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ab234624 Collagenase (Collagen Degradation/Zymography) Assay Kit (Fluorometric)

For the measurement of collagenase activity in biological samples.

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

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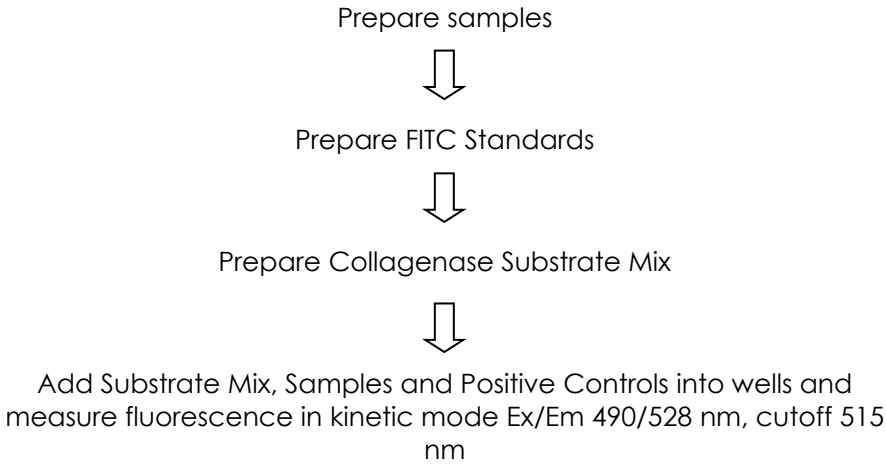
1. Overview

Collagenase (Collagen Degradation/Zymography) Assay Kit (Fluorometric) (ab234624) employs a highly quenched collagen substrate which upon cleavage by a suitable collagenase releases a fluorophore, which can be easily quantified using a conventional microplate reader. This method is substrate-specific, simple, fast, high-throughput adaptable and amenable to the sensitive detection of collagenase activity (as low as 0.6 mCDU for bacterial collagenase) in biological samples.



2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

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

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Lysis Buffer VI	25 mL	-20°C	-20°C
Assay Buffer 38	25 mL	-20°C	-20°C
Collagenase Substrate	1 vial	-20°C	-20°C
Enzyme Positive Control	100 µL	-20°C	-20°C
FITC Standard	10 µL	-20°C	-20°C

PLEASE NOTE: Assay Buffer 38 was previously labelled as Assay Buffer XXXVIII and Collagenase Assay Buffer, and FITC Standard as FITC Standard (5 mM), and Lysis Buffer VI as Cell Lysis Buffer. The composition has not changed.

3. 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 = 490/528 nm, cutoff 515 nm
- 96 well plate with black flat bottom
- Dounce homogenizer

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Assay Buffer 38

Ready to use as supplied. Bring to room temperature before use.

5.2 Lysis Buffer VI

Ready to use as supplied.

5.3 Enzyme Positive Control

Ready to use as supplied. Thaw on ice before use.

5.4 Collagenase Substrate

1. Reconstitute in 220 µL of deionized water.
2. Mix well by pipetting up and down. Vortex if necessary.
3. Unused substrate can be stored at -20°C by covering it with aluminum foil or transferring it to an amber vial.

5.5 FITC Standard

Ready to use as supplied.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Prepare a 50 µM FITC standard by diluting 2 µL of 5 mM FITC Standard with 198 µL of Assay Buffer 38. Mix well by pipetting up and down, vortex vigorously for 30 seconds.
2. Using the 50 µM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	FITC Standard (µL)	Assay Buffer 38 (µL)	Final volume standard in well (µL)	End amount of FITC standard in well (pmol/well)
1	0	200	100	0
2	4	196	100	100
3	8	192	100	200
4	12	188	100	300
5	16	184	100	400
6	20	180	100	500

Each dilution has enough standard to set up duplicate readings (2 x 100 µL).

3. Mix well and measure the fluorescence at Ex/Em 490/528 nm, cutoff 515 nm in end-point mode at 37 °C.

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware, however, that this might affect the stability of your samples and the readings can be lower than expected. Avoid multiple freeze-thaws.

7.1 Fresh/frozen tissue and cells:

1. Homogenize fresh or frozen tissue (~5-10 mg) or cells (1-2 x 10^6) with 100 µL Lysis Buffer VI and incubate on ice for 5 minutes.
2. Centrifuge the homogenate at 16,000 X *g*, 4°C for 10 minutes.
3. Transfer the clarified supernatant to a fresh pre-chilled tube and keep on ice.
4. Measure the amount of protein in the lysate or purified enzyme using BCA protein assay kit reducing agent compatible (microplate) (ab207003).
5. Add 1-50 µL of lysate or purified enzyme into desired well(s) in a black 96-well plate. If necessary, dilute the lysate with Assay Buffer 38.
6. For the Positive Control, dilute 2 µL of Enzyme Positive Control with 18 µL of Assay Buffer 38 and use 1-10 µL/well.
7. Adjust the volume of Samples and Positive Control to 50 µL/well with Assay Buffer 38.

