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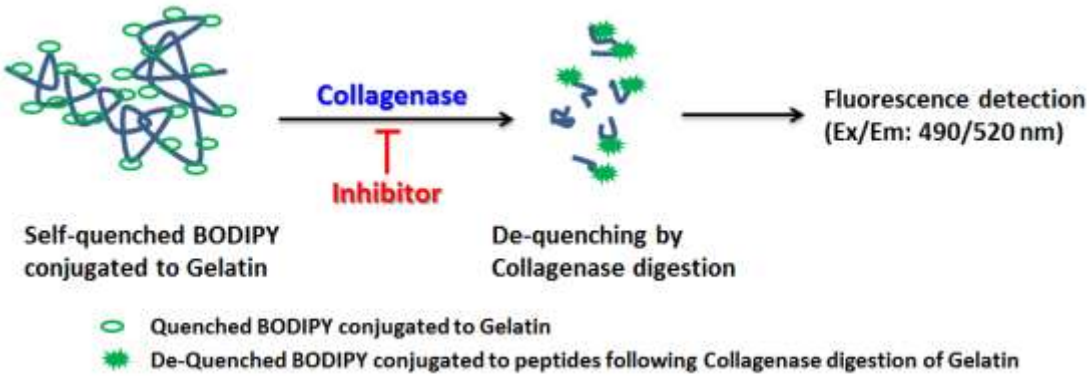
Collagenase Inhibitor Screening Kit (Fluorometric)

(Catalog # K833-100; 100 assays; Store at -20°C)

I. Introduction:

Collagenase (EC 3.4.24.3) is an enzyme in the matrix metalloproteinase (MMP) family that breaks down collagen, assisting in degradation of the extracellular matrix, a key step in the pathogenesis of bacteria and tumor cell invasion. 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. BioVision's Collagenase Inhibitor Screening Kit provides a quick and sensitive way for screening, studying and characterizing potential inhibitors of Collagenase. The kit uses Self-Quenched BODIPY conjugate of Gelatin (Type B) as a fluorogenic substrate to monitor the activity of Collagenase. Gelatin consists of a heterogeneous mixture of proteins of high average molecular weight derived from collagen Upon proteolytic digestion of the highly quenched BODIPY-labeled gelatin by Collagenase, the de-quenched BODIPY yields bright green fluorescence that can be assayed using a fluorometer or a fluorescence microplate reader (Ex/Em: 490 nm / 520 nm, 515 nm cutoff). In the presence of a Collagenase inhibitor, the gelatin is not digested, the dequenching of BODIPY does not occur and the fluorescent signal is not produced.



II. Application:

- Screening/studying/characterizing potential Collagenase inhibitors

III. Kit Contents:

Components	K833-100	Cap Code	Part Number
Collagenase Assay Buffer	25 ml	WM	K833-100-1
Collagenase Substrate	1 vial	Orange	K833-100-2
Collagenase	30 µl	Red	K833-100-3
Inhibitor (1,10)-Phenanthroline (400 mM)	50 µl	Yellow	K833-100-4

IV. User Supplied Reagents and Equipment:

- 96-well white plate with a flat bottom.
- Multi-well spectrophotometer (Fluorescence plate reader).

V. Storage Conditions and Reagent Preparation:

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

- **Collagenase Assay Buffer**: Bring to room temperature before use. Store at -20°C or 4°C.
- **Collagenase Substrate**: Reconstitute with 210 µl Assay Buffer. Incubate the vial at 37°C for 10 min. Tap gently to mix and ensure that it is completely dissolved. Store the reconstituted substrate at -20°C.
- **Collagenase:** Aliquot and store at -20°C. Avoid repeated freeze/thaw.
- **Inhibitor (1,10)-Phenanthroline (400 mM):** Aliquot and store at -20°C. To make 100x working solution, dilute 2 µl of 1,10-Phenanthroline stock solution with 8 µl ethanol.

