For life science research only.
Not for use in diagnostic procedures.

Dispase® I (neutral protease, grade I)
lyophilizate from *Bacillus polymyxa*, filtered through 0.2 µm pore size membrane

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Cat. No. 04 942 086 001	10 x approx. 2 mg ≥ 20 U

Store the lyophilizate at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Dispase® I (neutral protease, grade I)	Filtered through 0.2 µm pore-size membrane prior to lyophilization.	10 vials, approximately 2 mg (≥20 U)

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the lyophilizate is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Dispase® I (neutral protease, grade I)	Store at +2 to +8°C.

Storage Conditions (Working Solution)

The working solution diluted in HEPES-buffered saline is stable at +2 to +8°C for 3 days.

Reconstitution

For a 10 mg/ml stock solution, dissolve the lyophilized enzyme in HEPES-buffered saline (50 mM HEPES/KOH pH 7.4, 150 mM NaCl).
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.
⚠ ***Avoid repeated freezing and thawing.***

1.3. Additional Equipment and Reagent required

For preparation of stock and working solutions

- HEPES-buffered saline
- Culture medium

For disaggregation of tissue

- PBS*

1.4. Application

Dispase® is used for the preparation of cells from a wide variety of different tissues and organs:
- 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.
- Harvest and transfer of normal diploid cells and cell lines.
- Subculture cells and prevent unwanted clumping of cells cultured in suspension.
- Detach epidermal cells as confluent intact sheets from the surface of culture dishes without dissociating the cells.
  ℹ *Suitability of the enzyme for detaching and dissociating a particular cell line, however, must be determined empirically.*

2. How to Use this Product

2.1. Before you Begin

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

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.

Do not use concentrations >2.4 U/ml.

2.2. Protocols

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.

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.

2.3. Parameters

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.

EC-Number

EC 3.4.24.4

Inhibition

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

pH Optimum

pH 6.0 to 8.5

Specific Activity

≥20 U/vial (+37°C, casein as substrate, pH 7.5).

Specificity

Dispase® is a nonspecific protease.

Unit Definition

One U 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 U of Roche 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.
- A practical comparison of Roche units of Dispase® with those cited in the Japanese literature (where concentrations of 1,000 to 2,000 U Dispase®/ml are not uncommon), suggest one Roche U of Dispase® I, grade I equals approximately 416 Japanese units of Dispase®.

3. Additional Information on this Product

3.1. Test Principle

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. The enzyme is especially suitable for tissue disaggregation and subcultivation 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.
- 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.

3.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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