How to Prepare a Single Cell Suspension from a Frozen Cell Sample

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ABSTRACT

Preparing a single cell suspension from a frozen starting sample is critical for optimizing cell isolations by avoiding additional cell loss and enabling the maximum labeling of target cells. Performing cell separation on clumpy cell samples can result in lower recovery and may interfere with proper labeling of the target cells. Samples may sometimes appear "clumpy" when they have been exposed to repeated freeze/thaw cycles or enzymatic tissue dissociation. These cell clumps occur because environmental stresses can accelerate the rate of cell death within the sample, resulting in the release of "sticky" DNA molecules from the dying cells that can clump neighboring cells together. Adding the endonuclease deoxyribonuclease I (DNase I) into your sample can minimize the presence of free-floating DNA fragments and cell clumps. This protocol describes a method to harvest cells and prepare a clump-free single cell suspension from a frozen cell sample prior to performing cell separation.

MATERIALS

NAME	CATALOG #	VENDOR
Falcon® Conical Tubes 50 mL	38010	Stemcell Technologies

STEPS MATERIALS

NAME	CATALOG #	VENDOR
DNase I Solution (1 mg/mL)	07900	Stemcell Technologies

BEFORE STARTING

This video outlines how to harvest cells from a frozen starting sample, and prepare a single cell suspension prior to performing cell isolation.

Preparation of a Single Cell Suspension

1 Thaw the vial of cells quickly by swirling it in a **37 °C** water bath.

2 Transfer thawed cells into a sterile 50 mL conical tube.

Optional: Add 0.25 to 0.5 mL of a 1 mg/mL DNase I solution directly to the tube prior to transferring thawed cells

3 Slowly add 10-15 mL of medium or buffer containing 10% FBS drop-wise, while gently swirling the tube.

4 Rinse the vial with 1 mL of culture medium or buffer (i.e. PBS or HBSS) containing 10% FBS to recover any remaining cells, and transfer the medium to the new tube.

5 Top up the 50 mL tube with culture medium or buffer containing 10% FBS. Gently invert to mix.

6 Centrifuge the 50 mL tube at **300 x g for 10 minutes** at room temperature to collect the cells.

7 Carefully remove and discard as much of the supernatant as possible, taking care to not disturb the pelleted cells. Gently tap the tube to resuspend the pellet.

DNase I Treatment for Clumpy Cell Suspensions

8 If cells appear clumpy, calculate the volume of DNase I that should be added to the sample to yield a final concentration of 100 µg/mL DNase I. Add DNase I drop-wise to the cell suspension while gently swirling the tube. Incubate for 15 minutes at room temperature.

DNase I Solution (1 mg/mL)
by Stemcell Technologies
Catalog #: 07900

9 To wash the cells, add 25 mL of culture medium or buffer containing 2% FBS. Gently invert to mix and centrifuge at **300 x g for 10 minutes** .
Discard as much of the supernatant as possible and gently resuspend the pellet.

10 If cells still appear clumpy, pass the sample through a 30 - 70 µm mesh cell strainer into a fresh conical tube. Rinse the sample tube three times with culture medium or buffer containing 2 % FBS and pass through the strainer. Repeat this step 3 times.

The cell suspension is now ready for cell counting and further downstream applications such as cell isolation with an EasySep™ Cell Separation Kit.