STEMprep™ Mouse Liver Dissociation Kit

For processing 50 livers using the STEMprep™ Tissue Dissociation System

Catalog #100-2136 50 Preparations

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Product Description

STEMprep™ Mouse Liver Dissociation Kit provides an optimized enzymatic dissociation cocktail for processing liver tissues into single-cell suspensions, designed for high yields of non-parenchymal cells, including liver sinusoidal endothelial cells (LSECs) and Kupffer cells, while preserving cell surface markers. When used with the STEMprep™ Tissue Dissociator (Catalog #100-2112), mechanical dissociation is combined with enzymatic degradation of the extracellular matrix, efficiently breaking down tissue structure while preserving cellular integrity. Following dissociation, an optional density gradient centrifugation step can be performed to remove parenchymal cells and debris prior to downstream use. The resulting single cells are immediately suitable for downstream applications, such as cell separation, culture, or various analyses.

For best results, use with STEMprep™ Tissue Dissociator and STEMprep™ Sample Tubes (Catalog #200-0800).

Product Information

The following components are sold as part of STEMprep™ Mouse Liver Dissociation Kit (Catalog #100-2136) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMprep™ Enzyme A	100-2131	1 x 2.5 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMprep™ Enzyme B	100-2149	1 x 1.25 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMprep™ Enzyme C	100-2163	1 x 2.5 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMprep™ Enzyme Diluent Z	100-2134	1 x 125 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
50 mL Conical Tubes	e.g. 38010
70 µm Cell Strainer	e.g. 27260
Culture Dish, Non-Treated	e.g. 38045
D-PBS (Without Ca++ and Mg++)	37350
EasySep™ Buffer OR PBS containing 2% FBS and 1 mM EDTA	20144
STEMprep™ Sample Tubes	200-0800
STEMprep™ Tissue Dissociator	100-2112

Preparation of Reagents and Materials

Thaw STEMprep™ Enzyme A, Enzyme B, and Enzyme C at room temperature (15 - 25°C) for immediate use or overnight at 2 - 8°C. Do not thaw at 37°C.

NOTE: Once thawed, use immediately or aliquot and store at -20°C until the expiry date as indicated on the label. After thawing the aliquots, use immediately. Do not re-freeze.

LIVER DISSOCIATION ENZYME COCKTAIL

Prepare liver dissociation enzyme cocktail fresh before use.

For one liver, prepare 2.5 mL of liver dissociation enzyme cocktail by combining the following in a STEMprep™ Sample Tube:

- 2.375 mL of Enzyme Diluent Z
- 50 µL of Enzyme A
- 25 µL of Enzyme B
- 50 µL of Enzyme C

Sample Preparation

Dissect mouse liver into a non-treated culture dish containing cold PBS.

Directions for Use

1. Transfer the liver tissue (0.9 - 2 g) into the STEMprep™ Sample Tube containing 2.5 mL of liver dissociation enzyme cocktail.

   NOTE: Plastic Rack for Centrifuge Tubes, 50 mL (Catalog #200-0651) is provided with each STEMprep™ Tissue Dissociator System to manage STEMprep™ Sample Tubes while loading or removing tissues.
2. Tightly close the STEMprep™ Sample Tube lid (ensuring proper closure) and insert it into a slot on the STEMprep™ Tissue Dissociator. For complete instructions on using the instrument, refer to the Technical Manual: STEMprep™ Tissue Dissociator (Document #10000030598), available at www.stemcell.com, or contact us to request a copy.

   NOTE: For best results, avoid pinning the tissue with the rotor in the center of the STEMprep™ Sample Tube. Ensure total sample volume containing tissue does not exceed the “MAX” fill line on the tube.
3. Select and run the “Mouse Liver” dissociation protocol on the STEMprep™ Tissue Dissociator.

