



# Type I collagenase assay kit

(Code No. PMC-AK37-COS)

\*For Research Use Only.

## Introduction

Type I collagenase has an important role on the collagen metabolism and cleaves Type I collagen, which one of the collagen family comprising 9 members, yielding 1/4 and 3/4 collagen fragments. Type I collagenase Assay Kit (PMC-AK37-COS) is designed to quantify Type I collagenase activity using a fluorescent-labeled collagen as a substrate. This kit provides a convenient system for studying, for example, arthritic synovial in which Type I collagenase activity can be detected.

## Components

Component	Quantity	Storage
Fluorescent-labeled collagen	6 mL ×2	4-10°C
Buffer A	100 mL×1	4-10°C
Buffer B	150 mL×1	4-10°C

One kit contains reagents for 240 samples using a fluorometer or 480 samples using a fluorescent microplate reader.

## Additional Materials Required

- distilled water
- Fluorometer or fluorescent microplate reader

## Prepare reagents

- Substrate Solution

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

**Note:** This working solution should not be stored. Ready to use.

- Sample Diluent

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

## Protocol <Fluorometer>

Prepare tubes for samples, blanks, and Substrate Solution.

1. Add 100  $\mu$ L of Substrate Solution into each tube.
2. Add 100  $\mu$ L of sample into each tube and mix thoroughly. For Total Reaction Substrate Solution and Blank, add 100  $\mu$ L of Sample Diluent into each tube. In the case that collagenase activity will be high, the samples must be diluted with Sample Diluent.



3. Incubate sample and blank tube at 35°C for 1-3 hours and Total Reaction Substrate Solution tube at 80°C for 3 minutes.
4. Add 600 uL of cool Buffer B into each tube and mix thoroughly. Keep on ice for 15 minutes, and then centrifuge at 10,000 rpm for 10 minutes at 4°C.
5. Collect the supernatant and examine the sample by fluorometer with an excitation filter of 4985 nm and an emission filter of 520 nm.

**Note:** For microtiter plate wells, use half the amount of reagents for each well.

## ACTIVITY VALUE

One unit of collagenase activity is defined as the cleavage of 1 ug of collagen per minute. Since 0.5mg/ml of collagen is used as a substrate in this assay kit, collagenase activity is calculated by the following equation:

(Substrate Solution; 100 uL, Samples; 100 uL)

Collagenase activity (units/ml) =

$$\frac{(\text{Fluorescence of samples} - \text{Fluorescence of blank})}{(\text{Fluorescence of Substrate Solution} - \text{Fluorescence of blank})} \times \frac{50 \text{ ug}}{\text{Reaction time}} \times \frac{1}{0.1 \text{ mL}}$$

### Notice to Purchaser

This product is to be used for Research Purposes Only. It is not to be used for Drug or Diagnostic Purposes, nor is it intended for Human Use. Primary cell products may not be resold, modified for resale, or used to manufacture commercial products without the express written consent of Primary Cell Co., Ltd.

Except as otherwise expressly set forth in this use manual, Primary Cell does not make any representation or warranties or conditions of any kind, either express or implied, with respect to the products, or information disclosed hereunder, including, but not limited to, the implied warranties of merchantability, fit for a particular purpose, or noninfringement of the intellectual property rights of third parties.



COSMO BIO CO., LTD.  
Inspiration for Life Science

TOYO EKIMAE BLDG. 2-20, TOYO 2-CHOME, KOTO-KU, TOKYO 135-0016 JAPAN  
TEL: (81)3-5632-9617 / FAX: (81)3-5632-9618 / e-mail: export@cosmobio.co.jp / URL:www.cosmobio.com