Instructions for use

ROTI®Sep

Ready-to-use and sterile separation medium based on polysucrose 400, for efficient isolation of lymphocytes from human blood by density gradient centrifugation.

Lymphocyte separation medium, Polysucrose solution
Density 1.077 g/ml or 1.086 g/ml
Best alternative to Ficoll

0634, 0642, 0624, 0622

- Optimised for isolation of lymphocytes from non-coagulated whole blood
- Produces distinct, compact layers
- Results in high recovery rates of viable cells
- Maintains the representative ratio of B- to T lymphocytes
- May be directly applied to all protocols using Ficoll®

1. Description

ROTI®Sep solution excels due to the superior separation power, forming very distinct, easy to pipet, lymphocyte layers during centrifugation. The ready to use solution is very easy in handling, resulting in high cell recovery rates, while cell viability and the representative ratio of B- to T lymphocytes are maintained. Osmolality as well as pH value are strictly kept in the physiological range.
Suitable for application with human non-coagulated peripheral blood.

In all respective protocols originally using Ficoll® this reagent may be replaced by ROTI®Sep solution without any adjustment.

ROTI®Sep 1077 **human**: Optimised for isolation of PBMCs from human blood
ROTI®Sep 1077 **human/tube**: Optimised for isolation of PBMCs from human blood. Prefilled in 50 ml centrifugation tubes, 15 ml each**.** With permeable membrane for easy pipetting of diluted blood.
ROTI®Sep 1077 **animal**: Optimised for isolation of PBMCs from mammalian blood in general.
ROTI®Sep 1086 **mouse**: Optimised for isolation of PBMCs from murine blood.

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2. Method

For lymphocyte separation blood is used which has been defibrinated or treated with anticoagulants (Heparin, EDTA, Citrate). Generally and depending on the haematocrit level of the blood sample, this blood is then diluted one to three-fold with the required volume of a physiological saline solution.
For preparation of lymphocytes, ROTI®Sep solution is prepared in 50 or 15 ml centrifuge tubes and then covered with a layer of diluted blood, while very carefully avoiding to mix the phases. During a short centrifugation step, the lymphocytes (with monocytes and platelets) gather in the white blood cells layer between the plasma sample and the ROTI®Sep solution.
In subsequent washing steps the lymphocytes are washed to remove remaining platelets, serum and ROTI®Sep solution. As a result of this process a highly purified suspension of viable lymphocytes and monocytes (PBMC) is obtained.

3. Mechanism

During centrifugation, all cells of the blood sample migrate to the ROTI®Sep layer where they get in contact with the polysaccharide contained therein. At room temperature, the red blood cells immediately aggregate by this substance, increasing the sedimentation rate of the red blood cells which sediment at the bottom of the centrifuge vial together with the granulocytes.
Due to their lower weight, lymphocytes, monocytes and platelets cannot enter pass the ROTI®Sep layer, but are forced downwards nevertheless, gathering in a more or less sharp band on top of the ROTI®Sep layer, where they can be harvested quite easily by pipetting.

4. 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.

5. Notes for application (see also 9. Trouble shooting)

- Tube/sample size: Use 50 ml tubes with 15 ml ROTI®Sep for sample sizes of 15-30 ml. Use 15 ml tubes with 3 ml ROTI®Sep for sample sizes of 3-8 ml. Samples of 8-15 ml should be diluted to 15 ml and be centrifuged using 50 ml tubes.
- Temperatures: Always keep the separation solution under cooled conditions.
  Vials prepared for use have to be brought to room temperature before use.
- Assembling of the separation gradient: The layer of diluted blood sample MUST NOT MIX with the layer of ROTI®Sep separation solution.
  Tip: Use prefilled 50 ml tubes 'ROTI®Sep 1077-human/tube' (15 ml solution each) with application aiding membrane.
- Centrifuge: Use a centrifuge with a swing-out rotor. The break has to be switched off during the preparation.
- Yield of lymphocytes: It is important to remove all the lymphocyte band ('interphase') in as little volume as possible. If too much ROTI®Sep (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 will occur.

6. Application.

1.) Transfer 15 ml (3 ml) cooled ROTI®Sep under sterile conditions into a suitable 50 ml (15 ml) sterile centrifuge tube. Bring the tube to room temperature.
Alternatively use prefilled 50 ml tubes 'ROTI®Sep 1077-human/tube' (15 ml solution each) with application aiding membrane.

2.) Dilute the blood sample 1x to 3x with physiologic saline solution.

3.) Carefully cover the ROTI®Sep separation solution with a layer of the diluted blood sample.
Important: Do not mix the blood sample with ROTI®Sep solution!

4.) Centrifuge at 800 g at 20 °C for 20 minutes. **SWITCH BRAKE OFF!**

5.) After centrifugation, carefully remove the upper phase (containing plasma and platelets) using a pipette, without mixing the interphase with the lymphocytes.