   NOTE: If the tube will not be removed immediately after protocol completion, add a 4°C hold to preserve sample integrity.
4. After the protocol is complete, remove the STEMprep™ Sample Tube from the slot. Place a 70 µm cell strainer in a 50 mL conical tube. Pre-wet the strainer with 5 mL of EasySep™ Buffer. Carefully pour the sample over the strainer.
5. Rinse the STEMprep™ Sample Tube with 10 mL of cold EasySep™ Buffer and pour the wash over the strainer. Repeat this wash step once more. Top up the conical tube to 50 mL with EasySep™ Buffer and screw on the cap. Discard the strainer and STEMprep™ Sample Tube.

   NOTE: Small tissue fragments may remain in the filter after dissociation; these typically do not affect yield. Larger tissue pieces can be gently pushed through the strainer using the rubber end of a syringe plunger.
6. Centrifuge the conical tube at 300 x g for 10 minutes at room temperature with the brake on low. After centrifugation, carefully aspirate the supernatant.
7. OPTIONAL: If desired, perform red blood cell lysis by adding 5 mL of Ammonium Chloride Solution (Catalog #07850) to the cell pellet. Thoroughly mix the cell suspension by pipetting up and down. Incubate on ice for 10 minutes. Top up sample to 50 mL with EasySep™ Buffer. Centrifuge at 300 x g for 10 minutes at room temperature with the brake on low. After centrifugation, carefully aspirate the supernatant.

   NOTE: If proceeding to EasySep™, perform red blood cell lysis followed by debris removal.
8. OPTIONAL: If desired, perform parenchymal cell and debris removal with OptiPrep™ (Catalog #07820). See below for instructions.
   a. Prepare 10 mL of 28% v/v OptiPrep™ solution per sample by adding 2.8 mL OptiPrep™ to 7.2 mL EasySep™ Buffer. Gently invert the tube to mix, do not shake or vortex. Store at room temperature until use.
   b. Add 10 mL of 28% OptiPrep™ solution to the cell pellet and gently pipette up and down; transfer to a 15 mL conical tube. Avoid bubbles and foam.
   c. Gently overlay 4 mL of cold EasySep™ Buffer on top of the cell suspension.

      NOTE: Slowly add EasySep™ Buffer down the wall of the tube without disturbing the interface.
   d. Centrifuge at 1300 x g for 15 minutes at room temperature with no brake.
   e. Using a Pasteur or serological pipette, carefully collect the interface containing non-parenchymal cells (~2.5 mL) and transfer to a new tube (Figure 1).

f. Top up the new tube to 50 mL with EasySep™ Buffer and centrifuge at 300 x g for 10 minutes. Carefully aspirate the supernatant.

9. Resuspend cells in the desired volume of EasySep™ Buffer or medium of your choice. Cells are ready for downstream use.

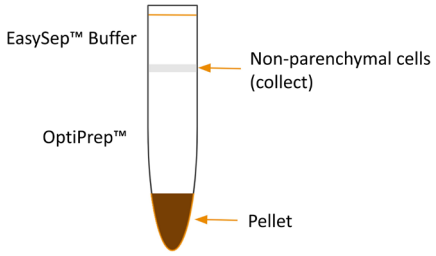

Figure 1. Parenchymal Cell and Debris Removal

Data

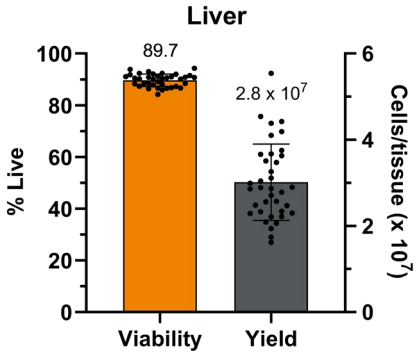

Figure 2. Cell Viability and Yield Obtained Using the STEMprep™ Mouse Liver Dissociation Kit
Livers from healthy C57BL/6 mice were dissociated into single-cell suspensions using the STEMprep™ Mouse Liver Dissociation Kit on the STEMprep™ Tissue Dissociator. Following STEMprep™ processing (without OptiPrep™ treatment), viability and yield of single-cell suspensions were assessed by flow cytometry. Data are presented as mean ± SD.

Related Products

For more information about STEMprep™ kits and protocols, visit www.stemcell.com/stemprep, contact us at techsupport@stemcell.com.