Δ Note: *The kit is designed to work with active Collagenase enzymes only. If the sample contains inactive zymogen forms of collagenase, it can be activated with p-aminophenylmercuric acetate (APMA) or other activators. The conditions for activation of each enzyme should be determined empirically by following an appropriate testing protocol (Shapiro et. al., J. Bio. Chem. 1995, 270 (11), 6351-6356).*

Δ Note: *To induce higher collagenase expression, cells can be grown in the presence of Phorbol myristate acetate (10-50*

ng/ml), lysed and tested directly in the assay (Hersh et. al., Biochem., 1986, 25 (17), 4750-4757).

Δ Note: For unknown samples, we suggest doing pilot experiment and testing 3-5 different amounts of samples to ensure the readings are within the Standard Curve range.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate, apart from Positive Control, keep this on ice.
- Assay all standards, controls and samples in duplicate.

Δ Note: *If you suspect your samples contain substance that can generate background, set up Sample Background Controls to correct for background noise.*

8.1 Collagenase Substrate Mix:

1. Prepare 50 µL of Collagenase Substrate Mix per well. Prepare a master mix to ensure consistency.
2. Dissolve the Substrate Mix by vigorous vortexing.

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

3. Add 50 µL of Substrate Mix into each Sample and Positive Control well.
4. Mix well and measure the fluorescence at Ex/Em 490/528 nm, cutoff 515 nm in kinetic mode at 37 °C for 1-2 hours.

   Δ Note: *Do not add Substrate Mix to the sample Background Control and Standard wells.*

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Choose two time points (t1 and t2) where the corresponding RFUs (RFU1 and RFU2) are in a linear range.
2. Calculate ΔRFU and Δt and obtain ΔRFU/Δt as RFU/minute for each Sample including Background Control.
3. Subtract the value of RFU/minute of Background Control from each Sample to obtain net RFU/minute (B).
4. Subtract the RFU of the zero standard from the RFU of the other standards.
5. Plot the RFU against pmol of FITC standard. The plot should be linear. Determine the slope A (RFU/pmol) of the curve.
6. Using RFU/min of each Sample, calculate Sample Collagenase activity using following equation:

$$\text{Collagenase activity } (X, \frac{U}{ml}) = \frac{B \text{ x } 1000}{A \text{ x } C} * D$$

$$Collagenase\ activity\ \left(\frac{U}{mg}\right) = X/P$$

Where:

B = Sample Collagenase Activity as calculated (RFU/minute)

A = Slope of the FITC standard curve (ΔRFU/pmol)

C = µl of Sample used in the assay

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

P = Protein concentration in the lysate (mg/mL)

1000 = Conversion Factor (1000 µL ≡ 1 mL)

Unit Definition: 1 U is the amount of Collagenase required to cleave the Collagenase Substrate and release 1 pmol of Fluorescein per minute under the conditions of the assay.

10. Typical Data

Data provided **for demonstration purposes only.**

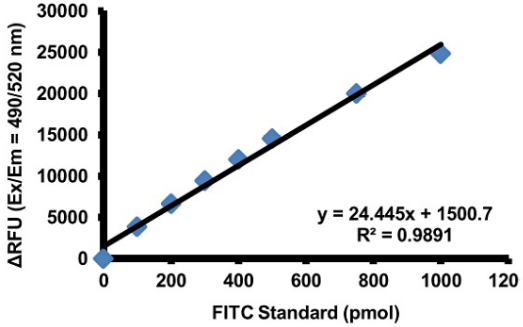


Figure 1. Example Standard Curve.

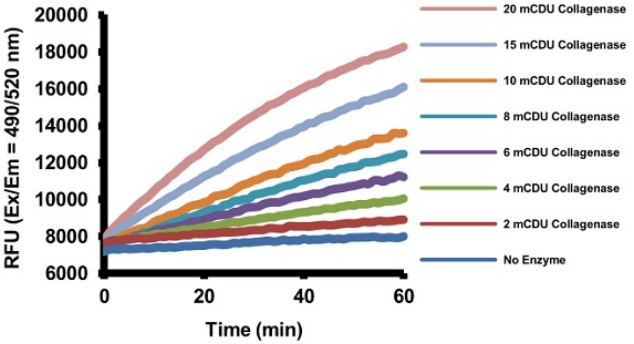


Figure 2. Collagenase activity with different amounts of Enzyme Positive Control.

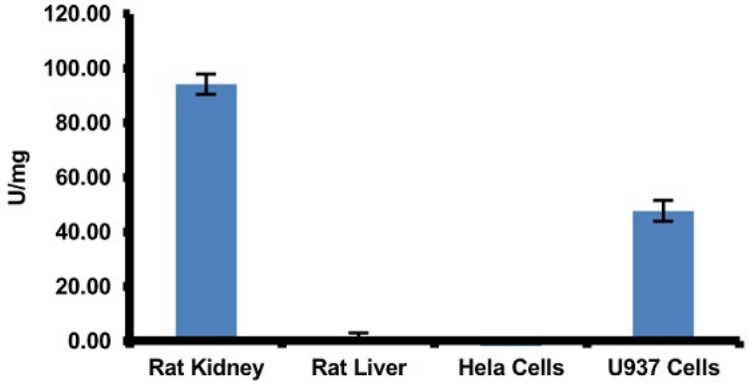


Figure 3. Collagenase activity in rat kidney, liver lysates along with Hela and U937 cell lysates. n=3.

11. Notes

Technical Support

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