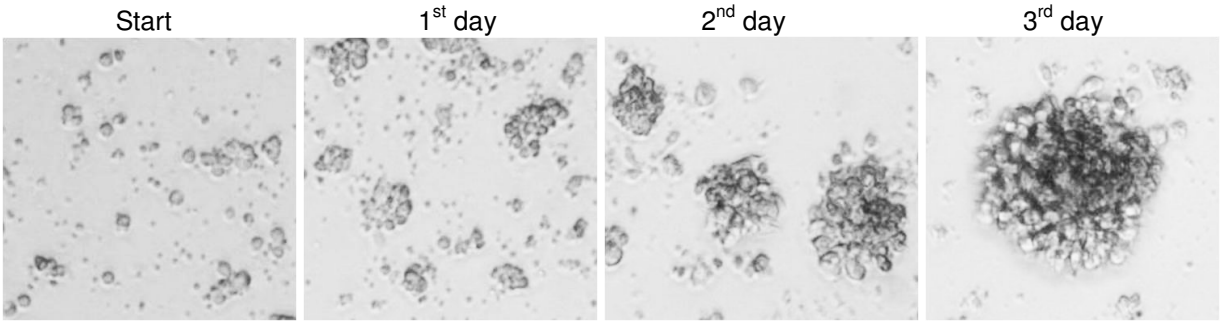
After centrifugation 4 phases are created:

- top phase plasma
- opaque whitely band, buffy coat (lymphocytes)
- separating medium
- pellet with erythrocytes and granulocytes

Stimulation of lymphocytes and resuspension in Panserin 413

Panserin 413 is developed for serum-free cultivation of lymphocytes from whole blood.
Cells from peripheral blood are arrested in G0 and will die in the *in vitro* cell culture relatively fast. To start and maintain several divisions in culture in primary lymphocytes, the cells need to be stimulated with mitogens. These mitogens are mostly plant lectins like phytohemagglutinin (PHA) or antibodies like anti-CD3.

- For stimulation and proliferation of T-cells adjust the cells to a cell density of approx. $1x10^5$/ml and add PHA according to the manufacturer's recommendation (generally 1-5µg/ml)
- 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 whole blood is limited.



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Cultivation and stimulation of Cytokine-induced killer cells

Cytokine-induced killer cells, initially described by Schmidt-Wolf *et al.* in 1991, are efficiently expanded *in vitro* from PBMCs by the timely addition of IFN-γ, , mAb anti-CD3 (OKT3) and IL-2.

After 2–3 weeks of *in vitro* culture the expansion of CIK cells is described to range from few to more than 1000-fold.

The isolation of *peripheral blood mononuclear cells* (PBMCs) is usually the first step to obtain cells of the immune system. Due to the different densities of granulocytes, PBMCs und red blood cells (RBCs) separating them via a density gradient like Pancoll is easy.

First, as described above, lymphocytes are isolated through a density gradient centrifugation, the buffy coat is isolated and the cell pellet is washed 2-3 times in 1x DPBS (without Ca^{2+}/Mg^{2+}).

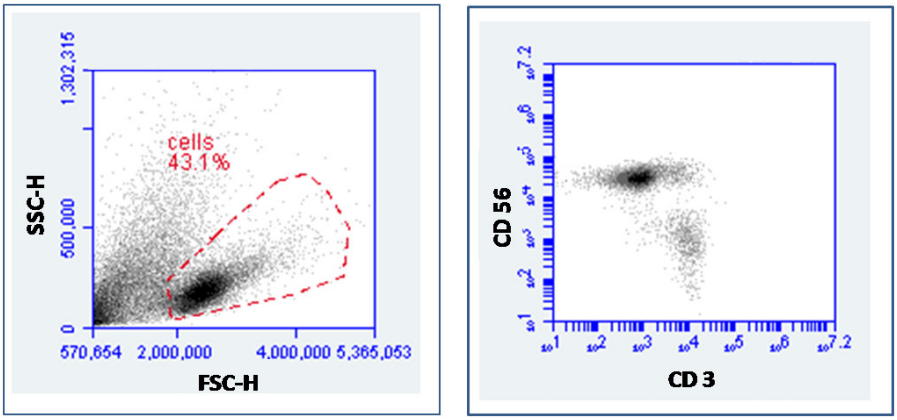
Cultivation of CIK

To isolate CIK (cytokine induced killer) cells from whole blood you need:

- Lymphocytes medium: Panserin 413
- PBMCs
- Anti-CD3
- IL-2 1000IU/ml, (PAN-Biotech Cat. No. CB-2130203, CB-2130202)
- IFNγ 2000 IU/ml, (PAN-Biotech Cat. No. P-2060020, P-2060100)
- If required 5% autologous serum

Use of anti-CD3 according to the manufacturer's recommendation

- Day 0: (optional) Coating: Dilute anti CD3 (OKT3) with sterile DPBS (without Ca^{2+}/Mg^{2+}) to 5 µg/ml. 10 to 15 ml are necessary for T175 cm² flask. Incubate at 4°C, overnight (maximal for 3 days). Wash with sterile DPBS (without Ca^{2+}/Mg^{2+}) and subsequently with Panserin 413 before use.
- Day 1: Inoculate the cells into to a T175 flask (OKT3 coated, optional) at a density of 1.5 – 2.0 x 10^6 cells/mL in 25 – 30 mL Panserin 413 supplemented with IFNγ and IL-2 at a concentration of 2000 IU/ml and 1000 IU/ml respectively. Add 100 ng/ml anti CD3 (OKT3) if the flask is not coated.
- Cultivation at 37°C with 5% CO2.
- Day 3: 100 µL sample for counting.
  Add 25 – 30 mL fresh Panserin 413 containing 1000IU/ml IL-2, 2000 IU/ml IFNγ, 100ng/ml CD3 (OKT3) (Not necessary if the flask is coated). (Total volume 50 -60 ml)
- Day 5/6: 100 µL sample for counting.
  Add 60 mL fresh Panserin 413 containing 1000IU/ml IL-2 (Total volume 120 ml).
- Day 7 to day 14: Add fresh Panserin 413 containing 1000IU/ml IL-2 according to the cell concentration and pH (or add fresh medium every 2 or 3 days).



Flow cytometry analysis of leukocytes. Enrichment of cells CIK from whole blood after stimulation and cultivation in Panserin 413

IF-staining with

- CD3-PE
- CD56-FITC

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.