



KAMIYA BIOMEDICAL COMPANY

Type 1 Collagenase Assay Kit

For the detection of collagenase 1 activity.

Cat. No. KT-701

For Research Use Only. Not for use in diagnostic procedures.

Product Information
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Introduction

Type 1 collagenase (MMP-1) is a member of the matrix metalloproteinases (MMPs) family which degrades extracellular matrix components. Our Type 1 Collagenase Assay Kit is optimized to detect collagenase activity using a fluorescent-labeled collagen as a substrate. Collagenase activity can be detected in arthritic synovia.

Storage

Store kit components at 4 °C

Components

Fluorescent-labeled collagen	12 ml
Buffer A	100 ml
Buffer B	125 ml

1 kit can assay 240 reactions using a fluorometer or 480 reactions using a fluorescent microplate reader

Materials required but not provided

- Pipettes
- Incubator set at 35 °C
- Fluorometer or fluorescent microplate reader
- Tubes
- Refrigerated centrifuge
- Distilled water

Reagent Preparation

1. Substrate Solution

a. Mix equal volumes of fluorescent-labeled collagen and Buffer A. Keep on ice until needed.

2. Sample Diluent

a. Dilute Buffer A with an equal volume of distilled water. Keep on ice until needed.

Protocol

Procedure for use with microtubes. For microtiter plate wells, use half the amount of reagents for each well.

1. Add 100 µl of Substrate Solution into each microtube. There should be enough tubes for samples, blanks, and Substrate Solution.
2. Add 100 µl of sample into microtube and mix thoroughly. For Blank and Substrate Solution, add 100 µl of Sample Diluent. If high collagenase activity is expected, dilute samples with Sample Diluent.
3. Incubate the tubes for samples and blank at 35 °C for 1-3 hours. Incubate the Substrate Solution tube at 80 °C for 3 minutes.
4. Cool Buffer B on ice, add 600 µl into all the tubes, and mix thoroughly. Keep on ice for 15 minutes.
5. Centrifuge at 10000 rpm for 10 minutes at 4 °C.
6. Save supernatant and measure fluorescence. Ex: 495nm, Em: 520nm

Calculation of collagenase activity

$$\text{Collagenase activity (units/ml)} = [(Fs - Fb) / (Ft - Fb)] \times [50 \text{ ug} / \text{Time}] \times [1 / 0.1]$$

Fs: Fluorescence of samples

Fb: Fluorescence of blank

Ft: Fluorescence of Substrate Solution

Time: Incubation time (minutes)

* Substrate Solution contains 0.5 mg/ml of collagen

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