Beijing Solarbio Science & Technology Co., Ltd
Tel: 400-968-6088
Fax: 010-56371281
Http://www.solarbio.com

Dispase II

Cat No.: D6430
Size: 100mg/1g
Storage: Store at 2-8°C, valid for 1 year.

Product Parameter:

CAS Number: 42613-33-2
EC-Number: EC 3.4.24.4
Appearance: White powder
pH Optimum: pH 6.0 to 8.5
Specific Activity: ≥0.8 U/mg (37°C, casein as substrate, pH 7.5)
Source: Derived from Bacillus polymyxa
Enzyme activity/efficacy: 0.5 units/mg solid
Solubility: 10mM NaAc(pH 7.5)and 5mM CaAc
Activator: Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Al^{3+} Optimal Ca^{2+} concentration is 2 mM. The enzyme preparations contain enough Ca^{2+} for optimal activity.
Inhibition: EDTA, EGTA, Hg^{2+}, and other heavy metals. Dispase is not inhibited by serum.

Product Description:

Dispase is used for the preparation of cells from a wide variety of different tissues and organs.

• Proven to be a rapid and effective, yet gentle agent for separating intact epidermis from the dermis, and intact epithelial sheets in culture from the substratum by cleaving the basement membrane zone region while preserving the viability of the epithelial cells.

• Used to subculture cells and prevent unwanted clumping of cells cultured in suspension.

• Used to detach epidermal cells as confluent intact sheets from the surface of culture dishes without dissociating the cells.

Note: Suitability of the enzyme for detaching and dissociating a particular cell line, however, should be determined empirically.

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 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.

Protocol:

I. Working Solution

Dilute the 10 mg/ml stock solution with the culture medium to be used for the isolated cells, at a final

concentration of 0.6 to 2.4 U/ml.

Note:

1) Concentrations higher than 2.4 U/ml are not recommended.

2) For best results, filter the working solution using a 0.22 μm pore-size membrane.

II. Disaggregation of Tissue

1. Fragment the tissue with a sterile scalpel or scissors.
2. Wash the tissue fragments in sterile PBS.
3. Incubate the fragments in the Dispase solution (0.6 U/ml to 2.4 U/ml) at 37°C.
   - Make sure that the tissue fragments are well covered by the solution.
4. 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.
   - One hour is required for hard compact tissue. The 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 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.
6. Spin the cells down and decant off the enzyme solution.
7. Resuspend the pellet in the culture medium and incubate under the normal predetermined conditions.

III. Subcultivation of Cells

1. Cover the cells with Dispase solution, prewarmed to 37°C.
   - Incubate for 5 minutes at 37°C.
2. Decant the solution and incubate for an additional 10 minutes at 37°C.
3. Control detaching using a microscope.
   - If necessary, incubate an additional 15 minutes.
4. Suspend the cells in culture medium.
   - Spin the cells down and wash the cells with culture medium.
5. Resuspend the cells in fresh culture medium.
6. Plate the cells as usual.

Note:

1．The product information is for reference only, if you have any questions, please call 400-968-6088 for consultation.

2．This product is for scientific research use only. Do not use in medicine, clinical diagnosis or treatment, food and cosmetics. Please do not store in ordinary residential areas.

3．For your safety and health, please wear a good lab coat and operate with disposable gloves and mask.