Collagenase Activity Colorimetric Assay Kit (#BN01008)

(Catalog # BN01008; 100 assays; Store at -20°C)

I. Introduction:

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 clinically for the treatment of Dupuytren's contracture, an affliction characterized by a thickening of connective tissue. Assay Genie's 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* type I-XI. In addition, it can also be used to screen/characterize collagenase inhibitors. The limit of detection for this assay is 0.02 mU Collagenase.

II. Application:

- Measurement of collagenase activity
- For screening/studying/characterizing collagenase inhibitors

III. Sample Type:

Purified Collagenase, bacterial extract

IV. Kit Contents:

Components	BN01008	Cap Code	Part Number
Collagenase Assay Buffer	20 ml	WM	BN01008-1
Collagenase (0.35 U/ml)	1 ml	Red	BN01008-2
Collagenase Substrate (FALGPA)	4 ml	NM	BN01008-3
Inhibitor (1,10-Phenanthroline) [1 M]	50 µl	Yellow	BN01008-4

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Store kit at –20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **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. Avoid repeated freeze/thaw. Keep on ice during use. Stable for two months.

VII. Collagenase Assay Protocol:

1. **Sample Preparation:** Dissolve test collagenase in cold dH_2O or Hank's Balanced Salt Solution (HBSS). Bacterial extract can be prepared in cold PBS. Suggested range of collagenase for measuring is 0.02-10 mU. Add 2-10 µl of test collagenase 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:

   a) For unknown collagenase activity, we suggest testing several amounts of collagenase or bacterial extract to ensure the activity is within the assay range.

   b) High activity samples will consume substrate within 3 min. Dilute enzyme and remeasure if necessary.

2. **Inhibitor Screening (optional):** To test Collagenase inhibitors, dissolve test inhibitor to 100X 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 min.

   Note:

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

3. **Reaction Mix:** 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 reaction mix:

Collagenase Substrate (FALGPA)	40 µl
Collagenase Assay Buffer	60 µl

   Mix and add 100 µl of the reaction mix into each well, mix well.

4. **Measurement:** Immediately measure absorbance kinetically at OD 345 nm in a microplate reader at 37°C for 5-15 min. Low activity samples can be measured for 1-3 hrs.

5. **Calculation:** Take the absorbance (A_{345nm1} and A_{345nm2}) 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 min. apart.

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To determine Activity, use the following equation:

$$\textbf{Sample Collagenase Activity} = \frac{\left(\frac{-\Delta A_{345nm}}{\Delta T}\text{Test} - \frac{-\Delta A_{345nm}}{\Delta T}\text{Reagent Background}\right)\text{X } (0.2) \text{ X DF}}{(0.53) \text{ X V}} \text{ U/ml}$$

Where: ΔA_{345nm} = Difference between A_{345nm2} and A_{345nm1}
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:

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

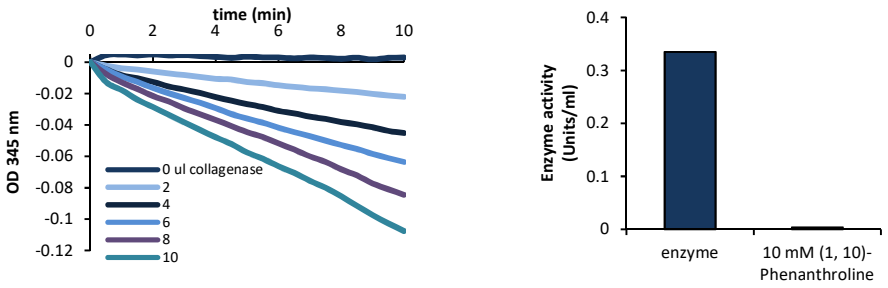

Figure: (a) Collagenase activity over 10 min. (b) Enzyme activity of collagenase and inhibition by 10 mM (1,10)-Phenanthroline. Assay was performed following the kit protocol.

MMP-17 Antibody (3537)

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