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ProductInformation

Collagenase
from *Clostridium histolyticum*

Product Number **C2799**
Storage Temperature -20 ºC

Product Description

Enzyme Commission (EC) Number: 3.4.24.3
CAS Number: 9001-12-1

This collagenase is obtained from the culture filtrate of *Clostridium histolyticum*. The culture filtrate is thought to contain at least 7 different proteases ranging in molecular weight from 68-130 kDa.[1]

This is a highly purified collagenase preparation that has low amounts of clostripain and neutral proteases. Highly purified collagenase is generally not as efficient at dissociating tissues as crude collagnease. It has been tested with cell lines to verify the product is not cytotoxic.

Collagenase is typically used to digest the connective components in tissue samples to liberate individual cells. The concentration for cartilage dispersal is 1-2 mg/ml, but literature searches should be performed for species specific and/or tissue specific concentrations.[2,3]

Many references exist for using collagenase to digest various tissues. The choice of one technique over another is often arbitrary and based more on past experience than on an understanding of why the method works and what modifications could lead to better results. Concentrations typically vary from 0.1 to 5 mg/ml, and digestion time should be experimentally monitored using a very gentle agitation system to check for tissue dissociation. Collagenase treatment can cause some cells to die. Satisfactory efficiency of cell dissociation without causing too much cell death typically is achieved from 15 minutes to several hours, but can fall outside of this range if the concentration is unusual. The preferred buffer to use is Krebs Ringer Buffer with calcium and BSA. Zn^{2+} is required for activity, but it is tightly bound to the collagenase during purification. Additional Zn^{2+} should not be necessary as long as no chelator is added to the solution during digestion.

When this enzyme is tested for suitability for the release of hepatocytes, the collagenase is used at approximately 1 mg/ml in a total volume of 100 ml for each rat liver.

If excessive cell death is observed with concentrations used with previous lots, the new lot used might have a higher specific activity. Lowering the enzyme concentration and/or adding BSA or serum (0.5% and 5-10%, respectively) is recommended. These components are added to stabilize the cells to futher digestion by the enzyme.

Radiolabeled gelatin has been used to measure the activity and mechanism of collagenase digestion.[4]

Mandl units have the same description as Sigma collagen digestion units. The conversion factor for Mandl units/Wuensch units to Sigma units is approximately 1000-2000 to 1.

Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

Preparation Instructions

For measurement of enzymatic activity, an enzyme stock solution is prepared by dissolving 0.05 - 0.1 mg/ml collagenase in 50 mM TES buffer, pH 7.4 (37 ºC), containing 0.36 mM calcium chloride. Final concentrations in the reaction mixture are 50 mM TES, 0.36 mM calcium chloride, 25 mg collagen (Product No. C 9879), and 0.005-0.01 mg collagenase.

For tissue culture applications, collagenase can be solubilized in calcium-free solutions such as Hank's Balanced Salts (Product No. H 2387) or Earles Balanced Salt Solution (Product No. E 6267).

To sterile filter solutions of collagenase, first centrifuge the solution or filter through a 0.8 µm filter to remove insolubles. This will remove particulates and reduce the probability of clogging the 0.2 µm filter during sterile filtration.

Storage/Stability
Solutions at neutral pH and with adequate calcium ion concentration (0.3-0.5 mM) will retain activity for at least 5 hours at 37 ºC.

Solutions at -20 ºC are stable for several months.[3]

References

1. Angleton, E.L., et. al., Preparation and reconstitution with divalent metal ions of class I and class II *Clostridium histolyticum* apocollagenases. Biochemistry, **27**, 7406-7412 (1988).
2. Bassleer, C., et al., Human Chondrocytes in Tridimensional Culture, In Vitro Cell. Dev. Biol., **22**, 113-119 (1986).
3. Klagsbrun, M., Large-Scale Preparation of Chondrocytes, Methods in Enzymology, **58**, 560-564 (1979).
4. Mookhtiar, K. A., et al., Properties of Radiolabeled Type I, II, and III Collagens Related to their Use as Substrates in Collagenase assays, Anal. Biochem., **158**, 322-333 (1986).

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