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ProductInformation

Collagenase
from *Clostridium histolyticum*

Product Number **C7657**
Storage Temperature -0 °C

Product Description

CAS Number: 9001-12-1
Molecular Weight: This collagenase obtained from the culture filtrate of *Clostridium histolyticum*. The culture filtrate that is obtained is thought to contain at least 7 different proteases ranging in molecular weight from 68 -130 kDa.[1]

Many references exist for using collagenase to digest various tissue. The choice of one technique over another is mostly arbitrary and based more on past experience than on an understanding of why the method works and what modifications could lead to even better results. Concentrations typically vary from 0.1 mg/ml to 5 mg/ml, and digestion time is something that should be experimentally monitored using a very gentle agitation system to check for tissue dissociation. The concentration of collagense needed to isolate islets is dependent on the method used. A general recommendation is to start at a collagenase concentration of 2.5 mg/ml. Satisfactory dissociation versus viability 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 using concentrations tested in the past, the particular 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.

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.

Radiolabeled gelatin has been used to measure the activity and mechanism of collagenase digestion.[2]

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.

Most of the proteases in this preparation may be inhibited by a combination of benzamidine hydrochloride (Product No. B6506) and TLCK (Product No. T 7254) without affecting the activity of collagenase. However, there is no guarantee that all the proteases will be inhibited. For general applications, the working concentrations for benzamidine hydrochloride is about 1 mM and TLCK is 0.100-0.135 mM. If a collagenase with no significant protease activity is required, Product Number C0773 is recommended, since this product has been tested for any residual protease activity and found to contain less than 1 unit neutral protease.

Precautions and Disclaimer

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

Preparation Instructions

For in enzymatic activity measurement, an enzyme stock solution is prepared by dissolving 0.05 - 0.1 mg/ml collagenase in 50 mM TES buffer, pH 7.4, containing 0.36 mM calcium chloride at 37 °C. Final concentrations in the reaction mixture are 50 mM TES, 0.36 mM calcium chloride, 25 mg collagen (Product Number C9879), and 0.005-0.01 mg collagenase.

Storage/Stability

Solutions at neutral pH and with adequate calcium (0.3-0.5 mM) will retain activity for at least 5 hours at 37 °C.

Solutions at -20 °C 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(19)**, 7406-7412 (1988).
2. Mookhtiar, K.A., et al., Properties of radiolabeled type I, II, and III collagens related to their use as substrates in collegenase assays. Anal. Biochem., **158(2)**, 322-333 (1986).
3. 3. Schomberg, et al., Enzyme Handbook (Springer-Verlag, Berlin Heidelberg: 1991), p. 1-6.

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