VI. Collagenase Inhibitor Screening Protocol:

1. **Collagenase Control (EC):** Dilute the provided collagenase 1:50 with the Collagenase Assay Buffer. Prepare the enzyme Control (EC) by mixing 5 µl of the diluted Collagenase with 45 µl of Collagenase Assay Buffer.
2. **Background Control (BC):** Prepare a Background Control (BC) with 50 µl of Collagenase Assay Buffer alone.
3. **Screening Compound (S) and Inhibitor Control (IC) preparation:** Dissolve candidate inhibitors into an appropriate solvent to make a 100X stock solution. Prepare Inhibitor Samples (S) by mixing 1 µl candidate Inhibitor stock with 5 µl diluted Collagenase and 44 µl Collagenase Assay Buffer. Prepare an Inhibitor Reference Control (IC) by mixing 1 µl (1,10)-Phenanthroline with 5 µl diluted Collagenase and 44 µl Collagenase Assay Buffer.

Note: Solvents used to solubilize the inhibitors might affect the enzymatic activity. If solvent effect on the enzymatic activity is a concern, prepare a solvent control well(s) (SC) with the same final concentration of the solvent(s) as in the inhibitor sample(s) containing 5 µl of the diluted Collagenase as in the enzyme control.

4. **Reaction Mix Preparation:** Prepare 50 µl Reaction Mix for each well to be analyzed :

Collagenase Assay Buffer	48 µl
Collagenase Substrate	2 µl

Add 50 µl of the Reaction Mix into all the wells. Mix well.

5. **Measurement:** Measure the fluorescent signal Ex/Em = 490/520 nm, 515 nm cutoff) in a kinetic mode at 37°C for 30-60 min.
6. **Calculation:** Choose two time points T_1 & T_2, (at least 10 min. apart) in the linear range of enzyme kinetics and obtain the corresponding ΔRFU (RFU2 –RFU1) during the reaction time ΔT (T2 - T1) for Enzyme Control ($\Delta RFU_{(EC)}$), and Test Inhibitor ($\Delta RFU_{(Test\ Inhibitor)}$). Use these values to obtain the percentage of inhibition.

$$\textbf{\% Relative Inhibition} = \frac{\Delta RFU_{(EC)} - \Delta RFU_{(Test\ Inhibitor)}}{\Delta RFU_{(EC)}} \textbf{ X 100}$$

Notes:

- Subtract Background Control (BC) reading from the Enzyme Control (EC) and Inhibitor (S).
- If Solvent control (SC) values are significantly different from the EC, use these values in the equation above.
- Irreversible inhibitors that inhibit the collagenase activity completely at the tested concentration will have ΔRFU = 0 and thus the % Inhibition will be 100%.

Figure 1

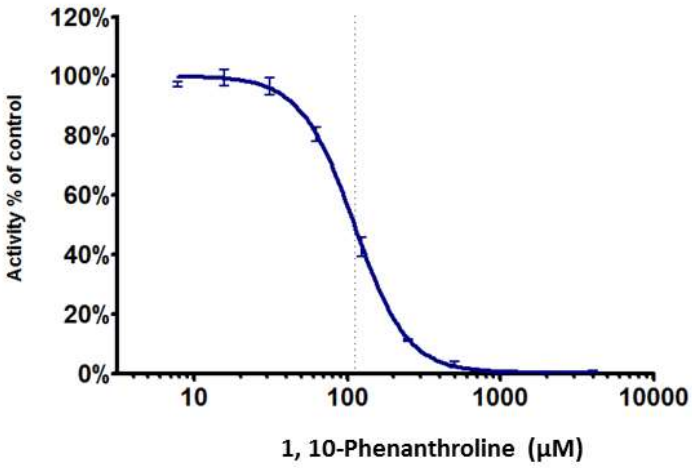


Figure: Inhibition of Collagenase activity by (1,10)-Phenanthroline (IC_{50} 110.5 µM), a Collagenase inhibitor. Assay performed following kit protocol.

VII. RELATED PRODUCTS:

Collagenase Activity Colorimetric Assay kit (K792)
Collagen-I, human recombinant (4796)
Collagen-III, human recombinant (4797)
MMP-1 Antibody (5781)
MMP-9 Antibody (3529, 3969, 5565)
MMP-11 Antibody (3531R)
Self-Quenched BODIPY FL Conjugate of BSA (Green) (7932)
Self-Quenched BODIPY Gelatin (7935-30)
MMP-1 Inhibitor Screening Kit (K794)
MMP-8 (rat) ELISA Kit (K4743)
MMP-2 Antibody (5562)
MMP-9, human recombinant (7789)
MMP-17 Antibody (3537)

FOR RESEARCH USE ONLY! Not to be used on humans