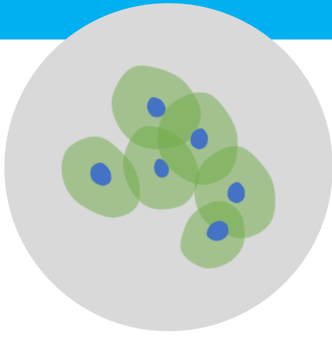
Davids Biotechnologie



Preparation of Cell Suspensions

From Whole Spleen and Lymph Nodes

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General Information

This protocol shows how to prepare a single cell suspension from whole spleens or lymph nodes that can be used in various experiments like flow cytometry, cell culture or functional assays.

Materials

Wash Buffer	Storage Buffer	Freezing Buffer	Cell Strainer
Sterile 1 x PBS	RPMI 1640	RPMI 1640 10% DMSO	70 µm or 100 µm

Preparation

- Place a cell strainer in a 50-ml conical centrifuge tube or place it in a petri dish
- Wet the cell strainer with cold Wash Buffer
- Use a sterile scissor 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
- When you used a petri dish, transfer the cells to a 50 ml tube
- Determine the cell concentration
- Centrifuge the cell suspension at 400 x g for 5 min 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
- When you want to freeze the cells, you may use Freezing Buffer with your desired cryoprotective agent

Not for diagnostic or therapeutic use. Not for human use. For research purposes only. This product is not a medical device. Not for use in in vitro diagnostic procedure for clinical diagnosis. Custom tests must be done to determine the ability for a specific use.

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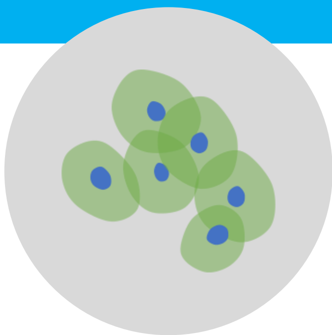

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Advices and Troubleshooting

Reason	Solution
RNA Isolation does not work	Use sterile DNAse and RNAse free material and solutions
Insufficient yield	When you rinse the cells in the cell strainer, you may use a plunger from a 5-ml or 10-ml syringe to carefully crunch the tissue. Be careful not to flush any debris from fat or connective tissue through the cell strainer.
Low viability of cells after thawing	You may use an alternative cryoprotective agent like 10% Glycerol or 40% Ethylene glycol. When RPMI 1640 disturbs in your downstream applications, you may want to use another buffer like DMEM, PBS or a special cryopreservation medium.
Cells do not resuspend after centrifugation	Loosen the pellet by tapping the 50 ml tube carefully before resuspending in a new buffer after centrifugation. You may decrease the speed of the centrifuge to 300 x g