

Uni-Sep and Uni-Sep Maxi : Catalog no. U-02 and U-10 : ready-to-use sterile tubes for density gradient separation of lymphocytes / WAK-Chemie Medical GmbH.

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WAK-Chemie Medical (Firm)

Publication/Creation

Bad Homburg : WAK-Chemie Medical, [1993]

Persistent URL

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Uni-Sep and Uni-Sep Maxi

Catalog No. U-02 and U-10

Ready-to-Use Sterile Tubes For Density Gradient Separation of Lymphocytes

Introduction

Density gradient centrifugation of diluted whole blood on a polysucrose - sodium metrizoate medium (e.g. Ficoll-Paque, Lymphoprep) 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 and UNI-SEP MAXI

are sterile plastic centrifuge tubes containing a solution of 5.6% polysucrose and 9.6% sodium metrizoate (density 1.077 g/ml, osmolality 280 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.

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Procedure

1. UNI-SEP and UNI-SEP MAXI are sterile and ready for use. Open only under aseptic conditions.
2. Best results are obtained when all steps are performed at 18-20 °C. Use anticoagulant treated or defibrinated blood which has been diluted with an equal volume of sterile saline or other sterile isotonic buffer.
3. Add diluted blood, either 4-8 ml for UNI-SEP or 25-35 ml for UNI-SEP MAXI, directly to the tube, cap and centrifuge (18-20 °C) 1000 x g for 20 min. Procedures carried out at lower temperatures may require longer centrifugation times. 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 polysucrose - sodium metrizoate.
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 4-25 °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.