（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F061

Specification：48T(30 samples)/96T(78 samples)

Measuring instrument：Fluorescence Microplate reader

（Ex/Em = 325 nm/393 nm）

Detection range：0.03-9.66 U/L

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

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tel: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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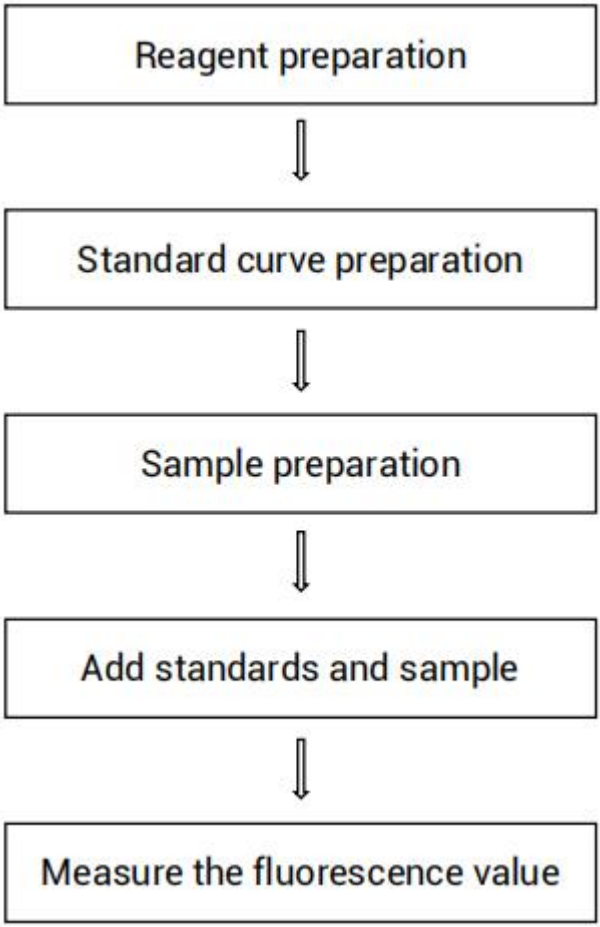
Assay summary



Intended use

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

Detection principle

Matrix metalloproteinase 3 (MMP-3) is an important member of the MMP family. The precursor of MMP-3 was 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 and 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.

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		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate working solution：
Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 909 μL of substrate working solution (mix well mix well 9 μL of substrate and 900 μL of buffer solution). The substrate working solution should be prepared on spot and it should be used up within 4 hours.

③ The 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 The 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 The 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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**20**	**30**	**40**	**60**	**80**	**90**	**100**
100 μmol/L standard (μL)	0	20	30	40	60	80	90	100
Double distilled water (μL)	100	80	70	60	40	20	10	0

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80 ºC 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 ºC.

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 (E-BC-K318-M).

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 a ultrasonic cell disruptor at 4 ºC.

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 (E-BC-K318-M).

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 well 50 μL of sample(tissue/cells supernatant or serum/plasma) and 1 μL of activator, incubated at 37 ºC for 10 min, and mixed with 10 μL of

proteinase inhibitor. The sample was diluted 1.22 times during this step.

② Dilution of sample

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

Sample type	Dilution factor
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 the dilution of other sample types, please do pretest to confirm the dilution factor

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.

Operating steps

The measurement of samples

① 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 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℃ 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.

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 absoluted F_1 value.

3. Plot the standard curve by using absoluted 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:

$$\text{MMP-3 activity (U/L)} = (\Delta F_{sample} - \Delta F_{control} - b) \div a \div T \times f$$

Activated sample:

$$\text{MMP-3 activity (U/L)} = (\Delta F_{sample} - \Delta F_{control} - b) \div a \div T \times f \times 1.22$$

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℃ is defined as 1 unit.

Unactivated sample:

$$\text{MMP-3 activity (U/gprot)} = (\Delta F_{sample} - \Delta F_{control} - b) \div a \div T \div C_{pr} \times f$$

Activated sample:

$$\text{MMP-3 activity (U/gprot)} = (\Delta F_{sample} - \Delta F_{control} - b) \div a \div T \div C_{pr} \times f \times 1.22$$

[Note]

ΔF_{sample}: Absolute fluorescence value of sample (F_2 - F_1).

