

# Dispase

# Description

Dispase, or neutral protease, is a metalloenzyme produced by *Bacillus polymyxa* which has been classified as an amino-endopeptidase. This enzyme has proven to be a rapid and gentle agent for the harvest and transfer of normal diploid cells and cell lines. In addition, Dispase has been shown to effectively separate cell clumps, as well as cells from intact tissue without significantly affecting cell membrane integrity or viability.

Cat. no.	Size	Store at 2°C to 8°C
17105-041	5 g	

## Product Use

For Research Use Only. Not for use in diagnostic procedures.

#### Shelf Life

Dispase is stable for 24 months when stored as directed.

#### Important Information

- Appearance: Yellowish powder.
- Dispase is inhibited by EDTA, EGTA, Hg<sup>2+</sup>, and other heavy metals.
- Dispase is supplied as a non-sterile product.
- Protect from moisture and light.

#### Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Avoid inhalation and skin contact.

#### **Unit Definition**

One unit of Dispase equals 181 protease units (release of folin-positive amino acids from casein equivalent to 1  $\mu$ mol of tyrosine per minute at pH 7.5 and 37°C).

Pub. no. MAN0007349

### Use

# Reconstitute Dispase

- 1. Dissolve the non-sterile enzyme in Dulbecco's Phosphate-Buffered Saline (DPBS) without calcium and magnesium to 10 mg/mL.
- Further dilute with DPBS without calcium and magnesium to a final concentration of 0.6–2.4 U/mL.
  Note: Concentrations higher than 2.4 U/mL are not recommended.
- 3. Filter sterilize through a 0.22 µm filter membrane.

# Dissociate Tissue

- 1. Mince tissue into 3–4 mm pieces with a sterile scalpel or scissors.
- 2. Wash the tissue pieces several times in sterile DPBS without calcium and magnesium.
- 3. Submerge tissue fragments in Dispase solution (0.6–2.4 U/mL) and incubate at 37°C.
- Stir slowly at 37°C until the tissue is sufficiently dissolved. For compact tissues, we recommend incubating for 1 hour. Cells will not be adversely affected even after several hours in Dispase.
- 5. If necessary, separate the dispersed cells from residual tissue by passing the mixture through a sterile stainless steel or nylon mesh 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.
- 6. Pellet cells by centrifugation and decant the enzyme solution.
- Resuspend the cell pellet in appropriate culture medium. Determine viable cell density using a Countess<sup>®</sup> Automated Cell Counter (alternate automated or manual methods may be used).
- 8. Seed cells into culture vessels containing appropriate culture medium and incubate under predetermined conditions.

**Note:** More efficient dissociation of tissue is obtained by mixing the Dispase at 0.3–0.6 U/mL with collagenase (60–100 U/mL).

## Subculture Cells

- 1. Aspirate culture medium and cover the cells with Dispase solution, prewarmed to 37°C. Incubate for 5 minutes at 37°C.
- 2. Decant the Dispase solution and incubate the cells for an additional 10 minutes at 37°C.
- 3. Monitor cell detachment using an inverted microscope. If necessary, incubate for an additional 15 minutes or until detachment is complete.
- 4. Suspend the cells in culture medium and pellet by centrifugation.
- 5. Resuspend the cells in fresh culture medium.
- 6. Plate the cells as usual.

#### Related Products

Product	Catalog No.
DPBS, without calcium or magnesium	14190
Collagenase Type I	17100
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227

#### Limited Product Warranty

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