

STEMprep™ Mouse Tumor Dissociation Kit

For processing 50 tumors using the STEMprep™ Tissue Dissociation System

Catalog #100-2137

50 Preparations



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Product Description

STEMprep™ Mouse Tumor Dissociation Kit provides an optimized enzymatic dissociation cocktail for processing tumor tissues into single-cell suspensions. 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. STEMprep™ Mouse Tumor Dissociation Kit facilitates this process, enabling the efficient and reliable generation of single-cell suspensions from implanted or induced mouse tumor tissues. This method preserves cell surface epitopes and yields high numbers of tumor cells, stromal cells, and tumor-infiltrating leukocytes (TILs). 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 Tumor Dissociation Kit (Catalog #100-2137) 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 | 2 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.

TUMOR DISSOCIATION ENZYME COCKTAIL

Prepare tumor dissociation enzyme cocktail fresh before use.

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

- 2.35 mL of Enzyme Diluent Z
- 50 µL of Enzyme A
- 50 µL of Enzyme B
- 50 µL of Enzyme C

Sample Preparation

Harvest mouse tumor tissue into a non-treated culture dish containing cold PBS. For medium (e.g. CT26 colon carcinoma) and hard (e.g. 4T1 mammary tumor) mouse tumor types, cut the tumor tissue into ≤ 5 mm pieces using a razor blade, scalpel, or scissors and immediately proceed to Directions for Use.

Directions for Use

1. Transfer the tumor tissue (without PBS; 160 - 1500 mg) into the STEMprep™ Sample Tube containing 2.5 mL of tumor 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 Tumor" 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 cell clumps are present, filter lysed sample over a pre-wet 70 µm cell strainer on a new 50 mL conical tube prior to centrifugation. Wash the old tube with EasySep™ Buffer and pour over the filter. Proceed with centrifugation.

NOTE: If proceeding to EasySep™, perform red blood cell lysis.

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

Data

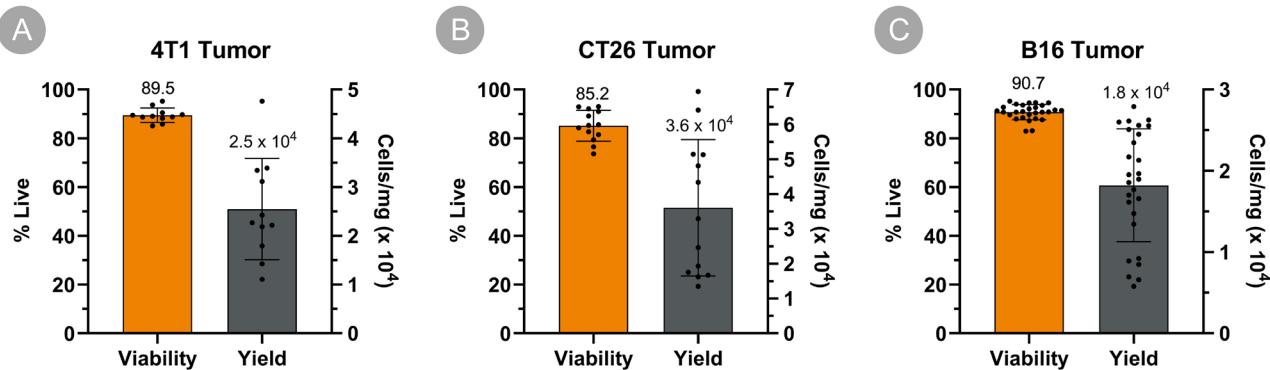


Figure 1. Cell Viability and Yield Obtained Using the STEMprep™ Mouse Tumor Dissociation Kit

Primary solid tumors were generated by subcutaneous injection of tumor cell lines into the flanks of mice. Mouse tumor tissues were dissociated into single-cell suspensions using the STEMprep™ Mouse Tumor Dissociation Kit on the STEMprep™ Tissue Dissociator. Processed cells from (A) hard 4T1 mammary tumors, (B) medium CT26 colon carcinoma tumors, and (C) soft B16 melanoma tumors were analyzed by flow cytometry to assess cell viability and yield. Data are presented as mean \pm SD.

Related Products

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

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