MABTECH

Splenocytes

Isolation, freezing, and thawing

Splenocytes are, at least for mouse and rat samples, the most used cells in ELISpot and FluoroSpot assays. Splenocytes can easily be isolated from a spleen with no need for Ficoll separation. The cells can be used directly after isolation, but it is often more convenient to cryopreserve the cells and use them at a later timepoint.

This is a detailed guide on how to best isolate, freeze, and thaw splenocytes.

Isolating splenocytes

Materials

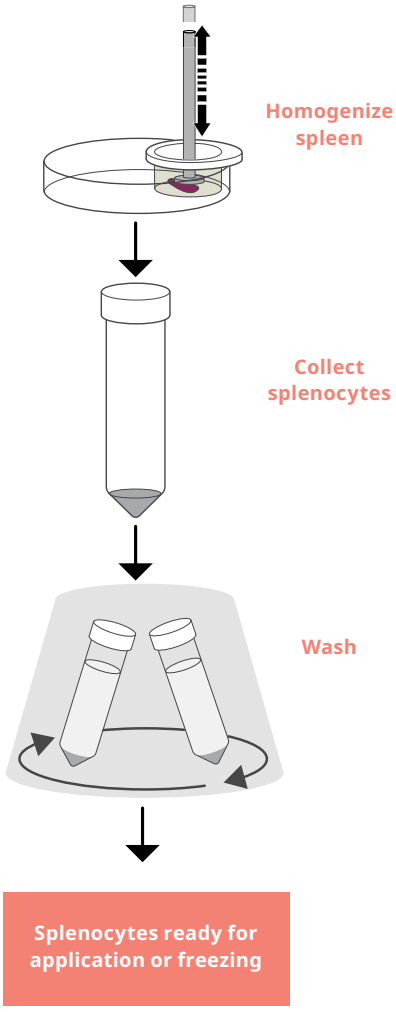
- Cell strainer, 70 µm nylon, sterile
- Scissors and tweezers or forceps, sterile
- Petri dish, sterile
- Pasteur pipette, sterile
- Sterile syringe, 3 ml or 5 ml (no needle)
- Polypropylene centrifuge tubes: 15 ml, sterile
- Medium: RPMI 1640 with 100 µg/mL penicillin + 100 µg/mL streptomycin

Before you begin

Spleen from sacrificed mouse or rat should be placed into a sterile centrifuge vial with 5 ml medium immediately after being harvested.

Steps

1. Pour out the spleen into a petri dish. If required, remove access fat and tissue using scissors and tweezers or forceps.
2. Place a cell strainer in a new petri dish and transfer the spleen to the strainer. Add 5 ml medium.
3. Use the flat plunger end of a syringe to homogenize the spleen through the cell strainer into the petri dish.
4. Rinse the cell strainer with 5 ml medium into the petri dish.
5. Transfer the splenocytes from the petri dish to a 15 ml centrifuge tube.
6. Leave the tube for one minute to let big clumps sediment or remove clumps and aggregates using a sterile Pasteur pipette. Transfer the splenocyte suspension to a new 15 ml centrifuge tube. Fill the tube with medium.
7. Centrifuge for 10 minutes at 300 x g.
8. Discard the supernatant and resuspend the cell pellet in 15 ml medium.
9. Note the volume and take a small aliquot to count the cells.
10. Centrifuge for 10 minutes at 300 x g.
    Tip! *Count the cells during this step.*
11. **If you want to use the cells right away** Discard the supernatant and resuspend the cell pellet in appropriate cell culture medium by gently pipetting up and down. The splenocytes are now ready to be used for cell-based immunoassays such as ELISpot, FluoroSpot and flow cytometry.



If you want to cryopreserve the cells
Continue to “Freezing of splenocytes”.

