Version 1a Last updated 27 April 2021

ab234057 Gelatin Degradation Assay Kit (Alternative to Zymography)

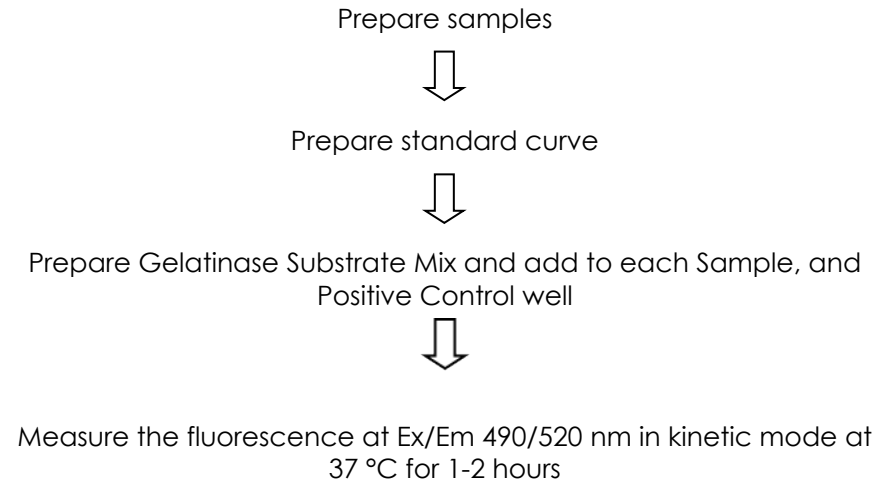
For the detection of gelatinase activity in biological samples such as tissue and cell lysates.

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

Table of Contents

1. Overview

Gelatin Degradation Assay Kit (Alternative to Zymography) (ab234057) utilizes a hybrid approach for the detection of gelatinase activity by employing a highly quenched gelatin substrate which upon cleavage by a suitable gelatinase 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 gelatinase activity (as low as 0.06 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)
Gelatinase Assay Buffer	25 mL	-20°C	-20°C
Cell Lysis Buffer	25 mL	-20°C	-20°C
Enzyme Positive Control	100 µL	-20°C	-20°C
Gelatinase Substrate	1 vial	-20°C	-20°C
FITC Standard (5 mM)	10 µL	20°C	20°C

3. Materials Required, Not Supplied

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

- Multi-well spectrofluorometer.
- 96-well Clear/Black/White well plate (The black plate will yield lowest background while white plate will yield highest background fluorescence).

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 Gelatinase Assay Buffer

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

5.2 Cell Lysis Buffer

Ready to use as supplied.

5.3 Enzyme Positive Control

Aliquot and store at -20°C. Thaw on ice before use. Avoid repeated freeze/thaw.

5.4 Gelatinase 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 (5 mM)

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 to 200 µL of Gelatinase Assay Buffer.
2. Mix well by pipetting up and down, vortex vigorously for 30 seconds.
3. Using the 50 µM FITC Standard, prepare standard curve dilutions as described in the table in a microplate or microcentrifuge tubes:

Standard #	FITC Standard (µL)	Assay Buffer (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).

7. Sample Preparation

General sample information:

We recommend that you use fresh samples for the most reproducible assay.

1. Homogenize fresh or frozen tissue (~5-10 mg) or cells (1-2 x 10^6) with 100 µL Cell Lysis Buffer 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) or equivalent.
5. Add 1-50 µL of lysate or purified enzyme into desired well(s) in a white 96-well plate. If necessary, dilute the lysate with Gelatinase Assay buffer.
6. Adjust the volume of Samples to 50 µL/well with Gelatinase Assay Buffer.
   Δ Note: *The kit is designed to work with active Gelatinase enzymes only. If the sample contains inactive zymogen forms of gelatinase, 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 appropriate testing protocol (Shapiro et. al., J. Bio. Chem. 1995, 270 (11), 6351-6356).*
   Δ Note: *We recommend using the tissue/cell homogenate immediately to measure the Gelatinase activity. If desired, snap freeze the lysate and store at -80°C.*
   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.*
   Δ Note: *To induce higher gelatinase expression, cells can also be grown in the presence of Phorbol myristate acetate (10-50 ng/ml), lysed and tested directly in the assay (Shin et. al., Exp. Mol. Med., 2003, 39 (1), 97-105).*

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 Positive Control:

1. Dilute 2 µL of Enzyme Positive Control with 18 µL of Gelatinase Assay Buffer and use 1-10 µL/well.
2. Adjust the volume to 50 µL/well with Gelatinase Assay Buffer.

8.2 Background Control (Optional):

1. For samples having background, prepare parallel sample well(s) as sample background control. Use the same amount of tissue/cell homogenate or purified enzyme as in the sample well.
2. Adjust the final volume to 100 µL with Gelatinase Assay Buffer.

