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ab211108 Collagenase Inhibitor Screening Kit (Fluorometric)

For the rapid, sensitive and accurate screening of potential Collagenase inhibitors.

This product is for research use only and is not intended for diagnostic use.

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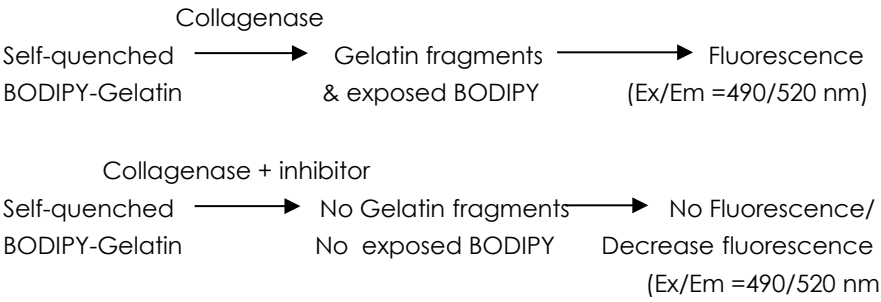
1. Overview

Collagenase Inhibitor Screening Kit (Fluorometric) (ab211108) provides a quick and sensitive way for screening, studying and characterizing potential inhibitors of Collagenase. The kit uses a self-quenched BODIPY conjugated to 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 and can be cleaved by collagenase. Upon proteolytic digestion by Collagenase, the de-quenched BODIPY yields bright green fluorescence that can be quantified using a fluorescence microplate reader (Ex/Em = 490/ 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 no fluorescent signal is produced.



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.

2. Protocol Summary

Screening compound & controls preparation

Enzyme and substrate solution preparation

Add enzyme solution to wells.
Incubate for 15 minutes at RT

Add Substrate Solution to wells

Measure fluorescence at Ex/Em = 490/520 nm in kinetic mode
for 30-60 minutes at 37°C

**For kinetic mode detection, incubation time given in this summary is for guidance only*

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer 38	25 mL	-20°C	-20°C
Collagenase Substrate (Gelatin)	1 vial	-20°C	-20°C
Active Collagenase	30 µL	-20°C	-20°C
Collagenase Inhibitor	50 µL	-20°C	-20°C

PLEASE NOTE: Collagenase Inhibitor was previously labelled as Inhibitor (1,10)-Phenanthroline (400 mM), and Active Collagenase as Collagenase, and Assay Buffer 38 as Assay Buffer XXXVIII and Collagenase Assay Buffer, and Collagenase Substrate (Gelatin) as Collagenase Substrate (40 µg). The composition has not changed.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring fluorescence at Ex/Em = 490/520 nm
- MilliQ water or other type of double distilled water (ddH_2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom, preferably white
- Ethanol

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer 38 (25 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C or -20°C.

9.2 Collagenase Substrate (Gelatin:

Reconstitute with 210 µl Assay Buffer. Incubate the vial at 37°C for 10 minutes. Tap gently to mix and ensure that it is completely dissolved. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.3 Active Collagenase (30 µL):

Ready to use as supplied. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.4 Collagenase Inhibitor:

Ready to use as supplied. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C.

Immediately prior to start the assay, make a 100X Working Solution by diluting 2 µL of Collagenase Inhibitor/Inhibitor stock solution in 8 µL ethanol.

10. Sample Preparation

General sample information:

– Always prepare a fresh set of samples and controls for every use.

10.1 Screening Compounds:

10.1.1 Dissolve test compounds into proper solvent to make a 100x stock solution.

Δ Note: We suggest using different concentrations of test compounds if effective concentration is unknown.

11. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.

Δ Note: preferred final solvent concentration should not be more than 5% by volume. If solvent exceeds 5%, include solvent control to test the effect of the solvent on enzyme activity.

11.1 Prepare collagenase:

11.1.1 Dilute provided Active Collagenase 1:50 in Assay Buffer 38. Prepare as much volume as needed.

11.2 Set up Reaction wells:

- Sample compound wells (S) = 1 µL test compound + 5 µL diluted Active Collagenase + 44 µL Assay Buffer 38.
- Inhibitor Control wells (IC) = 1 µL diluted Inhibitor + 5 µL diluted Active Collagenase + 44 µL Assay Buffer 38.
- Enzyme Control wells (EC) = 5 µL diluted Active Collagenase + 45 µL Assay Buffer 38.
- Background Control (BC): 50 µL Assay Buffer 38
- OPTIONAL: Solvent Control (SC) = 50 µL solvent.

