

Dispase (5 U/mL)

5 U/mL dispase in Hanks' Balanced Salt Solution

Catalog # 07913

100 mL



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Product Description

Dispase is a protease that is suitable for the gentle dissociation of a wide variety of tissues.

This product contains 5 U/mL Dispase II (neutral protease from *Bacillus polymyxa*) dissolved in Hanks' Balanced Salt Solution.

Properties

Storage: Store at -20°C.

Shelf Life: Stable until expiry date (EXP) on label.

Handling / Directions For Use

NOTE: If product is received partially thawed, place immediately at -20°C or thaw and aliquot as described below. Do not use Dispase past the expiry date as indicated on the label.

Thaw Dispase (5 U/mL) at 2 - 8°C overnight. Once thawed, use immediately or store at 2 - 8°C for up to 2 weeks. Alternatively, aliquot and store at -20°C until expiry date as indicated on label. After thawing the aliquots, use immediately or store at 2 - 8°C for up to 2 weeks. Do not re-freeze.

Incubation of minced tissue with pre-warmed Dispase and gentle agitation will liberate cells with minimal cell damage. Pre-warmed Dispase can also be used to harvest cells from tissue culture plastic. Unlike trypsin, Dispase is not inhibited by serum. Dispase activity is inhibited by EDTA and EGTA. Dispase should be removed from cell suspensions by centrifugation followed by washing the cells with buffer or culture medium.

GENERATION OF A SINGLE-CELL SUSPENSION FROM DISSOCIATED HUMAN AND MOUSE MAMMARY TISSUE

The following protocol is for generating a single-cell suspension from dissociated human and mouse mammary tissue using Trypsin-EDTA (0.25%; Catalog #07901), Dispase (5 U/mL), and DNase I Solution (1 mg/mL; Catalog #07900). More information can be found on the Product Information Sheets for EpiCult™-B Human Medium Kit (Document #29567), EpiCult™-B Mouse Medium Kit (Document #29568), Collagenase/Hyaluronidase (Document #29634), and Gentle Collagenase/Hyaluronidase (Document #29629), available at www.stemcell.com or contact us to request a copy.

1. Add 1 - 5 mL of pre-warmed Trypsin-EDTA to the collagenase-dissociated mammary tissue so that the clumps of epithelial cells are well suspended. Using a 1 mL pipette tip, gently pipette the suspension up and down for 1 - 3 minutes. The sample should become very stringy due to lysis of dead cells and the release of DNA.
2. Add 10 mL of cold HBSS with 10 mM HEPES, Without Phenol Red (Catalog #37150) supplemented with 2% fetal bovine serum (FBS). The Hanks' + FBS solution will now be referred to as HF. Centrifuge at 350 x g for 5 minutes.
3. Remove as much of the supernatant as possible. The cells may be a large, stringy mass floating in the HF.
4. Add 2 - 5 mL of pre-warmed Dispase (5 U/mL) and 200 µL of DNase I Solution, and pipette the sample for 1 - 2 minutes. The sample should now be cloudy, but not stringy. If still stringy, add more DNase I Solution.
5. Dilute the cell suspension with 10 mL of cold HF and filter the cell suspension through a 40 µm Cell Strainer (Catalog #27305) into a new 50 mL conical tube (e.g. Catalog #38010). Centrifuge at 450 x g for 5 minutes and discard supernatant.

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