

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



Collagenase/Dispase[®]

**Collagenase from *Vibrio alginolyticus*,
Dispase from from *Bacillus polymyxa***

 **Version: 19**

Content Version: December 2020

Cat. No. 10 269 638 001 100 mg

Cat. No. 11 097 113 001 500 mg

Store lyophilizate at +2 to +8°C.

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1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Collagenase/Dispase®	Lyophilized, nonsterile ⚠ The lyophilizate already contains Ca²⁺ as activator.	10 269 638 001	1 vial, 100 mg
			11 097 113 001	1 vial, 500 mg

1.2. Storage and Stability

Storage Conditions (Product)

Shipped at +15 to +25°C.

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

Vial / Bottle	Label	Storage
1	Collagenase/Dispase®	Store dry at +2 to +8°C.

Storage Conditions (Working Solution)

Store reconstituted solution in aliquots at –15 to –25°C.

⚠ Avoid repeated freezing and thawing.

Reconstitution

Reconstitute in water to a final concentration of 100 mg/ml.

i Further dilution with PBS (phosphate buffered saline) (Ca²⁺/Mg²⁺-free).

1.3. Additional Equipment and Reagent required

For reconstitution of lyophilizate

- Water

For preparation of working solution

- PBS (phosphate buffered saline)
- Filter membrane, 0.22 µm

1.4. Application

Collagenase/Dispase® provides a combination of collagenolytic and proteolytic enzymes required for tissue disaggregation.

- This highly effective enzyme mixture offers both the advantage of a highly purified collagenase from *V. alginolyticus* and gentleness of purified Dispase® from *B. polymyxa*.
- Collagenase/Dispase® is used for the isolation of cells from many tissues. However, the suitability of Collagenase/Dispase® for preparation of a particular cell type 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 reconstituted enzyme solution with PBS ($\text{Ca}^{2+}/\text{Mg}^{2+}$ -free) to a final concentration of 1 mg/ml and filter through a sterile 0.2 μm membrane filter.

Enzyme activity of the filtered solution as prepared above:

- Collagenase: ≥ 0.1 U/ml PBS
- Dispase[®]: ≥ 0.8 U/ml PBS

 Refer to the Certificate of Analysis for lot-specific data.

2.2. Protocols

Disaggregation of tissue

- 1 Make sure that tissue pieces are well covered with enzyme solution.
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- 2 Stir slowly for about 1 hour at +37°C.
 - If desired, decant off cells every 10, 15, or 20 minutes.
 - Cool cells to 0 to +5°C, wash, and continue as with other enzymes.
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 **Chelating reagents such as EDTA and EGTA inhibit both enzymes. However, serum does not affect enzyme activity.**

Subcultivation of cells

- 1 Pour off and discard medium.
 - Wash cell with PBS ($\text{Ca}^{2+}/\text{Mg}^{2+}$ -free), add enzyme solution, and incubate for 5 minutes at +37°C.
 - 2 Pour off the enzyme solution completely and incubate for another 10 minutes at +37°C, gently tapping on the side of the culture vessel to accelerate cell release.
 - 3 Resuspend cells in a small volume of PBS containing 10 μM Ca^{2+} .
 - 4 Dilute cell concentrate with media and subculture.
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2.3. Parameters

Activator

Ca²⁺

EC-Number

Collagenase: EC 3.4.24.3

Dispase[®]: EC 3.4.24.4

Inhibition

Collagenase (only): EDTA, EGTA, Cys

Collagenase/Dispase[®] is not inhibited by serum.

pH Optimum

pH 7.0 to 8.0

Specific Activity

Collagenase: >0.1 U/mg lyophilizate at +25°C; 4-phenyl-azobenzyl-oxycarbonyl-Pro-Leu-Gly-Pro-D-Arg as substrate (substrate according to Wunsch) under assay conditions (Wunsch E, Heidrich HG, 1963).

Dispase[®]: >0.8 U/mg lyophilizate (+37°C, casein as substrate, pH 7.5).

Unit Definition

1 U is the enzyme activity which liberates under assay conditions within 1 minute folin-positive amino acids and peptides corresponding to 1 µmol tyrosine.

3. Additional Information on this Product

3.1. Test Principle

How this product works

The extracellular matrix in animal tissues is a complex mixture of collagens and other extracellular matrix proteins, such as glycoproteins and proteoglycans. This matrix must be effectively broken down to isolate single cells, without alteration of cellular structures. Therefore, a combination of proteolytic enzymes is required for dissociating tissues. Crude collagenase from *C. histolyticum* is a mixture of collagenases and other proteolytic enzymes. Although collagenase is the most widely used enzyme preparation for this purpose, it has the disadvantage of having lot-to-lot variability with respect to its different proteolytic enzyme activities.

3.2. References

- Wunsch E, Heidrich HG. On the quantitative determination of collagenase. Hoppe Seylers Z Physiol Chem. 1963;333:149-151.

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.**

   etc. Stages in a process that usually occur in the order listed.

   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. Trademarks

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

4.4. License Disclaimer

For patent license limitations for individual products please refer to:

List of biochemical reagent products.

4.5. Regulatory Disclaimer

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

4.6. Safety Data Sheet

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

4.7. 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.

