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Product Information

Gelatinase (Gelatin Degradation/Zymography) Assay Kit

Catalog Number **MAK348**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Gelatinases are a type of matrix zinc-dependent metalloproteases (MMPs) that degrade gelatins and a variety of other extracellular matrix proteins. These enzymes are synthesized as latent zymogens that are activated by proteolysis and inhibited by tissue inhibitors of metalloproteases (TIMPs). Two mammalian gelatinases, Gelatinase A (MMP-2) and Gelatinase B (MMP-9), are critical for basement membrane degradation and are highly upregulated in variety of tumor cells.

Gelatinase activity is usually detected by small peptide-based activity assays which may suffer from lack of substrate specificity. Other methods for gelatinase activity include gelatin zymography where samples are electrophoresed on a gelatin-containing SDS-PAGE, and further renatured in a suitable buffer for 12–16 hours. The zymogram is subsequently stained, and areas of digestion appear as clear bands against a darkly stained background where the substrate has been degraded by the enzyme. Such methods are laborious, time-consuming, and may lead to the loss of enzymatic activity as renaturation may not be completely reversible.

The Gelatinase Activity Assay Kit 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.

Components

The kit is sufficient for 100 fluorometric assays in 96 well plates.

Gelatinase Assay Buffer Catalog Number MAK348A	25 mL
Cell Lysis Buffer Catalog Number MAK348B	25 mL
Enzyme Positive Control Catalog Number MAK348C	10 µL
Gelatinase Substrate Catalog Number MAK348D	1 vial
FITC Standard (5 mM) Catalog Number MAK348E	10 µL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Black flatbottom 96 well plates
- Fluorescence multiwell plate reader, capable of 37 °C temperature setting
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- BCA Protein Assay Kit - Reducing Agent Compatible

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at –20 °C, protected from light upon receiving. Briefly centrifuge small vials prior to opening.

Preparation Instructions.

Reagent Preparation

Gelatinase Assay Buffer: Warm to room temperature prior to use

Gelatinase Substrate: Reconstitute in 220 µL of ultrapure water. Mix well by pipetting up and down. Vortex if necessary. Unused substrate can be stored at –20 °C by covering it with aluminum foil or transferring it to an amber vial.

Enzyme Positive Control: Aliquot and store at –20 °C. Thaw on ice before use. Avoid repeated freeze/thaw.

Procedure

Sample Preparation

Notes:

- The kit is designed to work with active gelatinase samples 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.[1]
- It is recommended that the tissue/cell homogenate be used immediately to measure gelatinase activity. If desired, snap freeze the lysate and store at –80 °C.
- For unknown samples, perform pilot experiments by testing 3–5 different sample volumes to ensure the readings are within the Standard Curve range.
- 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.
- Optional: For samples having background, prepare parallel sample well(s) as sample background control. Use same amount of tissue/cell homogenate or purified enzyme as in the sample well. Adjust the final volume to 100 µL with Gelatinase Assay Buffer.

1. Homogenize fresh or frozen tissue (5–10 mg) or cells ($1–2 \times 10^6$) with 100 µL of Cell Lysis Buffer and incubate on ice for 5 minutes.
2. Centrifuge the homogenate at 16,000 × *g* at 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 a BCA Protein Assay Kit (must be reducing agent compatible).
5. Add 1-50 µL of lysate or purified enzyme into desired well(s) in a 96 well plate. If necessary, dilute the lysate with Gelatinase Assay buffer.
6. For Positive Control, dilute 2 µL of Enzyme Positive Control with 18 µL of Gelatinase Assay Buffer and use 1–10 µL /well.
7. Adjust the volume of Samples and Positive Control to 50 µL/well with Gelatinase Assay Buffer.

Standard Curve Preparation

Prepare 50 µM of FITC Standard by diluting 2 µL of 5 mM FITC Standard to 200 µL with Gelatinase Assay Buffer. Mix well by pipetting up and down, vortex vigorously for 30 seconds. Prepare FITC Standards according to Table 1. Mix well and measure the fluorescence at λ_{ex} = 490 nm/λ_{em} = 520 nm in end-point mode at 37 °C.