Freezing splenocytes

Materials

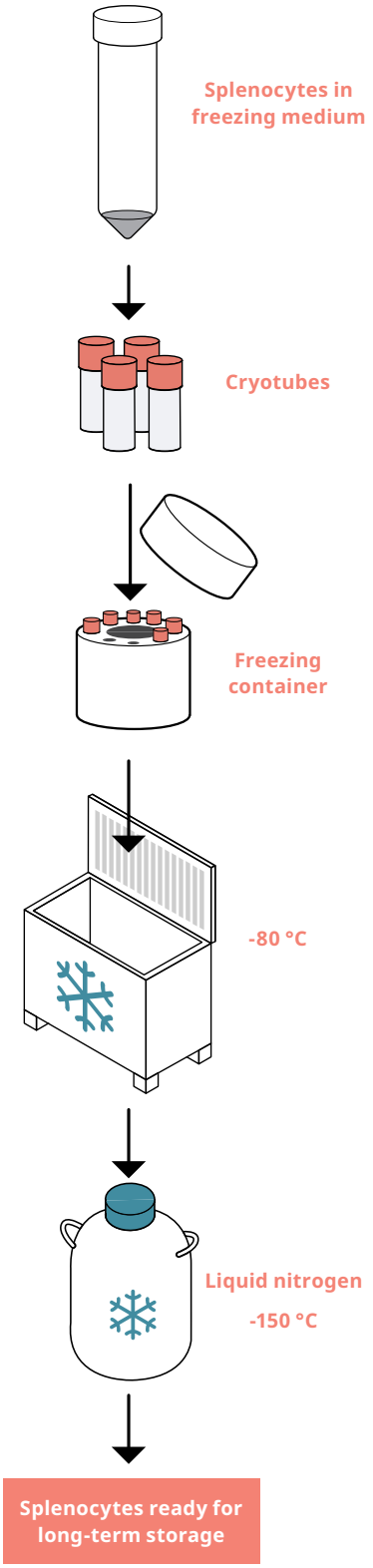
- Freezing medium: RPMI 1640 (with 2mM L-glutamine), + 20% FCS and 10% DMSO. If your cells are adapted to serum-free medium, use 7% DMSO
- Freezing container e.g., “CoolCell” or “Mr. Frosty”
- Polypropylene centrifuge tubes: 50 ml and/or 15 ml, sterile
- Automatic pipettes and sterile tips
- Cryotubes, e.g., 1.8 ml
- -80 °C freezer
- -150 °C freezer or liquid nitrogen tank

Before you begin
Label cryotubes and make sure to have the freezing container(s) ready. Leaving cells for too long in the freezing medium at room temperature will affect cell viability.

Steps

1. Discard the supernatant (from step 11 section 1) and dilute the cells in freezing medium to a concentration of 5 to 25 million cells/ml. If you are using serum-free freezing medium, aim instead for a cell concentration of 1 million cells/ml.
2. Aliquot e.g., 1 ml in each cryotube and transfer the cryotubes to the freezing container. Make sure to keep your cells in suspension by continuous resuspension.
3. Quickly place the freezing container in a -80 °C freezer, and store for a minimum of 4 hours, and a maximum of 1 week.
4. Transfer the cryotubes from the freezing container to a -150 °C freezer or liquid nitrogen tank for long-term storage.

! For 1.8 ml cryotubes, aliquot 1 ml cell suspension per tube. For other sizes of cryotubes, make sure to aliquot less than the maximum volume, as the liquid expands during freezing.