8.3 Gelatinase Substrate Mix:

1. Prepare 50 µL of Gelatinase Substrate Mix per well as given below:

Component	Reaction Mix (µL)
Gelatinase Assay Buffer	48
Reconstituted Gelatinase Substrate	2

2. Dissolve the Substrate Mix by vigorous vortexing.
3. Add 50 µL of Substrate Mix solution into each Sample, and Positive Control well.
   Δ Note: *Do not add Substrate Mix to the sample Background Control and Standard wells.*

8.4 Measurement:

1. Mix well and measure the fluorescence at Ex/Em 490/520 nm in kinetic mode at 37 °C for 1-2 hours.
2. Choose two time points (t_1 and t_2) where the corresponding RFUs (RFU_1 and RFU_2) are in a linear range.
3. Calculate ΔRFU and Δt and obtain $\Delta RFU/\Delta t$ as RFU/min for each Sample including background control.
4. Subtract the value of RFU/min of background from each Sample to obtain net RFU/min (B).

9. Data Analysis

9.1 FITC Standard Curve:

1. Obtain change in the RFU (ΔRFU) by subtracting fluorescence of the 0 Standard Controls from those containing all standards.
2. Plot the ΔRFU against pmol of FITC Standard. The plot should be linear; determine the slope A (ΔRFU/pmol) of the curve.

9.2 Samples:

Using RFU/min of each Sample, calculate Sample Gelatinase activity using following equation.

$$\text{Sample Gelatinase Activity } (X\frac{U}{mL}) = \frac{B * 1000}{A * C} * D$$

$$\text{Sample Gelatinase Activity } (\frac{U}{mg}) = \frac{X}{P}$$

Where:

B = Sample Gelatinase Activity as calculated (RFU/min).

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 Gelatinase required to cleave the Gelatinase Substrate and release 1 pmol of Fluorescein per min under the conditions of the assay.

10. Typical Data

Data provided **for demonstration purposes only.**

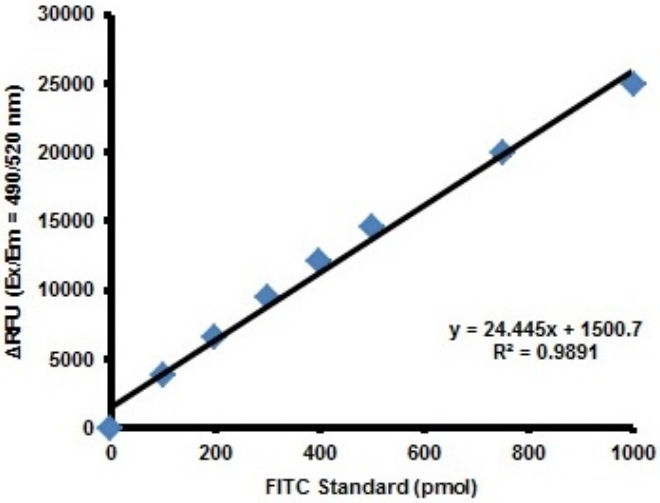


Figure 1. FITC Standard Curve.

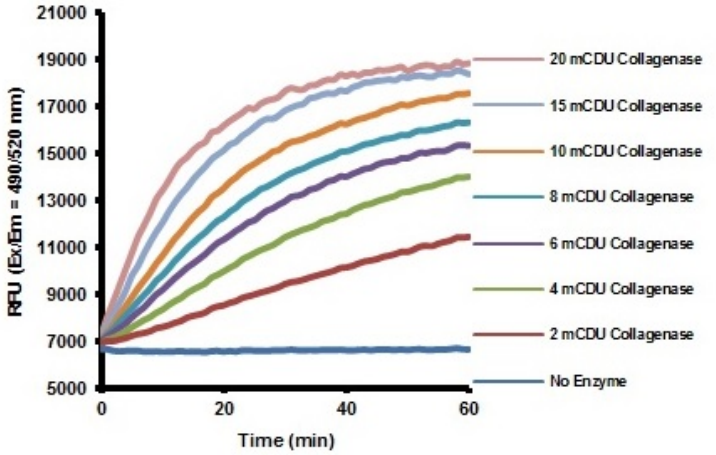


Figure 2. Gelatinase activity with different amounts of Enzyme Positive Control.

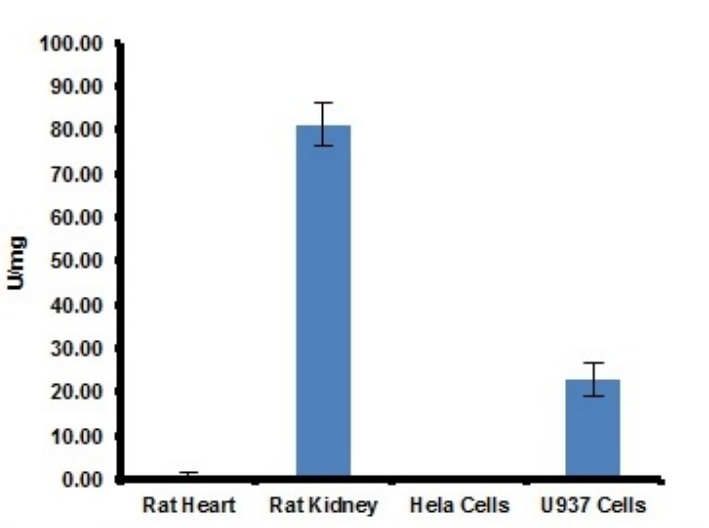


Figure 3. Gelatinase activity in rat heart and kidney lysates along with HeLa and U937 cell lysates.

11. Notes

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