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TECHNICAL INFORMATION

Catalog Number: 1692249, 1692254

Lympho Sep Lymphocyte Separation Medium

For in vitro isolation of lymphocytes from defibrinated , EDTA or heparinized treated human blood.

SUMMARY & EXPLANATION

The use of LymphoSep™ offers a quick, simple and reliable method for isolating lymphocytes from whole blood.

Boyum developed some of the early techniques of separation utilizing erythrocyte aggregating agents[1]. He subsequently noted that the low viscosity of Ficoll made it possible to isolate lymphocytes utilizing a procedure involving a short low speed centrifugation[2]. Other investigators developed modifications of the procedure by mixing Ficoll with sodium metrizoate[3,4] or sodium diatrizoate[5] for the preparation of viable lymphocytes in high yields.

LymphoSep™ is a mixture of Sodium Diatrizoate and Ficoll at a density of 1.077 ± 0.005 which aggregates the erythrocytes, thereby increasing their sedimentation rate allowing separation from lymphocytes .

PRINCIPLE OF THE PROCEDURE

Fresh blood (not more than 2 hours after collection) is defribinated or treated with anti-coagulant such as EDTA or Heparin. Defibrination has the advantage of removing most platelets from the blood sample.

The blood is then diluted 1:1 with a balanced salt solution. This controls the degree of aggregation of red blood cells so that they sediment easily with a minimum of lymphocyte entrapment . Various solutions can be used for this purpose, including RPMI 1640 or phosphate buffered saline. The diluted blood is layered on the LyphoSep™ in a siliconized glass centrifuge tube and the preparation centrifuged to achieve the separation of lymphocyte.

WARNINGS AND PRECAUTIONS

- For in Vitro Diagnostic Use.
- Dilution or adulteration of this reagent may result in inadequate lymphocyte separation .
- Do not use reagent beyond expiration date.
- Patient specimens and all materials coming into contact with them should be handled as if capable of

transmitting infection and disposed of with proper precautions.

- Never pipette by mouth and avoid contact with skin and mucus membranes.
- Do not expose reagent to strong light during storage.
- Avoid microbial contamination of reagents or incorrect results may occur.
- Centrifugation times or temperatures other than those specified may give erroneous results.
- If the fractions being isolated are to be used in cell culture, sterile equipment and aseptic techniques must be utilized throughout the procedure.

STABILITY AND STORAGE

LymphoSep™ is stable for 2 years provided the solution is kept sterile and protected from light. Direct sunlight exposure over long periods of time leads to a breakdown in Sodium Diatrizoate with release of iodine. Day to day working with this solution will not cause such a breakdown. LymphoSep™ should be stored at room temperature (15 to 30°C).

SPECIMEN COLLECTION AND HANDLING

Only fresh blood should be used to insure good separation and high viability of isolated cells. At least 3 ml of blood is collected fresh as a minimum working volume for this procedure. The blood should be kept at room temperature 15 - 30°C prior to and during centrifugation, and should be collected aseptically in the presence of EDTA or heparin. Blood should be processed within two hours of collection for maximum separation and functionability. However, acceptable separation can be obtained for up to six hours.

It has been shown that in the presence of ACD this product does not meet our specifications. The effect of other anticoagulants with the exception of EDTA and heparin has not been demonstrated.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Glass or plastic test tubes-siliconized

2. Balanced salts solution or RPMI 1640 tissue culture medium.

3. Tris Buffered saline :

   a.

Glucose	1.00 g/l
$CaCl_2, 2H_2O$	0.0074 g/l
$MgCl_2$	0.1992 g/l
KCl	0.4026 g/l
Tris	17.565 g/l

   Dissolve in 950 ml distilled, deionized H_2O and adjust pH 7.6 with HCl. Make up volume to 1 liter.

   b. NaCl, 8.19 g/l. Dissolve in 950 ml distilled, deionized H_2O. Make up volume to 1 liter. Mix 1 volume of (a) with 9 volumes (b) for final Tris buffered saline working solution.

