Manufactured for:

GIBCO®
invitrogen cell culture

DISPASE

Cat. No.: 17105 **1 g**
5 g
Custom pack sizes available upon request.
Storage: 2 to 8°C
Avoid: Moisture, inhalation, skin contact, and exposure to light.

This product is intended for cell isolation and culture use only.

Background
Dispase, or neutral protease, is a metalloenzyme produced by Bacillus polymyxa. It has been classified as an amino-endo peptidase. Dispase is supplied as a non-sterile product.
Dispase is suitable for tissue disaggregation and subcultivation procedures since it does not damage cell membranes. Since Dispase is from a bacterial source, it is free of mycoplasma and animal virus contamination. It is very stable with respect to temperature, pH and interference by serum components. Activity is greatly reduced by dilution, allowing suspension cultures to grow without difficulty. Dispase has even been added to cell suspension cultures to prevent unwanted cell clumping.
Dispase has been used to prepare many types of cells for culture. Dispase has proven to be a rapid, effective, but gentle agent for separating intact epidermis from the dermis and intact epithelial sheets in culture from the substratum. In both cases, it affects separation by cleaving the basement membrane zone region while preserving the viability of the epithelial cells. Dispase has been used to detach epidermal cells as confluent, intact sheets from the surface of culture dishes without dissociating the cells. Studies on the use of skin epithelial cell sheets detached from the culture substrate by Dispase for transplantation are in progress in many laboratories. Also, Dispase has been used for the harvest and transfer of normal diploid cells and cell lines.
Suitability of the enzyme for detaching and dissociating a particular cell line, however, should be determined empirically. A general observation is that fibroblast-like cells are detached by Dispase from the culture substrate as well as dissociated into a mono-disperse cell suspension while epithelial-like cells are detached, but not completely dissociated.

Specifications
Potency: One unit of Dispase equals 181 protease units (release of folin-positive amino acids from casein equivalent to 1 µmol of tyrosine per minute at pH 7.5 and 37°C). One unit of Dispase II equals approximately 600 Japanese units of Dispase.
Appearance: Yellowish lyophilisate

Instructions for Use

A. **Preparation of stock solution**
Dissolve the non-sterile enzyme in PBS (Ca^{2+}/Mg^{2+}-free) (Cat. No. 14190) to 10 mg/mL. Further dilute with PBS (Ca^{2+}/Mg^{2+}-free) to a final concentration of 0.6 to 2.4 U/mL. Concentrations higher than 2.4 U/mL are not recommended. Filter sterilize through a 0.22 µm filter membrane.

B. **Disaggregation of tissue**
- Fragment the tissue with a sterile scalpel or scissors.
- Wash the tissue fragments in sterile PBS.
- Incubate the fragments in the Dispase solution (2.4 U/mL to 0.6 U/mL) at 37°C. Make sure that the tissue fragments are well covered by the solution.
- Stir slowly at 37°C until the tissue is sufficiently dissolved. When using Dispase for the first time, determine the total reaction time by counting the cells. A time of one hour is required for hard compact tissue. The cells will not be adversely affected even after several hours in Dispase.
- If necessary, separate the dispersed cells from residual tissue by passing the mixture through a sterile stainless steel grid or simply decant the cells after larger fragments have settled. Fresh Dispase solution may be added to the remaining tissue fragments if further disaggregation is required.
- Pellet cells by centrifugation and decant the enzyme solution.
- Resuspend the pellet in the culture medium and incubate under predetermined conditions.
- More efficient dissociation of tissue is obtained by mixing the Dispase at 0.3 to 0.6 U/mL with collagenase (60-100 U/mL).

C. **Subcultivation of cells**
- Cover the cells with Dispase solution, pre-warmed to 37°C. Incubate for 5 minutes at 37°C.
- Decant the solution and incubate for an additional 10 minutes at 37°C.
- Monitor detachment using a microscope. If necessary, incubate for an additional 15 minutes or until detachment is complete.
- Suspend the cells in culture medium and pellet cells by centrifugation; wash the cells with culture medium.
- Resuspend the cells in fresh culture medium.
- Plate the cells as usual.

For further information on this or other GIBCO® products, contact Technical Services at the following:
United States TECH-LINE℠: 1 800 955 6288
Canada TECH-LINE: 1 800 757 8257
Outside the U.S. and Canada, refer to the GIBCO products catalogue for the TECH-LINE in your region. You may also contact your Invitrogen Sales Representative or our World Wide Web site at www.invitrogen.com.

For research use only.
CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

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