

FOR DENSITY GRADIENT SEPARATION OF LYMPHOCYTES

INTRODUCTION: Density gradient centrifugation of diluted whole blood on a polysucrose - sodium metrizoate medium is the method of choice for isolation of lymphocytes. The success of the procedure, i.e. the recovery of viable lymphocytes with the lowest proportion of contaminating granulocytes and erythrocytes, depends to a large extent on the careful layering of the blood sample onto the polysucrose - sodium metrizoate and the maintenance of a sharp interface between the two solutions prior to centrifugation. To this end UNI-SEP was developed. The UNI-SEP system allows the blood sample to be poured directly into the centrifuge tube with no special precautions required to prevent disruption of the polysucrose - sodium metrizoate layer. Thus, a large number of samples may be handled at the same time. The mechanism also reduces the length of centrifugation time required for separation of the lymphocytes.

PRINCIPLE: UNI-SEP products are sterile plastic centrifuge tubes containing an aseptically filled solution of 5.7% polysucrose and 9.1% sodium metrizoate (density 1.077 g/ml, osmolality 290 mOsm), which is sequestered in the bottoms of the tubes by one-way plastic inserts. Blood poured onto the insert does not disrupt the surface of the polysucrose - sodium metrizoate layer. During centrifugation, the insert opens to permit mixing of the blood and polysucrose - sodium metrizoate. Aggregated red blood cells and granulocytes are sedimented to the bottom of the tube while the white blood cells migrate to the interface between the plasma and polysucrose - sodium metrizoate layers. They are readily identified as a discrete white band located above the plastic insert.

INSTRUCTIONS:

1. UNI-SEP products are ready for use. Open under aseptic conditions.
2. Best results are obtained when all steps are performed at 18-20°C.
3. Use anticoagulant treated or defibrinated blood. Blood may be diluted with an equal volume of sterile saline or another sterile isotonic buffer or may be used undiluted. Add diluted or undiluted blood, according to Table I directly to the tube, cap, and centrifuge (18-20°C) 1000xg for 20 min. Procedures carried out at lower temperature may require longer centrifugation.

Table I.

Type of Tube	Cat. No.	Diluted Blood (1:1)	Undiluted Blood
Uni-Sep	U-02	4 - 8 ml	2 - 4 ml
Uni-Sep ₊	U-04	4-11 ml	2 - 5.5 ml
Uni-Sep _{MAXI}	U-10	20 -35 ml	10 -17.5 ml
Uni-Sep _{MAXI+}	U-16	not applicable	18.5-20 ml

Erythrocytes, dead cells and PMNs (polymorphonuclear leukocytes or granulocytes) are found at the bottom of the tube. The UNI-SEP insert separates the lymphocyte interface from the pellet of packed erythrocytes.

4. Remove the platelet-rich plasma and discard it.
5. Remove the mononuclear layer with the aid of a pipette. Alternatively, the entire contents of the tube above the plastic insert may be removed by decanting the solution.

STORAGE: Store at 15-25°C out of direct light. Deterioration of the polysucrose - sodium metrizoate is indicated by the appearance of a distinct yellow color or particulate material in the clear solution.

NOTE: *under normal shipping conditions the polysucrose - sodium metrizoate remains below the insert. Improper handling may cause some leakage to the top of the insert. In this case, centrifuge the tube for 1 minute at 400 x g to displace the liquid to the bottom of the tube before use. Plastic centrifuge tubes are somewhat brittle. Care should be taken to avoid dropping the tubes or striking them against hard surfaces.*

FOR RESEARCH USE ONLY!

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