

NycoPrep™ 1.077

PRODUCT DESCRIPTION

NycoPrep™ 1.077 is a ready made, sterile and endotoxin tested solution of Nycodenz, N.N'- Bis (2,3-dihydroxypropyl) -5- [N- (2,3-dihydroxypropyl) acetamido] - 2,4,6 - triiodo-isophthalamide, for the in vitro isolation of human mononuclear cells (lymphocytes and monocytes).

Composition

Nycodenz	14.1 % (w/v)
NaCl	0.44 % (w/v)
Tricine/NaOH pH 7.2	5 mM

Physical-chemical characteristics:

Density	1.077 ± 0.001 g/ml (20°C)
Osmolality	295 ± 15 mOsm

PRINCIPLE OF THE SEPARATION PROCEDURE

The most common technique up till now for separating leucocytes is to layer the blood sample on top of a solution containing an aggregating agent mixed with a compound of high density. Using a mixture of Sodium Metrizoate and Ficoll, Bøyum (1968) developed a one-step centrifugal technique for isolation of lymphocytes (Lymphoprep™). Blood separation media containing erythrocyte aggregating compounds may alter mitogen responsiveness of isolated lymphocyte preparations. Therefore, investigations have been done to avoid such compounds in the separation solution. Using a mixture of Nycodenz® and NaCl, Bøyum (1983) published a new method for isolation of mononuclear cells without any erythrocyte aggregating compound. As with Lymphoprep™ this is a reliable, simple and quick method suitable for preparation of lymphocytes from fresh blood and also superior to other techniques for lymphocyte preparations from cadaver blood, and from anticoagulated blood stored at room temperature for up to 6 hours.

STABILITY AND STORAGE

NycoPrep™ is stable for 3 years provided the solution is kept sterile and protected from light.

The solution should be stored at or below 20°C.

NycoPrep™ is autoclavable.

SEPARATION PROCEDURE

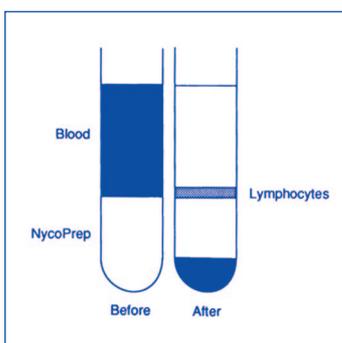
1. Collect blood into a tube containing anticoagulant (EDTA, heparin, ACD) or use defibrinated blood.

2. Dilute the blood by addition of an equal volume of 0,9 % NaCl.

3. Carefully layer 6 ml of the diluted blood over 3 ml NycoPrep™ in a 12 – 15 ml centrifuge tube. Avoid mixing of blood and separation fluid. Cap the tube to prevent the formation of aerosols.

4. Centrifuge at 800 x g for 20 min at room temperature (approximately 20°C) in a swingout rotor. If the blood is stored for more than 2 hours, increase the centrifugation time to 30 min.

5. After centrifugation the mononuclear cells form a distinct band at the interface between the sample layer and the NycoPrep™ solution, as shown in the figure. The cells are best removed from the interface using a Pasteur pipette without removing the upper layer.



6. Transfer the cell suspension to a smaller tube, and add some Tris-BSS (Tris-balanced salt solution) or similar physiological solution, and centrifuge at 400 x g for 10 min.

7. Resuspend the cells in Tris-BSS and spin down again.

8. Resuspend the cells in autologous or homologous fresh, normal human serum.

Tris-balanced salt solution (Tris-BSS) : 1:1 isotonic Tris buffer in balanced salt solution, or similar physiological solutions.

When ACD blood is defibrinated to be used for complement dependent tests, an additional washing is recommended. If heparinized or EDTA stabilized blood is used, most of the platelets are removed by the washing procedure. The centrifugation should then be carried out at 160 x g for 10 min.

PURITY AND VIABILITY

The described method has found to be rapid, simple and reliable and gives excellent results with blood samples from most normal individuals and patients. The contamination of erythrocytes in the lymphocyte suspension is usually between 1-5% of the total cell number. Some immature granulocytes may follow the lymphocytes during intense immunosuppressive therapy. When heparinized blood is used, it is essential to remove most of the platelets, in order to avoid inhibition in the cytotoxicity test. The described washing procedure is usually sufficient.

REFERENCES

1. Rickwood, D., Ford, T.C. and Graham, J., Nycodenz; a new nonionic iodinated density gradient medium. *Anal. Biochem.* 123, 23-31, (1982).
2. Ford, T.C. and Rickwood, D., Formation of isotonic Nycodenz gradients for cell separations. *Anal. Biochem.* 124, 293-298, (1982).
3. Bøyum, A., In Iodinated density gradient media. A practical approach. (ed. D. Rickwood), 1983, 147-171, IRL Press.