

Dissection of Mouse Spleen

Materials:

D's PBS or IsoMEM
disposable culture tubes
graduated centrifuge tube 12 cc.
curved end forceps 2 ea.
iris scissors
70% alcohol
100 mm disposable petri dish
wire mesh screen

Procedure:

1. Place mouse killed by neck dislocation on its right side and make incision in skin of left side overlying liver and spleen. The fur should be matted down with 70% alcohol to keep procedure clean. Separate skin to reveal muscle layer.
2. With forceps, lift muscle layer away from body cavity, and make a large incision across body wall.
3. Clean instruments of adherent fur or tissue, and draw spleen out of the body cavity, being careful not to damage the organ. Insertion of the scissors under the spleen (where it is attached to mesentery) and opening the blades will usually separate the organ from attached tissue.
4. Place the cleaned spleen on a rinsed (MEM) wire screen in a petri dish with 2-3 ml of medium. The petri dish may be placed on ice tray to keep cells iced.
5. Using clean curved forceps, force the contents out of the spleen by pressing down with the forceps in the center of the organ and drawing the forceps across the organ to its end. The cells should be extruded from the end of the organ wall by this method. When all the cells have been extruded, the spleen will be like an empty sack and can be discarded.
6. Pipet (Pasteur) the extruded cells through the wire screen to prepare a single cell suspension. Transfer cells to iced culture tubes and wash petri dish with 2 cc medium to obtain all cells available.
7. Disperse cells in iced culture tubes by pipeting and let stand 2 minutes. The larger chunks or material will sediment.
8. Draw off medium from the test tube (leaving behind any large material sedimented from the single cell suspension) into a graduated centrifuge tube and cap with parafilm.
9. Centrifuge at 1200 rpm for 6 minutes.
10. Resuspend spleen cells to 1 spleen per 1 ml and count a 1:100 dilution of this suspension.

Note: Wire screens and instruments must be washed immediately after use to keep debris from drying on. Wash wire mesh with toothbrush in tap water until no adherent tissue can be seen when looking through it.

1 spleen yields $\sim 1-2 \times 10^8$ cells before glass wool.