Rev. 0

ScienCell™
Research Laboratories

Collagenase Activity Assay (CAA)

Catalog #8818
100 Tests in 96-well plate

Product Description

Collagenase is an enzyme in the matrix metalloproteinase family that breaks down collagen. This assists in the degradation of the extracellular matrix and tissue remodeling, which is a key step in the pathogenesis of bacteria. In the ScienCell Collagenase Activity Assay, collagenase activity is measured using a synthetic peptide substrate, FALGPA, which mimics the structure of collagen. This assay is suitable for bacterial collagenases, such as those from Clostridium histolyticum, and for screening collagenase inhibitors. The peptide is cleaved by collagenase and the resulting activity can be quantified using a microplate reader at an absorbance of 345 nm in kinetic mode. The kit offers a detection limit of 0.02 mU and provides results within 5–15 minutes.

Kit Components

Cat#	# of vials	Reagent	Quantity	Storage
8818a	1	Assay buffer	10 mL	-20°C
8818b	1	Collagenase Positive Control	1 mL	-20°C
8818c	1	Substrate (FALGPA)	5 mg	-20°C
8818d	1	Reconstitution Solution	150 µL	4°C
8818e	1	Inhibitor (1,10-Phenanthroline)	50 µL	-20°C

Material Supplied by User

Microplate reader (measuring absorbance at OD 345 nm)
96-well plate
DI water

Product Use

Collagenase Activity Assay (CAA) can be used for rapid and sensitive measurement of collagenase activity in bacterial extracts or purifies collagenase. This kit can be used for screening collagenase inhibitor compounds as well. CAA is for research use only and is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Quality Control

CAA is applied to test collagenase activity in positive control, over a 10 minute-reading period (Figure 1). The suggested range for collagenase testing is 0.02-10 mU.

Shipping

All components are shipped on dry ice.

Procedure (96-well plate)

A. Reagent preparations

1. Briefly centrifuge tubes before opening.
2. Assay Buffer: Equilibrate the assay buffer (cat #8818a) to room temperature before use.
3. Collagenase: Aliquot the collagenase Positive Control (cat # 8818b) and avoid repeated freeze-thaw. Store at -20°C. Keep on ice while in use.
4. Substrate (FALGPA): Add 105 µL of Reconstitution Solution (cat # 8818c) in the substrate tube to make 100X substrate mix**.** Aliquot the substrate and avoid repeated freeze-thaw. Store at -20°C.

B. Preparation of test samples

1. Purified Collagenase: Reconstitute the test collagenase using cold DI water.
2. Bacterial lysate preparation: Lyse the bacterial cells in cold PBS and centrifuge the lysates at 13,000 x g for 5 minutes at 4°C. Collect the supernatant, transfer it to a new tube, and keep it on ice.
3. Inhibitor Screening compounds: Reconstitute the inhibitors into appropriate solvent to make 100x solution.

C. Plate setup:

1. For test samples, add 10 µL of test samples to each well.
2. For the Positive Control, add 10 µL of collagenase positive control.
3. For the Inhibitor Control, add 10 µL of collagenase positive control and 2 µL of inhibitor.
4. For Inhibitor Screening, add 10 µL of collagenase positive control and 2 µL of test inhibitor.
5. For Solvent Control, add 10 µL of collagenase positive control and 2 µL of solvent.

Note: We recommend to run the assay at least in duplicate.

D. Working reagent preparation and measurements

1. Calculate the total number of wells needed. Prepare 90 µL of working reagent for each well.
2. Prepare the working reagent by mixing 1 µL of substrate with 99 µL of assay buffer.
3. Add 90 µL of the working reagent to each well, including test samples, positive controls, inhibitor controls, and blanks.
4. Gently tap the plate to mix the contents, then immediately measure the absorbance at OD345 using a microplate reader in kinetic mode for 5–15 minutes at 37°C.

   Note: Low-activity samples can be measured for 1–3 hours, while high-activity samples may consume the substrate within 3 minutes. Dilute the enzyme if needed. We recommend measuring absorbance in kinetic mode and selecting two time points (T1 and T2) to calculate collagenase activity.

