Collagenase NB 7D from Clostridium Histolyticum

C278996

Introduction

Collagenases from Clostridium histolyticum are proteolytic enzymes that cleave peptide bonds in the triple helical collagen molecule of human or animal tissue in situ. They are also referred as crude collagenases, because they consist of collagenase class I and class II, neutral proteases and clostripain in different ratios. The ratio of enzymatic activities can be altered enabling cell isolation from various tissue types.

Description

Collagenase NB 7D from Clostridium Histolyticum is a partly purified collagenase that has slightly higher collagenase activity while containing lower tryptic activities. The product is intended for research use only.

Specification

Collagenase activity	≥ 0.2 U/mg (PZ acc. to Wünsch)
Trypsin-like activities	≤ 0.2 U/mg (BAEE)
Ratio Collagenase : Trypsin	≥ 3.0

Application

Collagenase NB 7D from Clostridium Histolyticum is suitable for dissociation of a broad variety of tissue types especially in cases where sensitive conditions are beneficial.

Storage conditions

Collagenase NB 7D from Clostridium Histolyticum is provided as a lyophilized powder. It should be stored at 2 to 8 °C in a dry environment. Under these conditions the product is stable until the minimum shelf life stated on the certificate of analysis if repeated opening and closing of the vial is avoided.

Documents

For each lot a specific certificate of analysis is provided. A certificate of origin is available.

Instructions for use

Collagenase NB 7D from Clostridium Histolyticum is especially designed for isolation of sensitive cells (e.g. dendritic cells, diverse tumor cells) from human or animal tissues. The

product exhibits slightly higher collagenase activity and low tryptic activities preserving cell-membrane receptors and other cell-surface proteins.

Tissue dissociation

Recommended starting concentrations for selected applications:

Skin (mouse): 0.18 - 0.22 PZ U/ml

Tumor cells (mouse): 0.50 - 0.60 PZ U/ml

In general, the appropriate collagenase concentration depends on tissue type and origin as well as on the isolation procedure.

Collagenase activity is at an optimum at 37 °C and pH 7.4.

Stock solution

Collagenase NB 7D from Clostridium Histolyticum can be dissolved in most buffers, which are used for cell isolation. The enzyme solution must be con- stantly stored on ice.

Since collagenase and some of the secondary proteases depend on calcium, it is recommended to use a buffer with ≥ 2 mM Ca^{2+} .

Absolutely no calcium chelating agents (e.g. EDTA) should be present at all.

For 0.22 µm filtration filters with low protein-binding properties (e.g. cellulose acetate, PVDF, or PES) are recommended.

Working solution

To prepare a working solution, the stock solution is diluted with buffer to achieve the required collagenase concentration. The working solution must be constantly stored on ice until use.

Inactivation and inhibitors

The dissociation process can be reduced, e.g. by cooling down or dilution of the enzyme solution.

Collagenase is reversibly inactivated at high pH values and irreversibly inactivated at low pH values. Inhibitors of collagenase include cysteine or chelating agents like EDTA.

Important note

Collagenase NB 7D from Clostridium Histolyticum is intended for research use only.