TECHNICAL MANUAL

Matrix Metalloproteinase 3 (MMP-3) Activity Fluorometric Assay Kit

- **SKU CODE:** MAES0310
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure fluorescence values

2. Intended use

This kit can be used to measure matrix metalloproteinase 3 (MMP-3) activity in serum (plasma), animal tissue and cell samples.

3. Detection principle

Matrix metalloproteinase 3 (MMP-3) is an important member of the MMP family. The precursor of MMP-3 is cleaved by plasmin, chymotrypsin and other serine proteases to remove the precursor peptide containing cysteine switch and activate MMP-3 with protease activity. MMP-3 can degrade or shear a variety of extracellular matrix components, precursor proteins or precursor enzymes, and can destroy the histological barrier of tumor cell invasion, release E-cadherin, promote tumor invasion and metastasis, and promote inflammatory response, which has received increasing attention in tumor research. In addition, MMP-3 is also involved in a series of physiological and pathological processes such as tissue morphogenesis, injury repair and inflammatory response, and plays an important role in the occurrence and development of diseases such as rheumatic arthritis and atherosclerosis.

This kit is detected by fluorescence resonance energy transfer (FRET) method. MCA and Dnp are connected to two ends on the natural substrate of MMP-3 enzyme. When MMP-3 protease does not cut the substrate, the two groups are close enough to undergo fluorescence resonance energy transfer, that is, Dnp can quench the fluorescence of MCA, resulting in undetectable fluorescence. When the substrate is cut by MMP-3 protease, the ends of the polypeptide are separated, the two groups are separated, the fluorescence of MCA is no longer extinguished by Dnp, and the fluorescence of MCA can be detected, so that the enzyme activity of MMP-3 protease can be detected very sensitively through fluorescence detection. MCA has a maximum excitation wavelength of 325 nm and a maximum emission wavelength of 393 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2 (96 T)	Storage
Reagent 1	Buffer Solution	7 mL × 1 vial	14 mL × 1 vial	-20°C, 12 months
Reagent 2	Activator	0.15 mL × 1 vial	0.3 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Proteinase Inhibitor	0.75 mL × 1 vial	1.5 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	Substrate	0.1 mL × 1 vial	0.2 mL × 1 vial	-20°C, 12 months shading light
Reagent 5	Standard	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months shading light
	Black Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Fluorescent Microplate reader (Ex/Em = 325 nm/393 nm)

Reagents:

Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate working solution:** Before testing, prepare sufficient substrate working solution according to the test wells. For example, prepare 909 µL of substrate working solution (mix 9 µL of substrate with 900 µL of buffer solution). The substrate working solution should be prepared fresh and used within 4 hours.
3. **Preparation of 10 mmol/L standard solution:** Dissolve one vial of standard with 1.7 mL of DMSO, mix well to dissolve. Store at -20°C for 7 days protected from light.

4. **Preparation of 100 µmol/L standard solution:** Dilute 10 µL of 10 mmol/L standard solution with 990 µL of double distilled water. Store at 2-8°C for a day protected from light.
5. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 100 µmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 20, 30, 40, 60, 80, 90, 100 µmol/L.

7. Sample preparation

Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold normal saline (0.9% NaCl).

3. Homogenize 20 mg tissue in 180 µL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

4. Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Cells (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

2. Wash cells with normal saline (0.9% NaCl).

3. Homogenize 1×10^6 cells in 200 µL normal saline (0.9% NaCl) with an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

To determine the total MMP-3 enzyme activity in the sample, the sample needs to be activated with an activator, and the steps are as follows:

Mix 50 µL of sample (tissue/cells supernatant or serum/plasma) with 1 µL of activator, incubate at 37°C for 10 min, and mix with 10 µL of proteinase inhibitor. The sample is diluted 1.22 times during this step.

Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

10% Rat liver tissue homogenization: 1-2

10% Rat lung tissue homogenization: 1-2

10% Mouse liver tissue homogenization: 1-2

1×10^6 CHO cell: 1

Rat serum: 1

Rat plasma: 1

Note: The diluent is normal saline (0.9% NaCl). For dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

If the fluorescence value of the control well is greater than the fluorescence value of the sample well, it may be that the enzyme activity of the sample is high, and the sample needs to be diluted for re-detection.

9. Operating steps

1. **Add reagents to wells:** Standard well: add 10 μL of standard solution with different concentration to the corresponding well. Sample well: add 10 μL of sample to the corresponding well. Control well: add 10 μL of normal saline (0.9% NaCl) to the corresponding well.
2. **Add buffer and substrate solutions:** Add 90 μL of buffer solution to the standard wells. Add 90 μL of substrate working solution to the control wells and the sample wells.
3. Mix well with microplate reader for 3 s. Measure the fluorescence values at the excitation wavelength of 325 nm and the emission wavelength of 393 nm, recorded as F_1.
4. Incubate at 37°C for 20 min. Measure the fluorescence values at the excitation wavelength of 325 nm and the emission wavelength of 393 nm, recorded as F_2. The standard curve is drawn according to F_1.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean F_1 value of the blank (Standard #1) from all standard readings. This is the absolute F_1 value.

3. Plot the standard curve by using absolute F_1 value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

Definition: The amount of MMP-3 enzyme required for per liter of serum or plasma to catalyze the substrate to produce 1 μmol of product per minute at 37°C is defined as 1 unit.

Unactivated sample:

MMP-3 activity (U/L) = (ΔF_sample - ΔF_control - b) ÷ a ÷ T × f

Activated sample:

MMP-3 activity (U/L) = (ΔF_sample - ΔF_control - b) ÷ a ÷ T × f × 1.22

2. Tissue and cells sample:

Definition: The amount of MMP-3 enzyme required for each gram of sample protein to catalyze the substrate to produce 1 μmol of product per minute at 37°C is defined as 1 unit.

