DATA SHEET

1-Step™1.077 A (Animal)

PRODUCT DESCRIPTION

1-Step™ 1.077 Animal 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-isophtalamide, for the isolation of mononuclear cells from most mammalian species.

Composition:

Nycodenz	14.1 % (w/v)
NaCl	0.30 % (w/v)
Tricine/NaOH pH 7.2	5 mM

Physical-chemical characteristics:

Density	1.077 ± 0.001 g/ml (20°C)
Osmolality	265 ± 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 onestep 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. In 1989 Bøyum modified this method to be able to isolate the mononuclear cells from other species than human blood.

Mononuclear cells from most mammalian species have a higher density than human mononuclear cells. If the osmolality of the solution is decreased, the cells will gain water and their density will decrease. Experiments have shown that a solution with density 1.077 g/ml and an osmolality of 265 mOsm gives the best results.

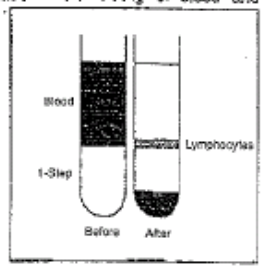
STABILITY AND STORAGE

1-Step™ 1.077 A is stable for 5 years provided the solution is kept sterile and protected from light.

The solution should be stored at or below 20°C.
1-Step™ 1.077 A is autoclavable.

SEPARATION PROCEDURE

1. Collect blood into a tube containing anticoagulant (EDTA, heparin, ACD).
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 1-Step™ A 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.
5. The mononuclear cells can be harvested from the interface between the plasma layer and the 1-Step™ A solution using a Pasteur pipette. See figure.



6. Transfer the cells to a smaller tube, and add some Tris-BSS (Tris-balanced salt solution) or a similar physiological solution, and centrifuge at 400 x g for 15 min.
7. Resuspend the pellet and spin down again.

PURITY AND VIABILITY

The described method has found to be rapid, simple and gives excellent results with blood samples from rabbits, rats and mice.

The following results are obtained with rabbit blood:

	Granulocytes	Mononuc. cells	Erythroc/ 100 cells
Upper fraction		93.7 ± 0.9	19 ± 5
Bottom fraction	94.2 ± 1.2	5.8 ± 1.2	

The lymphocytes responded by blastoid transformation in response to PHA. The response was weaker than in parallell cultures with human lymphocytes. Significant chemoluminiscence was observed when the granulocytes were stimulated with zymosan, but again the response was weaker than with human granulocytes.

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. Bøyum, A., Isolation of lymphocytes, granulocytes and macrophages. Scand. J. Immunol. 5, Suppl. 5, 9-15, (1976).
3. Bøyum, A., Personal communications.

ORDERING INFORMATION

1-Step™ 1.077 A prod. no. AN224510 1 x 100 ml.

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