Thawing splenocytes

Materials

- Cell culture medium: RPMI 1640 (with 2mM L-glutamine) + 10% FCS, 10 mM HEPES and 100 µg/mL penicillin + 100 µg/mL streptomycin
- Water bath at 37 °C
- Polypropylene centrifuge tubes: 50 ml and/or 15 ml, sterile
- Pipettes and pipette boy
- Automatic pipettes and sterile tips
- Centrifuge at room temperature
- CO_2 incubator at 37 °C

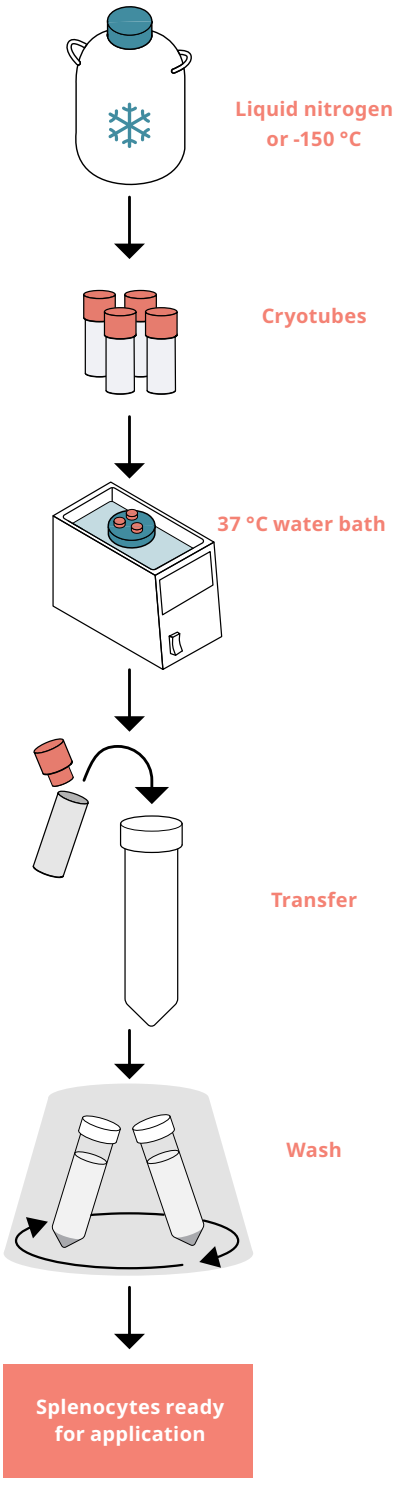
Before you begin
Heat a water bath to 37 °C. Pre-heat the cell culture medium to be used for step 2. It is fine if the medium used in later washing steps is of room temperature.

Steps

1. Transfer the cell ampoule rapidly from the freeze storage to a 37 °C water bath and thaw the cells until only a small ice crystal remains.

   If you intend to thaw multiple cryotubes, only take a few at a time. The freezing medium contains DMSO which is toxic to cells so proceed to step 2 as soon as possible

2. Slowly add 0.5-1 ml cell culture medium to the cryotube, resuspend and transfer the cells from the cryotube to a 15 ml tube. Rinse the cryotube with 1 ml of cell culture medium and transfer it to the 15 ml tube. Fill the tube with cell culture medium.
3. Centrifuge for 10 minutes at 300 x g.
4. Discard the supernatant and tap the tube to make the pellet less compact. Resuspend the cells in 1 ml of cell culture medium by slowly pipetting up and down. Add cell culture medium to a total volume of 15 ml.
5. Centrifuge for 10 minutes at 300 x g.
6. Discard the supernatant and resuspend the pellet in 1 ml of cell culture medium.
7. Place the cells in a CO2 incubator for one hour. Leave the cap slightly open.



Tip: *Utilize this one hour of downtime to wash and block your ELISpot/FluoroSpot plate.*

8. After incubation (cell resting), resuspend the cells and let any aggregated cell debris sediment (takes around 1 minute). Then carefully transfer the cell suspension, without the debris, to a fresh 15 ml tube.

Add a maximum of two cryotubes to each 15 ml tube (or around 30-40 million cells).

9. Count the cells and determine cell viability. Counting can be done with an automated cell counter or trypan blue staining using a microscope. Because only living cells will be able to secrete the analyte, make sure to exclude dead and, if possible, apoptotic cells from the cell count. If the cell concentration turns out to be lower than required, centrifuge the cells again, resuspend and dilute to correct volume.