4. Pasteur pipettes

5. Centrifuge tubes.

6. Centrifuge capable of producing at least 300 xG and maintaining 15 - 30°C temperature.

TEST PROTOCOL

The efficiency of separation, i.e. the yield of lymphocytes and the degree of contamination by erythrocytes, is chiefly dependent on the height of the blood layer in the centrifuge tube compared with the area of the interface between the blood and the separation medium. The following method will produce the most efficient separation.

1. Dispense 4 ml of LymphoSep™ into a 10 ml siliconized glass centrifuged tube.

2. Carefully layer 2 ml of blood sample diluted 1:1 with PBS or balanced salt solution.

3. Centrifuge for 30 minutes at 400xG. The temperature should be between 18 and 20°C.

Larger volumes of blood can be separated with the same efficiency, using identical centrifuge methods, by employing tubes of greater diameter, while maintaining the same depths of blood and separation medium layers. It is possible to obtain greater numbers of lymphocytes by layering up to double the amount of blood recommended above. This will result in some erythrocyte contamination which may not be prohibitive, depending on the use intended for the separated cells.

We recommend that the investigator determine experimentally the method most suited to the facilities available and the nature of the investigation.

WASHING OF LYMPHOCYTES

After centrifugation, the lymphocytes will have formed a gray colored layer at the interface of the blood plasma and the separation medium.

1. Using of Pasteur pipette, aspirate the supernatant blood plasma down to the upper surface of the lymphocyte layer, being careful not to disturb the lymphocytes. The plasma can be discarded.
2. Aspirate the layer of lymphocytes with a clean Pasteur pipette. Try to avoid aspirating any separation medium as this can interfere with subsequent washing and cause contamination with granulocytes.
3. Transfer the lymphocytes to a centrifuge tube containing at least three times the volume of balanced salt solution or PBS. Suspend evenly.
4. Centrifuge the lymphocyte suspension at 100xG for 10-15 minutes. Aspirate the supernatant and discard.
5. Resuspend the cell pellet in fresh balanced salt solution or PBS by gently drawing the cells in and out of a Pasteur pipette. Repeat step 3 and 4.

PERFORMANCE CHARACTERISTICS & EXPECTED RESULTS

MNC Fraction Composition : X%S.D.

Lymphocytes	55.0 ± 16.1%
Monocytes	43.0 ± 16.1%
Polycytes	1.6 ± 1.9%
Eosinophils	0.0 ± 0.0%
Basophils	0.2 ± 0.6%

MNC Viability :

98.8 ± 1.7%

A minimum of 30% ± 10% in recovered lymphocyte can be expected. The values were obtained using whole female blood with heparin from Interstate Blood Bank Inc. Blood from the 21 donors was washed at

least once with PBS to remove traces of the separation medium. Viability of each fraction, after separation, was tested using the Trypan blue dye exclusion method. Cell purity was assayed by differential cell counts on all fractions using rapid Wright 's stain.

LIMITATIONS OF PROCEDURE

Use of ACD anticoagulant is not recommended for this procedure.

The effects of other anticoagulants , except EDTA or heparin has not been demonstrated.

REFERENCES

1. Boyum, A., *Scan J Clin. Invest*, **v. 21** Suppl, 97, 1968.
2. Day, R.P., *Clinical Allergy*, **v. 2**, 205-212, 1972
3. Goldrosen, M.H., Gannon, P.J., Lutz, M., and Holyoke, E.D., *Immunol. Methods*, **v. 14**, 15-17, 1977
4. Loos, J.A. and Roos, D., *Exp. Cell Res.*, **v. 86**, 333-341, 1974
5. Mendelsohn, J., Skinner, A., and Kornfield, S., *J. Clin. Invest.*, **v. 50**, 818-826, 1971

TROUBLE SHOOTING

Trouble Shooting Guide		
Problem	**Possible Cause**	**Solution**
RBC Contamination	Saline diluted blood of low viscosity plasma.	Increase centrifuge speed
	Temperature not correct	Adjust to proper temperature
No defined or distinct mononuclear layer	Volume of blood to low.	Add more blood or dilute blood (1:2) with saline
	Centrifuge speed to low	Increase time or speed of centrifugation.