

Dispase II

Cat. No.: D1516122 | Pack size: 50 mg / 250 mg / 1 g | Storage: Store at 2-8°C

Overview

Dispase, a protease for tissue culture, is a metalloproteinase that cleaves the N-terminus of neutral and non-polar amino acids in peptide chains. It can detach epithelial cells from tissues in sheet form and has long been used for the isolation and dispersion of cells in primary culture. In recent years, it has also been applied in the field of regenerative medicine. Unlike proteases such as trypsin and collagenase, Dispase causes less cellular damage and can disperse cells gently, thus being used for cell dispersion and subculture in emerging fields such as ES and iPS cell research.

Product Information

Attribute	Dispase I	Dispase II
Product Code	D1516119	D1516122
Product Name	Dispase I	Dispase II
Form	Crystallized enzyme, sterile product	Powdered enzyme, non-sterile product
Enzyme Activity	10,000-13,000 PU/vial	300-360 PU/mg enzyme powder
Additives	Contains calcium acetate	Contains dextrin and calcium acetate
Storage & Shelf Life	Store at 2-8°C for 2 years (unopened). After reconstitution, store frozen at ≤ -20°C and use within 6 months.	Store at 2-8°C for 2 years (unopened). After reconstitution, store frozen at ≤ -20°C and use within 6 months.

Key Features

1. Dispase is a neutral metalloproteinase derived from *Paenibacillus* sp. (formerly *Bacillus polymyxa*), with Zn^{2+} in its active center and Ca^{2+} for stabilizing its activity.
2. Enzyme activity is hardly affected by serum and can be used in the presence or absence of serum. The enzymatic reaction can be terminated by adding chelating agents such as EDTA or washing cells with fresh medium or buffer.

3. It can effectively decompose type IV collagen and fibronectin that constitute the basement membrane, detaching epithelial cells from tissues in sheet form.
4. Unlike trypsin, collagenase and other proteases, it causes less cellular damage and enables gentle cell dispersion.
5. It has a wide range of working conditions and can function under different concentrations, treatment times, temperatures and pH values.
6. It is highly stable at the general cell culture temperature (37°C). For some cell types, suspension culture can be achieved by adding this product to the medium.
7. This product is animal-origin free and mycoplasma-free.

Usage Method

Dispase I: Dissolve and prepare under sterile conditions. Remove the aluminum cap, disinfect the rubber stopper and its surrounding area with sterile ethanol, draw the required volume of medium or buffer with a sterilized syringe, and inject it into the vial for dissolution. Note safety during operation as the aluminum cap may cause finger scratches.

Dispase II: Weigh the required amount of Dispase II powder and dissolve it with medium or buffer. Perform 0.2 µm filtration sterilization in a clean environment.

Working Conditions

Condition	Recommended Range / Description
Enzyme Concentration	100 ~ 2000 PU/mL
Dissolution Buffer	Ca ²⁺ -containing buffer or medium (serum may be included)
Reaction Time	30 minutes ~ several days
Reaction Temperature	Room temperature ~ 37°C
Reaction pH	6.5 ~ 9.0
Inhibitors	EDTA, Fe ³⁺ , Fe ²⁺ , Ni ²⁺ , Cu ²⁺ , Al ³⁺ , Zn ²⁺ . The active center of this enzyme contains Zn ²⁺ ; excessive Zn ²⁺ in the reaction solution may inhibit the enzyme activity.

Activity Assay Method

Add 1 mL of enzyme solution (50 PU/mL, 50 mM Tris-HCl buffer containing 2 mM calcium acetate) to 5 mL of 0.6% casein aqueous solution (pH 7.5, 50 mM Tris-HCl buffer containing 2 mM calcium acetate), and react at 30°C for 10 minutes. After the reaction, add 5 mL of trichloroacetic acid reagent to terminate the reaction. Let it stand at 30°C for 30 minutes, filter it, and detect the absorbance at 275 nm.

Under these conditions, 1 PU (Protease Unit) is defined as the amount of enzyme that hydrolyzes casein to produce 1 µg of tyrosine per minute.

Note: Trichloroacetic acid reagent is prepared by dissolving 1.8% (w/v) anhydrous sodium acetate, 1.8% (w/v) trichloroacetic acid and 1.98% (w/v) acetic acid in distilled water, and adjusting the pH to 4.0.

