

## INTENDED USE

ZeptoGel is a sterile, isotonic, pyrogen-free solution of salts plus gelatin. When added to whole blood, ZeptoGel separates the leukocytes by taking advantage of the differential sedimentation rates of blood components. Gentle mixing of ZeptoGel (1 part) and blood (4 parts), followed by settling of the blood components for 15 to 60 minutes at 20 to 37 °C, results in the formation of layers. The leukocyte fraction may be harvested by aspirating the uppermost layer.

**\*Research Purposes Only. Not For *in vitro* Diagnostic Use.**

## STORAGE

Store ZeptoGel at 15-30 °C.

**Note:** If ZeptoGel is gelled upon receipt, heat at 37 °C for 15 minutes and then store at 15-30 °C.

## CAUTION

This procedure may require the use of human blood or blood products. All human blood and blood products must be handled as if capable of transmitting infectious agents. All potentially biohazardous wastes generated in this procedure must be handled in a manner that will assure inactivation of infectious agents. Solid waste can be inactivated by autoclaving for 60 minutes at 121 °C. Liquid wastes can be inactivated by adding sodium hypochlorite (household bleach) to achieve a final concentration of 1.0% for a period of at least 30 minutes.

## PROCEDURE

### Small Scale Isolation:

1. Add 200 units of heparin and 2.5 mL of ZeptoGel to test tube containing 10 mL of freshly drawn, heparinized blood. The blood may also be collected in a syringe containing acid citrate dextrose (ACD) solution (1 part ACD to 7 parts blood), in which case heparin and ZeptoGel should still be added as above.
2. Mix the combination of blood and ZeptoGel gently but thoroughly, then let the blood settle for 30-60 minutes at 37 °C or until optimum settling is achieved.
3. Remove the uppermost layer containing leukocyte-rich plasma from the test tube and transfer to a conical centrifuge tube.
4. At room temperature, centrifuge the leukocyte-rich plasma for 10 minutes at 40 g, followed by 20 minutes at 200 g. The centrifuge should not be stopped after the initial 10 minute period, but turned up to run at 200 g for the remaining 20 minutes.
5. Remove and discard the plasma supernatant.
6. Resuspend the leukocyte pellet in 5 mL of RPMI 1640 medium containing 20 % fetal bovine serum (FBS), heparin (2 units/mL), penicillin (100 units/mL), and streptomycin (50 µg/mL) or other suitable medium.

### Large Scale Isolation:

1. Empty the blood from its container. When working with a unit of blood, swab the outlet of the container with alcohol. Pour the blood into graduated cylinders (not more than 200 mL of blood per 250 mL cylinder).
2. To each 200 mL of blood, add 4000 units of heparin and 50 mL ZeptoGel. Mix well. Allow the mixture to settle for 30-60 minutes at 37 °C or until optimum settling is achieved.
3. Optimum settling is achieved when the top, leukocyte-rich plasma layer constitutes about 60% of the total volume of the contents of the graduated cylinder. Remove this layer by aspiration into 1 liter aspirator bottle.
4. Determine the total volume of leukocyte-rich plasma collected into the aspirator bottle. Add an equal volume of serum-free RPMI 1640 medium containing streptomycin and penicillin. Mix well.

**This product was manufactured in a facility which has a Quality Management System that is ISO 13485 certified.**

**ZeptoMetrix Corporation**  
878 Main Street  
Buffalo, NY 14202  
800-274-5487  
[www.zeptometrix.com](http://www.zeptometrix.com)