E. Calculations

1. For all reaction wells, including background controls, select two time points (T1 and T2) within the linear phase of the reaction progress curve and record the corresponding OD values (OD1 and OD2).
   Note: Ensure there are at least two readings between T1 and T2, and the time points are more than 1 minute apart.
2. Calculate the change in OD for the sample using the formula:

$$\Delta OD345nm = OD1 - OD2$$

3. Correct the ΔOD for each sample by subtracting the ΔOD value of the background control well.

4. Use the corrected ΔOD (ΔODc) values to calculate collagenase activity (U/mL) in the test samples, positive control and inhibitor control is calculated as:

$$Collagenase\ activity = \frac{(\frac{\Delta ODc}{\Delta T}) \times 0.1 \times D}{0.53 \times V}$$

ΔODc = Difference in OD reading between two time points, corrected for background.
ΔT = Time difference between two readings during the linear phase of the reaction (in minutes).
0.1 = Reaction volume in mL.
D = Sample dilution factor.
0.53 = Millimolar extinction coefficient of substrate FALGPA.
V = Sample volume added to the reaction (10 µL or 0.01 mL).

Collagenase activity can also be expressed as U/mg of total protein in the sample.

5. For inhibitor screening, calculate percentage of inhibition using the following calculation:

$$Inhibition\% = \frac{Activity_{Enzyme} - Activity_{Inhibitor}}{Activity_{Enzyme}} \times 100$$

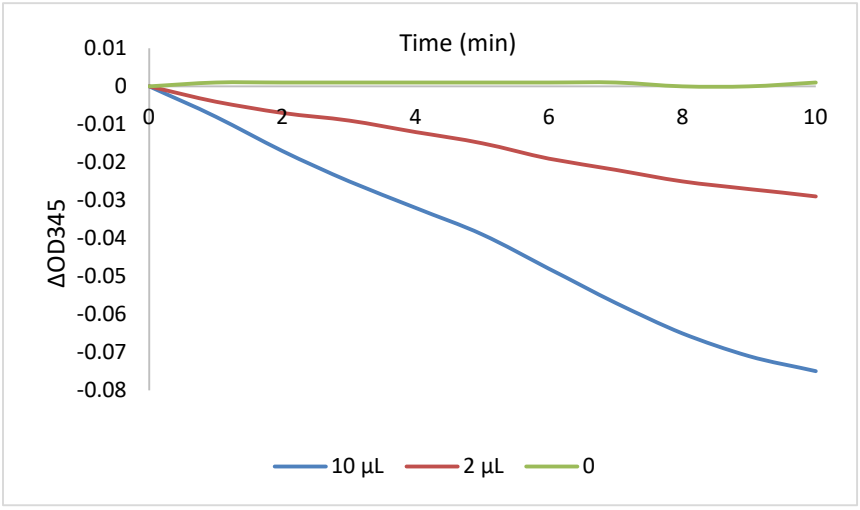

Figure 1. Typical collagenase activity from collagenase positive control, obtained over a 10 minute-reading period.

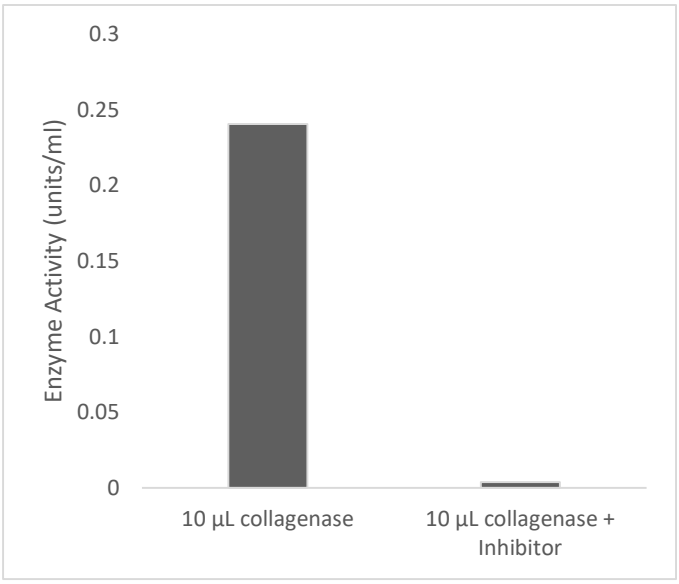

Figure 2. Example of enzyme activity of provided collagenase positive control and inhibitor.