$\Delta F_{control}$: Absolute fluorescence value of control (F_2 - F_1).

T: Reaction time, 20 min.

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

f: Dilution factor of sample before test.

1.22: Dilution of the activated sample.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

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.

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

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 103%.

	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

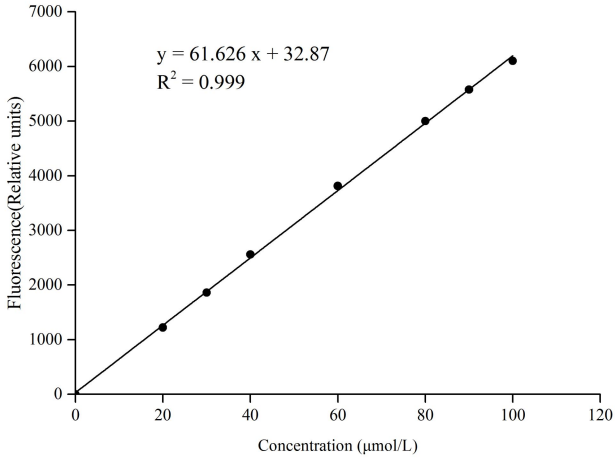
Sensitivity

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

2. Standard curve:

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

Concentration (μmol/L)	**0**	**20**	**30**	**40**	**60**	**80**	**90**	**100**
Fluorescence value (F_1)	238	1469	2091	2786	3975	5153	5739	6355
	249	1460	2121	2820	4135	5337	5902	6337
Average fluorescence value	243	1465	2106	2803	4055	5245	5821	6346
Absoluted fluorescence value	0	1222	1863	2560	3812	5002	5577	6103



Appendix Π Example Analysis

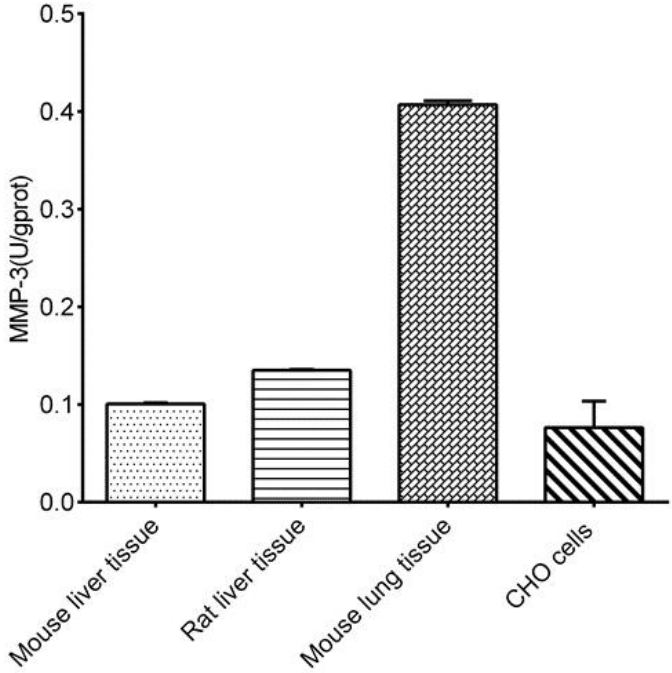
Example analysis：

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

standard curve: y = 61.626 x + 32.87, the F_1 of the sample well is 878, the F_2 of the sample well is 2304, $\triangle F_{sample}$ = 2304 - 878 = 1426, the F_1 of the control well is 700, the F_2 of the control well is 685, $\triangle F_{control}$ = 685 - 700 = -15, the concentration of protein in sample is 11.25 gprot/L, and the calculation result is:

$$\text{MMP-3 activity (U/gprot)} = (1426 - 32.87 + 15) \div 61.626 \div 20 \div 11.25 = 0.10 \text{ U/gprot}$$

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


0.5
0.4
0.3
0.2
0.1
0.0
MMP-3(U/gprot)
Mouse liver tissue
Rat liver tissue
Mouse lung tissue
CHO cells

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.