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How to Prepare a Single Cell Suspension from Mouse Spleen

STEMCELL Technologies[1]

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STEMCELL Technologies
Tech. support email: **techsupport@stemcell.com**

ABSTRACT

The spleen is an important organ of the immune system responsible for filtering blood and initiating immune responses to blood-borne antigens. Various blood, lymphoid, and hematopoietic cell types may be isolated from spleen samples for further study of the immune system. Preparing a true single cell suspension of the primary tissue sample will optimize cell separation by avoiding additional cell loss and enabling maximum labeling of the target cells. This protocol describes how to harvest cells from a spleen sample, and prepare a single cell suspension prior to performing cell isolation.

STEPS MATERIALS

NAME	CATALOG #	VENDOR
35 mm Culture Dishes	27100	Stemcell Technologies
EasySep™ Buffer	20144	Stemcell Technologies
RoboSep™ Buffer	20104	Stemcell Technologies
3 cc Syringes	28230	Stemcell Technologies
Reversible Strainers	27260	Stemcell Technologies

BEFORE STARTING

This video demonstrates how to harvest cells from a spleen sample, and prepare a single cell suspension prior to performing cell isolation. Preparing a true single cell suspension of the primary tissue sample will optimize cell separation by avoiding additional cell loss and enabling maximum labeling of the target cells.

Mechanical Digestion of a Spleen Sample

1 Transfer the spleen to be processed into a sterile 35 mm culture dish containing 5 mL of recommended dissociation medium for downstream isolation. If you are not using an isolation kit following dissociation please use phosphate buffered saline (PBS) + 1 mM EDTA.

35 mm Culture Dishes
by Stemcell Technologies
Catalog #: 27100

Recommended Medium:

EasySep™ Buffer
by Stemcell Technologies
Catalog #: 20144

RoboSep™ Buffer
by Stemcell Technologies
Catalog #: 20104

Note: If processing multiple spleens please use a 100 mm culture dish containing 10 mL of recommended medium.

100 mm Culture Dish, Non-Treated
by Stemcell Technologies
Catalog #: 38045

2 Trim any extra connective tissues or fat from the spleen, take note of any necrotic regions, and inspect the spleen for any other phenomena such as enlargement, discolouration, or lesions. It is important to note the condition of your starting sample as this may be taken into consideration when evaluating your cellular fraction.

3 Take the piston/plunger out of a sterile 3 cc syringe. Use the flat back end of this device to mince the spleen by crushing the spleen 5 times in gentle circular motions. This action will burst the spleen, disrupt the pulp, and release the splenocytes.

3 cc Syringes
by Stemcell Technologies
Catalog #: 28230

Prepare a Single Cell Suspension

4 Over a sterile 50 mL conical tube, place a 70 µm cell strainer and pass 2 mL of recommended medium through to prime the filter.

Reversible Strainers
by Stemcell Technologies
Catalog #: 27260

Note: Priming the filter reduces cell adhesion that may occur if the filter is dry when cells are passed through.

5 Triturate the released splenocytes to a homogeneous mixture with a primed 5 or 10 mL serological pipette.

6 Transfer the supernatant and tissue from the culture dish to the primed 70 µm cell strainer. Using a fresh piston/plunger out of a sterile 3 cc syringe gently pass the cells and the dissociated tissue through the cell strainer membrane by pressing in a circular motion with the plunger/piston end of the syringe. Wash the strainer with 3 mL of recommended medium. Repeat this step and then discard the leftover tissue and strainer.

7 Top up the tube containing the cell suspension with PBS, and collect the cells by centrifugation of the tube at **300 x g for 10 minutes.**

8 Carefully discard the supernatant without disturbing the pellet.

9 Gently tap the tube to resuspend cells in the residual volume.

Optional: A second wash step can be performed at this stage.

Splenocytes are now ready for downstream use.