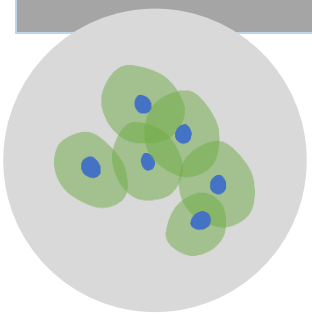

Spleen Cell Isolation

www.davids-bio.com (Custom Antibodies)
www.davids-science.de (Lab Material)

- 1 - Introduction

Splenocyte isolation from the intact spleen is a common preparative step in immunology and cell biology. The spleen contains a heterogeneous mix of immune cells — including T and B lymphocytes, macrophages, dendritic cells, and innate immune cells — organized within red and white pulp regions. Preparing a single-cell suspension from spleen tissue enables downstream applications such as flow cytometry, cell culture assays, functional immune assays, and cell sorting.

Material

Cell Strainer (70 µm)	Surgical Blade
1 x PBS (Sterile and cold (4°C))	50-ml tube
Storage buffer	Microscope for cell counting
Red Cell Lysis Buffer (Optional)	Sterile bench
Plunger from a 2-ml Syringe	

- 2 - Method

Spleen Collection

- Obtain spleen tissue in accordance with ethical approvals
- Transfer the spleen under sterile conditions in a petri dish containing 1 x PBS

Tissue Handling

- Cut the spleen into 5 – 10 small pieces with a surgical blade
- Place a cell strainer on top of a 50-ml tube
- Insert the small spleen pieces in the cell strainer (70 – 100 µm) and add 5 ml cold 1 x PBS
- Use a plunger of a 2-ml syringe to carefully grind the cells through the cell strainer
- Repeat the addition of 1 x PBS and the grinding with the cell strainer 2 – 4 times until all cells are transferred from the spleen to the 50-ml tube (the spleen turns pale)

Centrifugation

- Centrifuge the 50-ml tube containing the spleen cells at 400 x g for 10 minutes and 4°C
- Discard the supernatant

Red Lysis Buffer (OPTIONAL)

- With this step the red blood cells are removed. This step is only recommended, when the red blood cells disturb in your applications.
- Add RBC lysis buffer according to the manufacturers manual (usually 1 – 10 ml depending on the size of the spleen)
- Incubate the cells with the lysis buffer for 5 minutes at room temperature
- Pass the incubated cells through a new cell strainer (70 µm), collecting them in a new 50-ml tube
- Centrifuge at 400 x g for 10 minutes and 4°C
- Discard the supernatant

Storage

- Resuspend the cell pellet in 25 ml cold 1 x PBS and dilute them to your required concentration
- Centrifuge at 400 x g for 10 minutes and 4°C
- Discard supernatant
- Resuspend the pellet in 10 ml 1 x PBS
- Count the cells (for example using a Neubauer Counting Chamber). You may need to dilute the cell suspension for counting
- Centrifuge at 400 x g for 10 minutes and 4°C
- Discard supernatant
- Add storage buffer to the cells to acquire the required cell density

- 3 - Trouble Shooting

Reason	Solution
Low cell viability or yield	Minimize delays, choose gentler dissociation strategies when preserving fragile populations is critical, and ensure that handling throughout preserves viability.
High debris or particulate contamination in suspension	Include appropriate clarification/filtration steps conceptually and avoid excessive force that fragments tissue into fine debris.
Altered cell phenotype or activation during processing	Adopt handling strategies that minimize activation (e.g., reduce processing duration and harsh manipulations).
Inconsistent results between samples	Standardize collection and processing workflows conceptually and include experimental controls; batch samples when feasible.