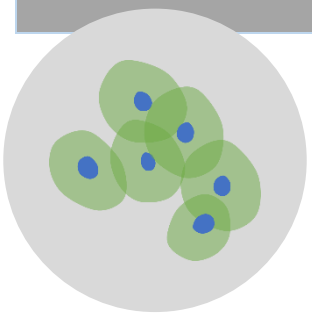

Preparation of Cell Suspensions

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

- 1 - Introduction

This protocol provides an established and efficient method for isolating cells from freshly prepared spleen tissue. The spleen plays a vital role in immune responses and contains diverse cell populations, including lymphocytes, macrophages, and dendritic cells. By isolating these cells, researchers can further investigate their functions, phenotypes, and molecular characteristics. The protocol may be adapted to lymph nodes, bone marrow and other organs.

The isolation procedure involves mechanical dissociation and filtration through a cell strainer, followed by appropriate washing and resuspension steps to obtain a purified cell suspension. The isolated cells can then be utilized for downstream applications such as flow cytometry, cell culture or isolation of RNA.

- 2 - Material

Material	
Wash Buffer	Sterile 1 x PBS
Storage Buffer	Cell culture media, like RPMI 1640 or DMEM
Freezing Buffer	10% DMSO Cell culture media
Cell Strainer	70 µm or 100 µm
Sterile Tools	Scissors and Forceps

- 3 - Method

Sample Preparation

- Use freshly isolated spleen for the isolation procedure to achieve the best results
- Place a cell strainer either in a 50-ml conical centrifuge tube or in a petri dish
- Wet the cell strainer with cold Wash Buffer

Isolation

- Use sterile scissors and forceps to mince the spleen into small pieces

- Pour the minced tissue through the cell strainer
- Rinse the cell strainer with 0.5 - 1 ml Storage Buffer to recover all cells
- If you used a petri dish, transfer the cells to a 50 ml tube
- Determine the cell concentration with a counting chamber

Storage

- Centrifuge the cell suspension at 400 x g for 5 minutes at 4°C
- Carefully remove the supernatant and resuspend the pellet in 5 ml Storage Buffer
- Adjust the concentration of your cells to fit your downstream applications
- If you wish to freeze the cells, you may use Freezing Buffer with your desired cryoprotective agent

- 4 - Trouble Shooting

Reason	Solution
Low Cell Yield	Ensure that the spleen tissue is fresh and has not been subjected to prolonged storage Verify that the cell strainer is properly positioned and securely attached to the collection tube Adjust the mincing technique to obtain smaller tissue fragments Optimize the washing steps to recover all cells, ensuring that the cell strainer is thoroughly rinsed with Washing Buffer
Contamination	Maintain strict aseptic conditions throughout the isolation process, including working in a laminar flow hood, using sterile instruments, solutions and containers Clean the work surface and instruments with appropriate disinfectants before starting the isolation procedure
Cell Aggregation	Ensure that the Wash Buffer are properly prepared Gently pipetting or swirling of the cell suspension during washing and resuspension steps disperse clumped cells Consider enzymatic digestion methods to achieve better cell dispersion
Cell Viability	Maintain the tissue and all solutions at appropriate temperatures Avoid extended exposure to excessive heat or cold Consider an alternative cryoprotective agent like Glycerol or Ethylene glycol if DMSO disturbs in your applications
Cell Purity	Utilize an appropriate cell strainer size that allows efficient capture of the desired cell population Consider additional purification steps such as density gradient centrifugation