Table 1.
Preparation of FITC Standards

Well	50 µM Premix	Gelatinase Assay Buffer	FITC (pmol/well)
1	0 µL	100 µL	0
2	2 µL	98 µL	100
3	4 µL	96 µL	200
4	6 µL	94 µL	300
5	8 µL	92 µL	400
6	10 µL	90 µL	500

Gelatinase Substrate Mix

Prepare 50 µL of Gelatinase Substrate Mix per well as stated below:

48 µL of Gelatinase Assay Buffer
2 µL of Reconstituted Gelatinase Substrate

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

Measurement

1. Mix well and measure the fluorescence at λ_{ex} = 490 nm/λ_{em} = 520 nm in kinetic mode at 37 °C for 1–2 hours.
2. Choose two time points (t_1 & t_2) where the corresponding RFUs (RFU_1 and RFU_2) are in a linear range.
3. Calculate ΔRFU and Δt and obtain ΔRFU/Δt as RFU/minute for each sample including background control.
4. Subtract the value of RFU/minute of background from each sample to obtain net RFU/minute.

Results

FITC Standard Curve

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

Samples

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

$$\text{Sample Gelatinase Activity}\left(X, \frac{\text{U}}{\text{ml}}\right) = \frac{B \times 1000}{A \times C} \times \textit{Dilution Factor}$$

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

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
P = Protein concentration in the lysate (mg/mL)
1,000 = Conversion Factor (1,000 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 minute under the conditions of the assay.

Figure 1.
Typical FITC Standard Curve

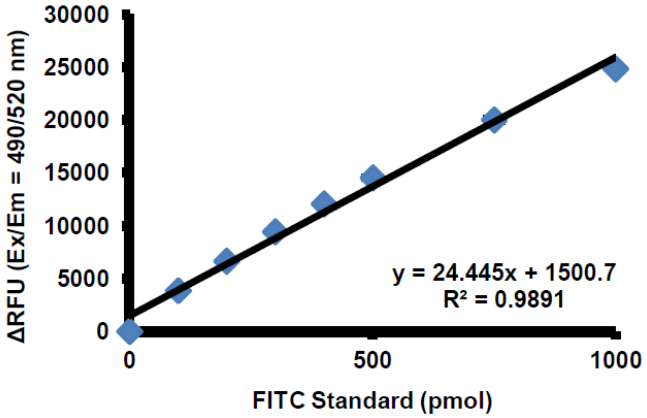


Figure 2.
Gelatinase Activity with Different Amounts of Enzyme Positive Control

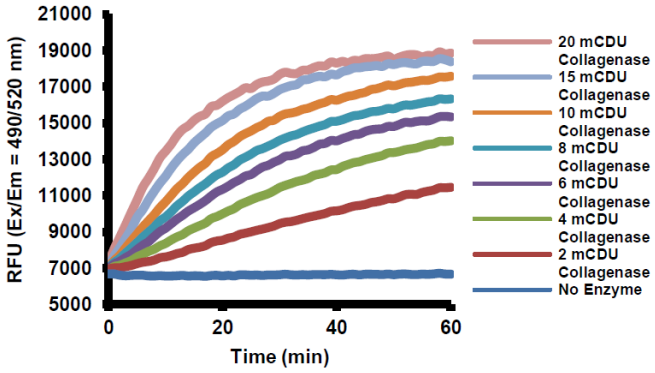
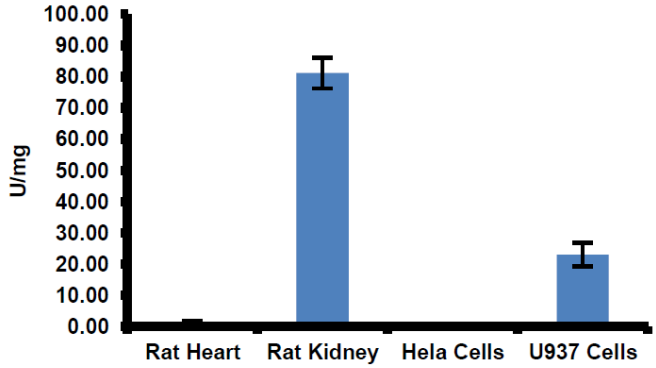


Figure 3.
Gelatinase Activity



Rat heart and kidney lysates along with Hela and U937 cell lysates (n = 3).

References

1. Shapiro et. al., J. Bio. Chem., **270**(11), 6351-6356 (1995).
2. Shin et. al., Exp. Mol. Med., **39**(1), 97-105 (2003).

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