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Product Information

Collagenase Activity Colorimetric Assay Kit

Catalog Number **MAK293**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Collagenase (EC 3.4.24.3) is an enzyme in the matrix metalloproteinase family that breaks down collagen, assisting in degradation of the extracellular matrix, a key step in the pathogenesis of bacteria. Collagen is an abundant structural protein present in the connective tissue of animals. Collagenase has been used for the treatment of Dupuytren's contracture, an affliction characterized by a thickening of connective tissue.

This Collagenase Activity Assay Kit provides a quick and easy way to determine activity of collagenase. The kit measures collagenase activity using a synthetic peptide (FALGPA) that mimics collagen's structure. It is suitable for measuring activity of bacterial collagenases such as from *Clostridium histolyticum*. In addition, it can also be used to screen/characterize collagenase inhibitors. The limit of detection for this assay is 0.02 mU of collagenase.

Components

The kit is sufficient for 100 assays in 96 well plates.

Collagenase Assay Buffer Catalog Number MAK293A	20 mL
Collagenase (0.35 U/mL) Catalog Number MAK293B	1 mL
Collagenase Substrate (FALGPA) Catalog Number MAK293C	4 mL
Inhibitor (1,10-Phenanthroline, 1 M) Catalog Number MAK293D	50 µL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for this assay.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Collagenase Assay Buffer - Bring to room temperature before use. Store at –20 °C or 4 °C.

Collagenase, Collagenase Substrate (FALGPA), and Inhibitor - Ready to use as supplied. Aliquot and store at –20 °C. Keep on ice during use. Use within 2 months.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate. Use ultrapure water for the preparation of samples.

Sample Preparation

Dissolve collagenase samples in cold water or Hank's Balanced Salt Solution (HBSS). Bacterial extract can be prepared in cold PBS. Suggested range of collagenase for assay is 0.02–10 mU.

Table 1.
Preparation of Sample and Controls

Reagent	Samples	Positive Control	Inhibitor Control
Test Collagenase or Bacterial Extract	2–10 µL	–	–
Kit Collagenase	–	10 µL	10 µL
Inhibitor (1 M, 1,10-Phenanthroline)	–	–	2 µL
Collagenase Assay Buffer	90–98 µL	90 µL	88 µL
Total Volume	100 µL	100 µL	100 µL

Add 2–10 µL of collagenase sample or bacterial extract into desired well(s) in 96 well plate and adjust the volume to 100 µL with Collagenase Assay Buffer. For positive control, add 10 µL of provided Collagenase (0.35 U/ml). For Inhibitor Control, add 10 µL of provided Collagenase (0.35 U/ml) and 2 µL of Inhibitor (1,10-Phenanthroline) into desired well(s). Adjust the volume of positive control and inhibitor control wells to 100 µL with Collagenase Assay Buffer. For reagent background control, add 100 µL of Collagenase Assay Buffer.

Notes: For unknown collagenase activity, testing several amounts of collagenase or bacterial extract to ensure the activity is within the assay range is suggested.

High activity samples will consume substrate within 3 minutes. Dilute enzyme and re-measure if necessary.

Inhibitor Screening (optional)
To test collagenase inhibitors, dissolve test inhibitor to 100× final test concentration in an appropriate solvent. Add 2 µL of test inhibitor and 10 µL of provided Collagenase (0.35 U/mL) into Test Inhibitor well(s). Prepare a parallel well as Enzyme Control (EC) by adding 10 µL of provided Collagenase. Adjust the volume of Test Inhibitor and Enzyme Control wells to 100 µL with Collagenase Assay Buffer. Incubate at room temperature for 10 minutes.

Note: Prepare a solvent control well by adding the same volume of solvent as the test inhibitor to test the solvent effect on collagenase activity. Provided Inhibitor (1,10-Phenanthroline) does not require a solvent control.

Assay Reaction
1. Prepare enough Reaction Mix for the number of wells (test collagenase, positive control, inhibitor control, reagent background, test inhibitor, and enzyme control) to be analyzed. For each reaction, prepare 100 µL of Reaction Mix, containing 40 µL of Collagenase Substrate (FALGPA) and 60 µL of Collagenase Assay Buffer.

   Mix and add 100 µL of the Reaction Mix into each well. Mix well.

2. Immediately measure in kinetic mode, the absorbance (A) at 345 nm in a microplate reader at 37 °C for 5–15 minutes. Low activity samples can be measured for 1–3 hours.

Results
Calculations
Take the absorbance (A_1 and A_2) at two time points (T_1 and T_2) in the linear range. There should be at least two readings in between and at least 1 minute apart.

To determine collagenase activity (U/mL), use the following equation:

$$\frac{\left(\frac{-\Delta A_{345\ \text{Test}}}{\Delta T} - \frac{-\Delta A_{345\ \text{Reagent Background}}}{\Delta T}\right) \times (0.2) \times DF}{(0.53) \times V}$$

ΔA_{345} = Difference between A_2 and A_1
ΔT = Difference between T_2 and T_1
0.2 = Reaction volume (mL)
DF = Dilution Factor
0.53 = millimolar extinction coefficient of FALGPA
V = Enzyme volume (mL)

For inhibitor screen, calculate percent inhibition using the following equation:

$$\%\ \text{Inhibition} = \frac{\text{Activity}_{\text{(Enzyme)}} - \text{Activity}_{\text{(Inhibitor)}}}{\text{Activity}_{\text{(Enzyme)}}} \times 100$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	It is recommended to use clear plates for this assay.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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