11.3 Prepare Reaction Mix:

11.3.1 Prepare 50 µL of Reaction Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency:

Component	Collagenase Reaction Mix (µL)
Assay Buffer 38	48
Collagenase Substrate (Gelatin)	2

11.3.2 Add 50 µL of the Reaction Mix into each well. Mix well.

11.4 Measurement:

Measure immediately fluorescence at Ex/Em = 490/520 nm (515 nm cutoff) on a microplate reader in kinetic mode, for 30-60 minutes at 37°C protected from light.

12. Calculations

– Use only the linear rate for calculation.

12.1 Plot readings for each sample test compound (S), inhibitor control (IC) and enzyme control (EC).

12.2 Draw the line of the best fit to construct the curve (most plate reader software or Excel can do this step). Calculate the trend line equation (use the equation that provides the most accurate fit).

12.3 Average the duplicate reading for each sample test compound (S), inhibitor control (IC) and enzyme control (EC).

12.4 Subtract Background Control (BC) reading from the Enzyme Control (EC) and Inhibitor (S). If the data obtained from the solvent control(s) is significantly different from the EC use this data instead of EC data in the equation below.

$$\%\ Relative\ Inhibition = \frac{RFU(EC) - RFU(S)}{RFU(EC)} * 100$$

Δ Note: Irreversible inhibitors that inhibit NOS activity completely at the tested concentration will have RFU = 0 and thus % Relative Inhibition will be 100%.

Δ Note: If RFU of SC < RFU of EC = make a higher stock of test inhibitor, or dissolve the inhibitor in lower concentration of the solvent; or use a different solvent if possible.

If RFU of S < RFU of EC = treat as 100% inhibition and further dilute the test inhibitor and repeat the assay.

13. Typical Data

Data provided **for demonstration purposes only.**

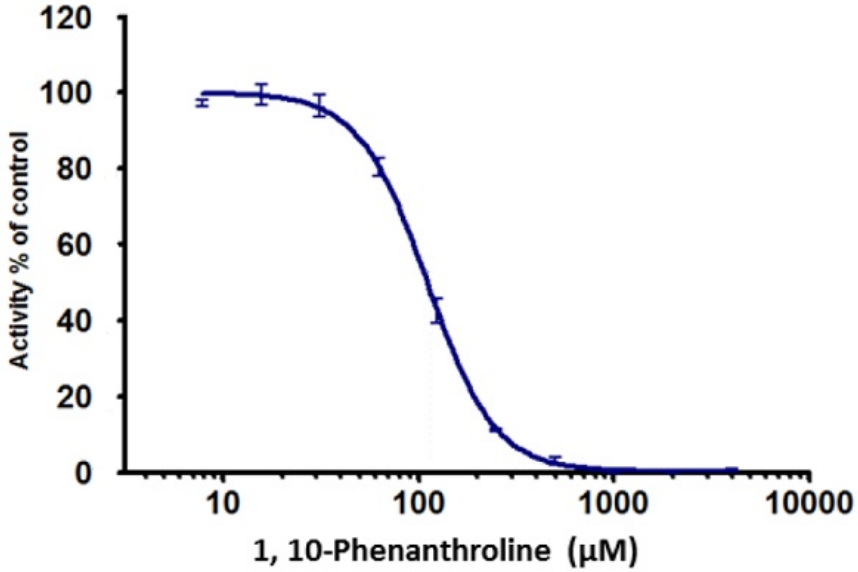


Figure 1. Typical inhibition curve of Collagenase activity by collagenase inhibitor. IC_{50} of (1,10)-Phenanthroline was determine to be 110.5 µM. Assay was performed following the kit protocol.

14. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare reagents and aliquot; get equipment ready.
- Prepare test compounds in suitable solvents; dilute if appropriate.
- Dilute Active Collagenase 1:50 in Assay Buffer 38.
- Prepare Reaction Mix (50 µL/well) by adding 2 µL of Collagenase Substrate (Gelatin) to 48 µL of Assay Buffer 38. Prepare a mix for all wells.
- Set up plate as follows:

Component	Sample (S) (µL)	Background Control (BC) (µL)	Enzyme Control (EC) (µL)	Inhibitor Control (IC) (µL)
Test Compound	1	0	0	0
Diluted Active Collagenase (1:50)	5	0	5	5
Assay Buffer	44	50	45	44
Diluted Inhibitor	0	0	0	1
Incubate 15 minutes at RT				
Add 50 µL Reaction Mix				

- Measure plate at Ex/Em= 490/520 nm in kinetic mode for 30 – 60 minutes at 37°C.

15. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Assay with erratic readings	Pipetting errors	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
No fluorescence above background in inhibitor wells	Inhibitor concentration is too high	Reduce concentration of inhibitor and re-do assay
No inhibition seen in test compound wells	Inhibitor concentration is not high enough	Increase concentration of inhibitor and re-do assay
	Compound is not an inhibitor	Use another compound for your test

16. Notes

Technical Support

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