Dispase® II (neutral protease, grade II)

From *Bacillus polymyxa*
Lyophilizate, non-sterile

Cat. No. 04 942 078 001 5 × 1 g

Version 5.0
Content version: October 2010
Store at +2 to +8°C

1. What this Product Does

Contents

5 bottles Dispase® II lyophilizate, non-sterile, 5 × 1 g

Storage and Stability

The lyophilizate is stable at +2 to +8°C until the expiration date printed on the label.

The reconstituted stock solution is stable at +2 to +8°C for 2 weeks. For longer storage times up to 2 months the stock solution should be stored frozen in aliquots. Repeated freezing and thawing should be avoided.

The working solution diluted with Hepes buffered saline is stable at +2 to +8°C for 3 days.

Application

Dispase® is used for the preparation of cells from a wide variety of different tissues and organs. Dispase® has 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. Dispase® is also used to subculture cells and prevent unwanted clumping of cells cultured in suspension.

2. How to Use this Product

2.1 Before you Begin

Preparation of Stock and Working Solution

Dissolve the non-sterile lyophilized enzyme in Hepes-buffered saline (50 mM Hepes/KOH pH 7.4, 150 mM NaCl) (10 mg/ml). Dilute further with the culture medium to be used for the isolated cells to a final concentration of 0.6 to 2.4 U/ml. Concentrations higher than 2.4 U/ml are not recommended. Filtered through 0.2 μM pore size membrane.

2.2 Procedures

Disaggregation of Tissue

Step	Action
❶	Fragment the tissue with a sterile scalpel or scissors.
❷	Wash the tissue fragments in sterile PBS.
❸	Incubate the fragments in the Dispase® solution (2.4 U/ml – 0.6 U/ml) at 37°C. Make sure that the tissue fragments are well covered by the solution
❹	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®.
❺	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
❻	Spin the cells down and decant off the enzyme solution
❼	Resuspend the pellet in the culture medium and incubate under the normal predetermined conditions

Subcultivation of Cells

Step	Action
❶	Cover the cells with Dispase® solution, prewarmed to 37°C, incubate for 5 min at 37°C.
❷	Decant the solution and incubate for a further 10 min at 37°C.
❸	Control detaching with the microscope, if necessary incubate for a further 15 min.
❹	Suspend the cells in culture medium and spin the cells down, wash the cells with culture medium.
❺	Resuspend the cells in fresh culture medium.
❻	Plate the cells as usual.

3. Additional Information on this Product

How this Product Works

Proteolytic enzymes such as trypsin, collagenase and pronase are used for dispersing tissues and cells. These enzymes, however, often injure the cells, are unstable during incubation, can be heterogeneous and also a source of mycoplasma contamination. The use of Dispase® overcomes all these difficulties. Dispase® is especially suitable for tissue disaggregation and subcultivation (1–7) procedures since it does not damage cell membranes. Since Dispase® is from a bacterial source, it is free of mycoplasma and animal virus contaminations. 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 (1,7). Dispase® has proven to be a rapid, effective, but gentle agent for separating intact epidermis from the dermis and intact epithelial sheet in culture from the substratum. In both cases, it effects separation by cleaving the basement membrane zone region while preserving the viability of the epithelial cells (2,4-6). 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. Dispase® has therefore been used to detach epidermal cells as confluent, intact sheets from the surface of culture dishes without dissociating the cells (2,4-6).

Product Description

EC 3.4.24.4

Specific Activity

≥0.8 U/mg (37°C, casein as substrate, pH 7.5).

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 Roche Applied Science Dispase® equals 181 protease units (PU) measured as release of amino acids equivalent to 1 μg tyrosine per min and ml at pH 7.5 and 37°C.

A practical comparison of RAS units of Dispase® with those cited in the Japanese literature (where concentrations of 1,000 to 2,000 units Dispase®/ml are not uncommon) suggests one Roche Applied Science unit of Dispase® II, grade II equals approx. 600 Japanese units of Dispase®.

Inhibitors

EDTA, EGTA, Hg^{2+}, other heavy metals (8,9). Dispase® is not inhibited by serum.

Activators

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.

Specificity

Dispase® is a non-specific protease.

pH Optimum

6.0 – 8.5

Quality Control

Protease activity of each lot is tested with casein as substrate.

References

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3 Mena I. et al. (2004) Infection of macrophage primary cultures by persistent and nonpersistent strains of Theiler's virus: role of capsid and noncapsid viral determinants. *Journal of Virology*, **78**, 13356-13361.

4 Brückener K. E. et al. (2003) Permeabilization in a cerebral endothelial barrier model by pertussis toxin involves the PKC effector pathway and is abolished by elevated levels of cAMP. *Journal of Cell Science*, **116**, 1837-1846.

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7 Khayyamian S et al. (2002) ICOS-ligand, expressed on human endothelial cells, costimulates Th1 and Th2 cytokine secretion by memory CD4+ T cells. *Proceedings of the National Academy of Sciences*, **99**, 6198-6203.

8 Griffin, P.J. & Fogarty, W. M. (1971) *Some properties of a protease from Bacillus polymyxa Biochem. J.* **125**, 109 P

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4. Supplementary Information

Changes to Previous Version

- New buffer for the preparation of Stock solution and Working solution
- Editorial changes

Text Conventions

To make information consistent and understandable, the following text conventions are used in this Instruction Manual:

Text Convention	Use
Numbered instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols

Symbols are used in this Instruction Manual to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.

Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, www.roche-applied-science.com

Product	Pack Size	Cat. No.
Dispase® I (neutral protease, grade I)	10 × approx. 2 mg	04 942 086 001

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