

Buffy Booster for *UNI-SEP*

Catalog no. U-15

DENSITY GRADIENT LYMPHOCYTE SEPARATION FROM BIOLOGICAL FLUIDS HAVING LOW RED CELL CONTENT

Critically important to the function of ready-to-use lymphocyte separation tubes (such as *UNI-SEP*) is the hematocrit, i.e. the volume of the blood fluid occupied by the red blood cells. It is this mass of cells that during the centrifugation procedure displaces the sodium metrizoate - polysucrose so that it rises above the centrifugation device to form a density layer at which the white cells collect. In cases where sufficient red cells are not present, the sodium metrizoate - polysucrose interface forms at or even below the level of the separation device. In this situation recovery of the white cell fraction may not be possible. Diluted whole blood, buffy coat (enriched white cell fraction), bone marrow, lymph and spinal fluid are all examples of this type of biological fluids.

When there is not sufficient red cell mass in the sample itself to displace the sodium metrizoate - polysucrose to the required level, extra mass must be added. **Buffy Booster**, a dense inert liquid, immiscible in water, is added prior to centrifugation. Centrifugal force causes **Buffy Booster** to sink to the bottom of the tube. Any red blood cells present form a sedimentation layer on top of the **Buffy Booster** (the red cell layer and **Buffy Booster** do not mix). The volume of sodium metrizoate - polysucrose displaced upwards is equal to the combined volumes of the **Buffy Booster** and the sedimented red cells. No contamination with red cells or **Buffy Booster** is possible even when the whole upper plasma layer is poured off. The red cells do not pass the separation device, while the **Buffy Booster** is blocked in by the red cell layer.

Instructions for Use

I. **Buffy Booster** is provided as a ready-to-use liquid in amber bottles. The liquid may be conveniently removed using a syringe with 21 g needle. Alternatively the cap may be removed and the liquid drawn off by pipette.

II. For use with the *UNI-SEP_{maxi}* centrifuge tube add 3 ml of **Buffy Booster** immediately before adding the sample. This amount is adequate for 15 ml of undiluted buffy coat. For use with *UNI-SEP* add 0.7 ml **Buffy Booster** immediately before adding the sample. This amount is sufficient for 3 ml of undiluted buffy coat. It may be necessary to establish the best amount for other fluids. Add sample and follow the instructions for *UNI-SEP*.

Technical Notes

Buffy Booster should be used whenever the volume of precipitated red cells is less than 30% of the volume of the sodium metrizoate - polysucrose supplied in the *UNI-SEP* tube.

If **Buffy Booster** was not used, it is still possible to improve lymphocyte yield by adding **Buffy Booster** after the initial centrifugation. Simply open the tube, add 3 or 0.7 ml **Buffy Booster**, close the tube, and centrifuge for 5 min at 1000 x g. The lymphocyte layer will now form several millimeters above the separation insert.

FOR RESEARCH USE ONLY!