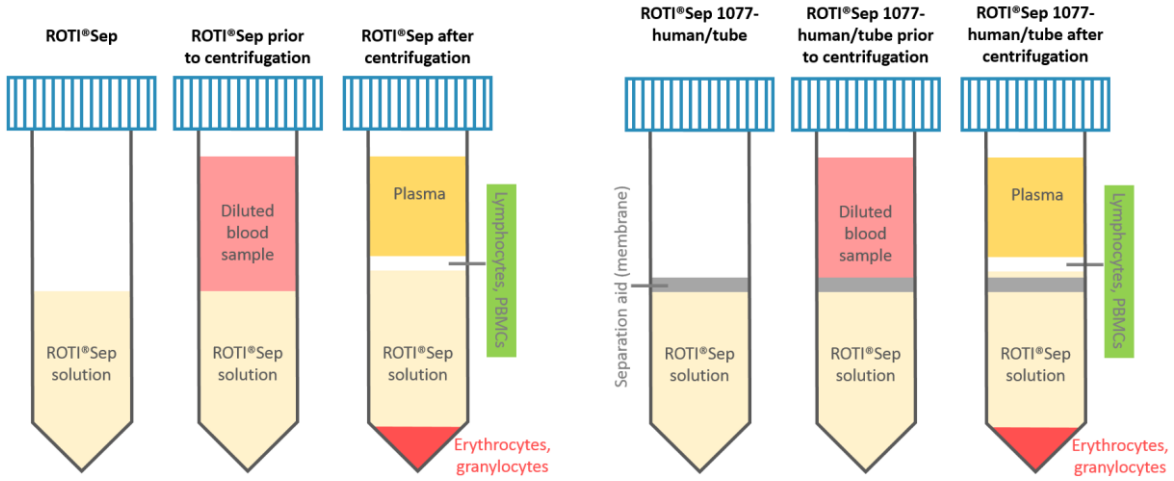
6.) Using a new pipette, transfer the lymphocyte band above the ROTI®Sep layer to a new centrifuge tube.
Note: Remove all the lymphocyte band of the interphase with as little volume as possible.

7.) Add at least 3 volumes of a physiological saline solution (e.g. ROTI®Cell DPBS) to the lymphocytes. 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 steps (7.-9.) 2x



7. Storage conditions

15 ml prefilled in 50 ml tubes.
Long-term storage at approx. 4 °C recommended.
Short-term storage at room temperature possible.
Storage protected from light.

8. Typical Results with ROTI®Sep solutions

LYMPHOCYTES	
60 ± 20 %	Yield of lmphocytes from original blood samples
95 ± 5 %	Mononuclear leukocytes (% of the lymphocyte fraction)
> 90 %	Living cells (trypan blue-exclusion)
OTHER CELLS	
3 ± 2 %	Granulocytes
5 ± 2 %	Erythrocytes
< 0.5 %	Total number of platelets (% of the original blood sample)

9. Additionally recommended products

ROTI®Cell 10x DPBS w/o Ca/Mg	Art.-No. 9130
ROTI®Cell DPBS w/o Ca/Mg	Art.-No. 9124
ROTI®Cell DPBS with Ca/Mg	Art.-No. 9131
ROTI®Cell Hanks' BSS w/o Ca/Mg, phenol red	Art.-No. 9117
ROTI®Cell Hanks' BSS with Ca/Mg, w/o phenol red	Art.-No. 9119

10. Trouble shooting

RESULT	PUTATIVE 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 ROTI®Sep is higher by lower temperatures. Thus the erythrocytes aggregate less and cannot penetrate the ROTI®Sep properly (also true for granulocytes) ➔ *Increase ROTI®Sep temperature to 20°C*
Low yield and viability of lymphocytes	Temperature too high	ROTI®Sep has a lower density at higher temperatures and lymphocytes are able to penetrate the ROTI®Sep layer. Adequate times and G-forces have to be kept to assure a complete sedimentation of non-lymphoid cells. ➔ *Decrease ROTI®Sep 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 diffuse lymphocyte band and of a diminished quality of cell separation ➔ *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	May happen with pathologic blood samples or when using samples of non-peripheral blood

ROTI®-Sep 1077 human	100 ml	**0642.1**
ROTI®-Sep 1077 human	500 ml	**0642.2**
ROTI®-Sep 1077 human/tube	25 x 50 ml, cardboard box	**0634.1**
ROTI®-Sep 1077 animal	100 ml	**0622.1**
ROTI®-Sep 1077 animal	500 ml	**0622.2**
ROTI®-Sep 1086 mouse	100 ml	**0624.1**
ROTI®-Sep 1086 mouse	500 ml	**0624.2**

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