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

Catalog No：E-BC-D003

Specification：96T

Measuring instrument：Fluorescence Microplate Reader

（Ex/Em=325nm/395nm）

Elabscience®Matrix Metalloproteinase 3 (MMP-3) Inhibitor Screening 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：

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

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Intended use

This kit is used for the determination of the inhibitory effect of matrix metalloproteinase 3 (MMP-3) inhibitors.

Detection principle

Matrix metalloproteinase 3 (MMP-3) is an important member of the MMP family. The MMP-3 precursor is cleaved by serine proteinases such as plasmin and chymotrypsin to remove the precursor peptide containing the cysteine switch and form 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 rheumatoid arthritis and atherosclerosis.

This MMP-3 inhibitor screening kit was detected by fluorescence resonance energy transfer (FRET) method. MCA and Dnp are linked to the two ends of the native substrate of MMP-3 enzyme. When MMP-3 protease does not cleave the substrate, the two groups are close enough to undergo fluorescence resonance energy transfer, that is, Dnp can quenthe fluorescence of MCA and cause no fluorescence to be detected. When the substrate is cut by MMP-3 protease, the both 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.

If the Inhibitor of MMP-3 protease is added to the reaction system, the generation of fluorescence will be inhibited, and the fluorescence intensity is inversely proportional to the inhibitory effect of the inhibitor, so that the inhibitory effect of MMP-3 protease inhibitor can be detected. MCA has a maximum excitation wavelength of 325 nm and a maximum emission wavelength of 393 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	15 mL × 1 vial	-20°C, 12 months
Reagent 2	Activator	0.05 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Stabilizer	1.5 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	Substrate	0.2 mL × 1 vial	-20°C, 12 months shading light
Reagent 5	0.1 mM GM6001	0.5 mL × 1 vial	-20°C, 12 months shading light
Reagent 6	MMP-3	0.05 mL × 1 vial	-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:

Fluorescence microplate reader (Ex/Em=325 nm/395 nm), Incubator（37℃）

Reagent preparation

① Keep MMP-3 on ice during use. Equilibrate other 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 23 μL of substrate working solution (mix well 3 μL of substrate and 20 μL of double distilled water). The substrate working solution should be prepared on spot.

③ The preparation of control working solution:
Before testing, please prepare sufficient control working solution according to the test wells. For example, prepare 530 μL of control working solution (mix well 525 μL of buffer solution and 5 μL of activator). Incubate at 37 °C for 10 min with shading light and keep it on ice for detection. The control working solution should be prepared on spot. Store at 2-8°C for 2 days protected from light.

④ The preparation of MMP-3 working solution:
Before testing, please prepare sufficient MMP-3 working solution according to the test wells. For example, prepare 105 μL of MMP-3 working solution (mix well 25 μL of MMP-3, 500 μL of buffer solution and 5 μL of activator). Incubate at 37°C for 10 min protected from light and keep it on ice for detection. The MMP-3 working solution should be prepared on spot. Store at 2-8°C for 2 days protected from light.

⑤ Use of GM6001:

Dilute the GM6001 with double distilled water to the desired concentration. (This kit provides GM6001 as a broad spectrum inhibitor of MMP-3, which is only used as a positive reference. The IC50 is about 100 nM, and the measured data may be different.)

The key points of the assay

① Prevent the formulation of bubbles when adding reagents. It is recommended to use micropipettor to suck two to one to add reagents.

② The volume of substrate and MMP-3 is small, centrifuge before use.

Operating steps

① Total enzyme well: Add 10 μL of MMP-3 working solution to the corresponding wells.
Sample well: Add 10 μL of MMP-3 working solution to the corresponding wells.
Positive control well: Add 10 μL of MMP-3 working solution to the corresponding wells.
Blank control well: Add 10 μL of control working solution to the corresponding wells.

② Add 90 μL of buffer solution into each well.

③ Add 10 μL of stabilizer into each well. Mix fully with microplate reader for 3 s and stand at room temperature for 3 min.

④ Add 10 μL of double distilled water to total enzyme wells and blank control wells, add 10 μL of sample to sample wells, add 10 μL of GM6001 to positive control wells. Mix fully with microplate reader for 3 s and stand at room temperature for 5 min.

⑤ Add 10 μL of substrate working solution into each well. Mix fully with microplate reader for 3 s and incubate at 37°C for 10 min.

⑥ Measure the fluorescence intensity of each well at the excitation wavelength of 325 nm and the emission wavelength of 393 nm.

Calculation

$$\text{Inhibition Rate (\%)} = (F_{total} - F_{sample}) \div (F_{total} - F_{blank}) \times 100\%$$

[Note]

F_{total}: The fluorescence intensity of total enzyme well.

F_{sample}: The fluorescence intensity of sample well.

F_{blank}: The fluorescence intensity of blank control well.

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.