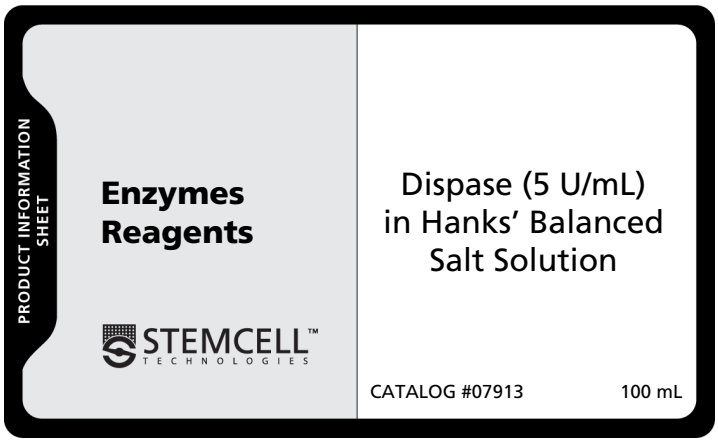
PRODUCT DESCRIPTION

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

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

This product (5 U/mL) has equivalent activity to the product previously provided at a concentration of 5 mg/mL.



STABILITY AND STORAGE

Product stable at -20°C until expiry date as indicated on label.

Product should be thawed, aliquoted into working volumes and refrozen. **Avoid repeated freeze-thaw cycles**.

Product is sterility tested.

DIRECTIONS FOR USE

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 of the cells followed by washing of the cells with buffer or culture medium.

The following is a protocol for generation of single-cell suspensions from dissociated human and mouse mammary organoids using Trypsin-EDTA (Catalog #07901), Dispase (5 U/mL) and DNase I (Catalog #07900). More information can be found on the product information sheets for EpiCult™-B Medium Kit (Human; Catalog #05601), EpiCult™-B Mouse Medium Kit (Catalog #05610) and Collagenase/Hyaluronidase (Catalog #07912) available on our website at www.stemcell.com.

1. Add 1 - 5 mL of pre-warmed Trypsin-EDTA to the mammary organoids such that the organoids are well suspended and gently pipette with a P1000 pipettor 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 Hanks' Balanced Salt Solution Modified (Catalog #37150) supplemented with 2% fetal bovine serum (now referred to as HF) and centrifuge at 350 x *g* for 5 minutes.
3. Remove as much of the supernatant as possible. The cells may be a big, stringy mass floating in the HF.
4. Add 2 - 5 mL of pre-warmed Dispase (5 U/mL) and 200 µL of 1 mg/mL DNase I and pipette the sample for 1 - 2 minutes. The sample should now be cloudy, but not stringy. If still stringy, add more DNase I.
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 centrifuge tube. Centrifuge at 350 x *g* for 5 minutes and discard supernatant.