Preparation of Murine Splenocytes

Reagents and Materials

Full Name	Short Name	Catalog Number
Mouse or rat of indicated strain		
Dissection board		
70% reagent alcohol	70% alcohol	
Dissection forceps		
Dissection scissors		
BD Falcon™ 50-mL conical centrifuge tube	conical tube	352098
PBS buffer	PBS	
Nylon cell strainer	strainer	352350
Aspirator		
BD Pharm Lyse™ lysing solution	lysing solution	555899
Water bath at 37°C		

Procedural Notes

- To ensure the humane handling and care of laboratory animals, all procedures involving animals must comply with current standards as outlined in the US Department of Health and Human Services publication *Guide for the Care and Use of Laboratory Animals*.
- This procedure is for the non-sterile acquisition of spleen cells. You can perform this procedure aseptically by using sterile media and sterile lysing solution under a culture hood. Sterilize dissecting instruments by washing them in 70% alcohol.

Procedure, tissue collection

1. Sacrifice the animal.
2. Place the animal on a clean dissection board and rinse the animal with 70% reagent alcohol.
3. Incise the abdominal cavity and remove the spleen, which is located to the left side of the abdomen, inferior to the stomach.
4. Place the tissue into a labeled conical tube containing PBS.
5. Dispose of the animal carcass in an appropriate freezer and store it at -20°C for disposal service pickup.

Procedure, preparation of cell suspension

1. Slice the excised spleen into small pieces.
2. Place the fragments onto a strainer attached to a 50-mL conical tube.
3. Press the excised spleen through the strainer using the plunger end of a syringe.
4. Wash the cells through the strainer with the excess PBS.
5. Centrifuge the cell suspension at 1,600 rpm for 5 minutes.
6. Aspirate the supernatant.
7. Resuspend the cell pellet in 2 mL of pre-warmed (at 37ºC) lysing solution.
8. Incubate the cells in a 37°C water bath for 2 minutes.
9. Add at least 30 mL of PBS and centrifuge the cells at 1,600 rpm for 5 minutes.
10. Discard the supernatant and resuspend the cells in PBS at 2 x 10^6 cells per mL.

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Procedure, performing a cell count and viability check

1. Perform a cell count and viability check using trypan blue and a hemacytometer.
2. If the viability is not acceptable, repeat the cell count or harvest another animal.
3. Continue with indirect or direct staining of murine leucocytes.