Unactivated sample:

MMP-3 activity (U/g_{prot}) = (ΔF_sample - ΔF_control - b) ÷ a ÷ T ÷ C_{pr} × f

Activated sample:

MMP-3 activity (U/g_{prot}) = (ΔF_sample - ΔF_control - b) ÷ a ÷ T ÷ C_{pr} × f × 1.22

[Note]

ΔF_sample: Absolute fluorescence value of sample (F_2 - F_1).

ΔF_control: Absolute fluorescence value of control (F_2 - F_1).

T: Reaction time, 20 min.

C_{pr}: Concentration of protein in sample (g_{prot}/L).

f: Dilution factor of sample before test.

1.22: Dilution of the activated sample.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.50	2.50	5.00
%CV	4.0	3.0	2.0

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.50	2.50	5.00
%CV	7.5	7.7	7.6

Recovery

Three samples of high concentration, middle concentration and low concentration were tested in parallel 6 times each to get an average recovery rate of 103%.

Recovery

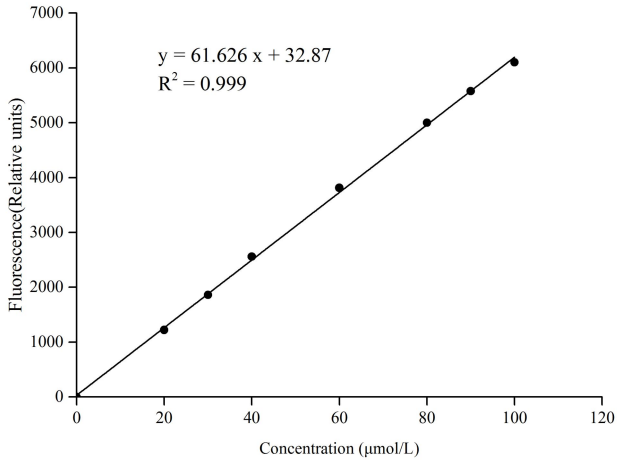
	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/L)	25	55	88
Observed Conc. (µmol/L)	23.0	64.9	103.0
Recovery rate (%)	92	118	117

Sensitivity

The analytical sensitivity of the assay is 0.03 U/L. This was determined by adding two standard deviations to the mean fluorescence value obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (μmol/L)	Fluorescence value (F1)		Average fluorescence value	Absolute fluorescence value
0	238	249	243	0
20	1469	1460	1465	1222
30	2091	2121	2106	1863
40	2786	2820	2803	2560
60	3975	4135	4055	3812
80	5153	5337	5245	5002
90	5739	5902	5821	5577

Concentration (μmol/L)	Fluorescence value (F1)		Average fluorescence value	Absolute fluorescence value
100	6355	6337	6346	6103

12. Appendix II Example Analysis

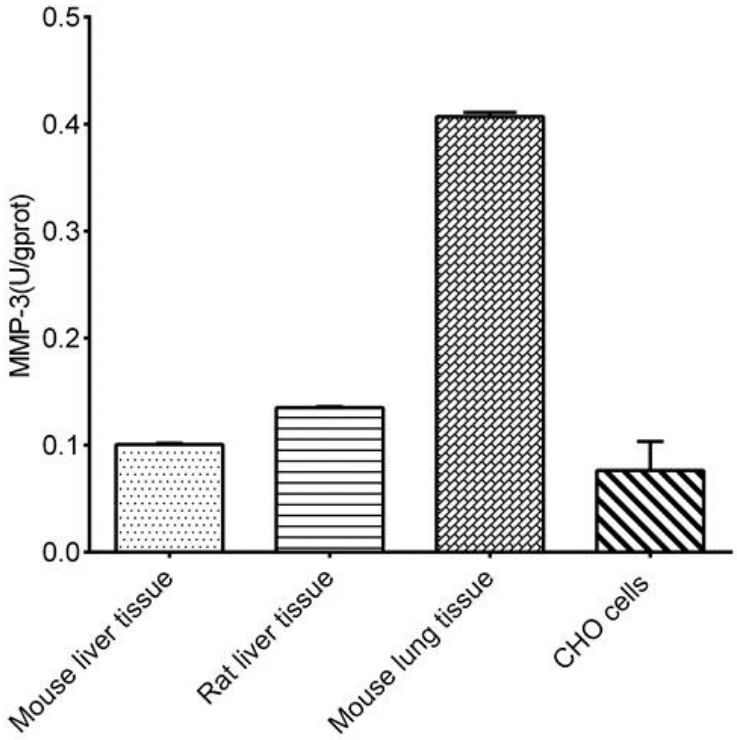
Example analysis:

Take 10% mouse liver tissue homogenate, and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 61.626x + 32.87, the F_1 of the sample well is 878, the F_2 of the sample well is 2304, ΔF_sample = 2304 - 878 = 1426, the F_1 of the control well is 700, the F_2 of the control well is 685, ΔF_control = 685 - 700 = -15, the concentration of protein in sample is 11.25 g_{prot}/L, and the calculation result is:

MMP-3 activity (U/g_{prot}) = (1426 - 32.87 + 15) ÷ 61.626 ÷ 20 ÷ 11.25 = 0.10 U/g_{prot}

Detect 10% mouse liver tissue homogenate (the concentration of protein is 11.25 g_{prot}/L), 10% rat liver tissue homogenate (the concentration of protein is 13.73 g_{prot}/L), 10% mouse lung tissue homogenate (the concentration of protein is 3.66 g_{prot}/L) and CHO cell (the concentration of protein is 1.53 g_{prot}/L) according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.