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Protocol for the isolation of splenocytes from murine spleens

Materials and Equipment

- Murine spleen
- Disposable Pasteur pipettes (sterile)
- Polystyrene Petri dish
- Clean scalpel (optional)
- Cell strainer (70 µm Nylon, e.g. Falcon® #2350)
- 2 ml syringe
- Sterile tubes (30 ml and 50 ml).
- Serological pipettes of appropriate volumes (sterile)
- Ammonium chloride lysing reagent (0.15 M NH4Cl, 1 mM KHCO3, 0.1 mM EDTA, or a commercial preparation e.g. PharM Lyse™, BD Biosciences #555899)
- Phosphate buffered saline (PBS)
- Wash buffer (0.1% sodium azide, 0.1% BSA in PBS)
- Benchtop centrifuge (NOT refrigerated) with swing-out rotor and appropriate carriers
- Hemacytometer and microscope for cell counting

Procedure

1. **Prepare ammonium chloride lysing reagent.** Leave at room temperature.
2. **Place a cell strainer in the Petri dish. Transfer the spleen and 1 ml PBS directly into the cell strainer.** If desired, score the outer membrane of the spleen with a clean scalpel before mashing it, but take care to avoid cutting through the strainer mesh.
3. **Remove the plunger from a 2 ml syringe and use the black rubber end to mash the spleen and release the splenocytes into the Petri dish.** Use grinding circular movements to homogenize the tissue. Periodically, draw up liquid from outside the strainer with a disposable pipette, and wash out the cells from within the strainer. Continue to mash the spleen until all that remains is the white connective tissue of the outer membrane.
4. **Transfer the homogenized cell suspension into a universal tube. Wash out the Petri dish a few times to maximize recovery of splenocytes. Make up to the full volume of the tube with PBS.**
5. **Centrifuge at 400 × *g* for 10 minutes at room temperature and aspirate supernatant.** The resulting cell pellet should be red in color. The supernatant may appear cloudy, due to smaller particles that cannot be centrifuged at this setting.
6. **Resuspend the cell pellet in 2 ml PBS per spleen. Add the appropriate amount of ammonium chloride lysing reagent (1 ml per 100 ml blood, or as defined by the manufacturer) and leave for 15 minutes in the dark at room temperature.** Ensure that the cells are fully resuspended before adding the lysis buffer in order to avoid excessive clumping.
7. **Centrifuge at 400 × *g* for 5 minutes and aspirate the supernatant.** Take care not to lose cells as the pellet will be loose. The cell pellet should be buff colored with minimal rbc contamination.
8. **Resuspend the cells completely and wash again with a full volume of PBS. Centrifuge at 400 × *g* for 5 minutes, aspirate supernatant, then resuspend cells in desired medium (e.g. wash buffer or RPMI-1640) to a final known volume.**
9. **Count live cells using a hemacytometer and light microscope.** If proceeding straight to staining for flow cytometry, distribute the cells equally between sample tubes. Otherwise, resuspend the cells in a few mls of wash buffer and use the same day.

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