Dispase II, Powder (Neutral Protease, Grade II)

Catalog	Unit Size
TBS2117-01	1G

DESCRIPTION

Dispase II is a neutral protease that hydrolyzes the N-terminal peptide bonds of non-polar amino acid residues. It can be used for separating many tissues and cells grown in vitro. The enzyme is very gentle and does not damage cell membranes. It can also be used to prevent clumping in suspension cultures. This protease cleaves fibronectin and type IV collagen, but not laminin, type V collagen, serum albumin, or transferrin. Dispase II is specific for the cleavage of Leucine-Phenylalanine bonds. Ca2+, Mg2+, Mn2+, Fe2+, Fe3+ and Al3+ activate the enzyme. EDTA, EGTA, Hg2+, and other heavy metals inhibit enzyme activity.

Tribioscience's Dispase II is a lyophilized powder, and stable at 2-8°C. It makes it easy to store and application.

MAIN FEATURES

- Rapid, effective, yet gentle agent that liberates cells with minimal cell damage.
- Maintains cell membrane integrity.
- Non-mammalian source - free of mycoplasma and animal virus contamination.
- Extremely stable to influences of temperature, pH, and interference by serum components.
- Easily inactivated by chelating agents or by dilution.
- Delivers higher activity and convenience.

PACKAGE SIZE

1G/bottle

ACTIVITY

1.0U/MG

STORAGE CONDITIONS

Dispase II is stored at +2 to +8°C for shelf-life of 1 year. The expiration date is printed on the label.

RECONSTITUTION

Stock solution of 10mg/mL: Dissolve the nonsterile lyophilized enzyme in DPBS. The reconstituted stock solution is stable at +2 to +8°C for 2 weeks. For storage up to 2 months, freeze the stock solution in aliquots.

Working Solution: Dilute the 10 mg/ml stock solution with DPBS or the culture medium to be used for the isolated cells, at a final concentration of 0.6 to 2.4 U/ml. Concentrations higher than 2.4 U/ml are not recommended. For best results, filter the working solution using a 0.22 μm pore-size membrane.

APPLICATION

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.

4. 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.

7. Resuspend the cell pellet in appropriate culture medium. Determine viable cell density using a Countess® 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, pre-warmed 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.

Unit Definition: One unit is defined as the amount of enzyme that liberates, under assay conditions, folin-positive amino acids and peptides from casein equivalent to 1 µM (181 µg) tyrosine per minute at pH 7.5 at +37°C. One unit of Dispase II equals 181 protease units (PU) measured as release of amino acids equivalent to 1 µg tyrosine per minute and ml at pH 7.5 and +37°C.

Research use only.