Precautions for Use

- This product is a research reagent and shall not be used for in vivo experiments on humans or animals, nor for diagnostic purposes.
- This product is a dispase and may cause inflammation if it comes into contact with eyes, mouth, skin or mucous membranes. In case of accidental direct contact, rinse immediately with a large amount of running water and seek medical attention as soon as necessary if needed.
- Calcium acetate is contained in this product to ensure enzyme stability. Therefore, white precipitation (calcium salt) may form when this product is used in a high-concentration phosphate buffer, which may lead to a decrease in enzyme activity and stability.
- For the enzyme solution and working conditions, it is recommended to use medium containing fetal bovine serum, which is conducive to cell dispersion and proliferation after dispersion.
- Note safety during operation as the aluminum cap may cause finger scratches when opened.
- Store this product under the specified storage conditions and do not use expired products.
- Store the Dispase solution at -20°C in a frozen state and use it up within 6 months.
- When discarding this product, comply with relevant waste disposal regulations and handle it properly.

Q&A

1. About Dispase I and Dispase II

Q1: What is the difference between Dispase I and Dispase II?

A1: Dispase I: Crystallized enzyme, sterile, contains calcium acetate. Dispase II: Powdered enzyme, non-sterile, contains dextrin and calcium acetate (perform filter sterilization after dissolution as required). Note: The purification degrees of I and II are slightly different (Dispase I has a higher purification degree).

Q2: What precautions should be taken when using Dispase I and Dispase II? Are they suitable for different experimental systems?

A2: They can be used for a variety of cell types and experimental systems, and no feedback of incompatible experimental systems has been received to date. Note that due to different packaging forms, the usage of the product after opening should be determined in accordance with the storage conditions. Dispase I: Vacuum-sealed, basically needs to be used up in one single operation. Dispase II: The powder can be aliquoted for use.

2. About Usage Method

Q1: What is the recommended concentration?

A1: The recommended concentration is as indicated in the product manual: 100-2,000 PU/mL. It is recommended to optimize the appropriate concentration within this range first.

Q2: What is the recommended concentration for detaching epithelial cells into sheets?

A2: The enzymatic effect depends on cell types and culture conditions, so a definitive recommended concentration cannot be provided. Please refer to the concentration range of 100-2000 PU/mL in the product manual to optimize the appropriate working concentration. In addition, when used at the same concentration, there is no significant difference between Dispase I and Dispase II in application.

Q3: Since the enzyme activity and stability are related to Ca^{2+} , can it still exert its effect in Ca^{2+} -free PBS (-) or HBSS?

A3: As stated in the product manual, white precipitation may occur or the enzyme activity and stability may decrease when used in phosphate buffer solutions. Therefore, the use of phosphate buffer solutions should be avoided as much as possible, and it is recommended to use this product under Ca^{2+} -containing conditions.

Q4: Can PBS (-) be used?

A4: It can be used, but the enzyme activity and stability may decrease. Please adjust the enzyme dosage (enzyme concentration) and use it immediately after dissolution.

Q5: What is the recommended concentration of Ca^{2+} for use?

A5: Only a few mM is sufficient for it to exert its effect fully.

Q6: It was mentioned earlier that calcium-containing buffer or medium is used as the enzyme dissolution solution. If a medium containing fetal bovine serum (FBS) is used, can its concentration be adjusted to 10%?

A6: Yes.

3. About Function

Q1: In addition to decomposing type IV collagen and fibronectin, can it decompose other basement membrane proteins?

A1: It has a slight decomposing effect on laminin.

Q2: Will treatment with Dispase affect cell viability or cellular genes?

A2: Cell viability varies greatly under different conditions. The effect on genes has not been confirmed yet.

Specifications

Attribute	Value
Product Name	Dispase II, CAS No.42613-33-2
Synonyms	Dispase II Neutral protease II Protease from Bacillus polymyxa
Grade	ActiBioPure™, Animal Free, Bioactive, High Performance, Mycoplasma free, Native, EnzymoPure™
Specifications & Purity	Animal Free, Bioactive, ActiBioPure™, Native, High Performance, Mycoplasma free, EnzymoPure™, from Paenibacillus polymyxa; 300-360 PU/mg enzyme powder
N-terminal Sequence	1 PU (Protease Unit) is defined as the amount of enzyme that liberates 1 µg of tyrosine per minute from casein.
CAS	42613-33-2
EC Number	255-914-4
Molecule Type	Enzyme
Concentration	from Paenibacillus polymyxa; 300-360 PU/mg enzyme powder
Reconstitution	Reconstitute in buffer or medium containing Ca ²⁺ (serum may be included).
Storage Conditions	Store at 2-8°C
Shipped In	Wet ice

Attribute	Value
Stability And Storage	Store at 2-8°C long term (24 months), unopened. Upon reconstitution, it is recommended to aliquot and store at -20°C (6 months).

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