Collagenase/Dispase®

From *Vibrio alginolyticus/Bacillus polymyxa*
Lyophilizate, nonsterile

Cat. No. 10 269 638 001 100 mg
Cat. No. 11 097 113 001 500 mg

Store at +2 to +8°C

1. What this Product Does

Contents
Lyophilizate, nonsterile.
Collagenase: *Vibrio alginolyticus* (formerly *Achromobacter iophagus*)
EC 3.4.24.3
Dispase®: *Bacillus polymyxa*, EC 3.4.24.4

Specific Activity
Collagenase: >0.1 U/mg lyophilizate (+25°C; 4-Phenylazobenzyl-oxy-carbonyl-Pro-Leu-Gly-Pro-D-Arg as substrate [1]).
Dispase®: >0.8 U/mg lyophilizate (+37°C; casein as substrate, pH 7.5.
One unit is the enzyme activity, which liberates under assay conditions within 1 minute folin-positive amino acids and peptides corresponding to 1 μmol tyrosine).

Inhibitors
EDTA, EGTA, Cys (collagenase only).
Collagenase/Dispase® is not inhibited by serum.

Activators
Ca²⁺

pH Optimum
7.0 – 8.0

Reconstitution
In water (to a final concentration of 100 mg/ml). Further dilution with PBS (phosphate buffered saline) (Ca²⁺/Mg²⁺-free).

Storage and Stability
Shipped at +15 to +25°C.
The lyophilizate is stable at +2 to +8°C when stored dry until the expiration date printed on the label. The reconstituted solution is stable at −15 to −25°C.
It is recommended to prepare appropriate aliquots and to avoid repeated freezing and thawing.

Application
The extracellular matrix in animal tissues is a complex mixture of collagens and other extracellular matrix proteins (glycoproteins, 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.

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 (2–19). However, the suitability of Collagenase/Dispase® for preparation of a particular cell type should be determined empirically.

2. How to Use this Product

Preparation of Working Solution
- Dissolve enzyme in water to a final concentration of 100 mg/ml.
- Dilute with PBS (Ca²⁺/Mg²⁺-free) to a final concentration of 1 mg/ml and filter through a sterile 0.22 μm membrane filter.

Disaggregation of Tissue
- Make sure that tissue pieces are well covered with enzyme solution.
- 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.

Subcultivation of Cells
- 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.
- Resuspend cells in a small volume of PBS containing 10 μM Ca²⁺.
- Dilute cell concentrate with media and subculture.
References

Symbols
In this document, the following symbol is used to highlight important information:

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<thead>
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<th>Symbol</th>
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<td>📜</td>
<td>Information Note: Additional information about the current topic or procedure.</td>
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Changes to Previous Version
- Updated information in sections “Reconstitution” and “Preparation of Working Solution”
- Editorial Changes

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