

FITC-labeled Gelatin-zymography Kit

Cosmo Bio-type

PMC-AK83-COS

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Background

Matrix metalloproteinases (MMPs) belong to the family of metalloproteinases, which consists of at least 20 members, and known to be involved in the metabolism of extracellular matrix proteins. MMPs are widely detected by zymography. The FITC-labeled Gelatin-Zymography Kit (Cat.No.PMC-AK83-COS) provides an easy system of the electrophoresis for zymography. This product is used for detecting ProMMP-2, MMP-2 and ProMMP-9 in blood, body fluid, secretion, cell lysate, cell culture medium, and other samples.

【 1-1 】 Kit Components

Materials Provided

Description	Quantity	Storage
Precast gel for 12 samples	5 pieces	4°C , Do not freeze
Electrophoresis Buffer (× 10)	100 mL × 2	4°C
Washing Buffer (× 10)	100 mL × 1	4°C
Reaction Buffer (× 10)	25 mL × 1	4°C
Sample Preparation Buffer	5 mL × 1	4°C
MMP markers (ProMMP-2, MMP-2, ProMMP-9)	0.2 mL	4°C

The size of the glass plate gel is 100 mm (W) × 100 mm (H) and the thickness is 1mm.

One kit contains reagents for 60 samples.

Additional Materials Required

- distilled water

【 1-2 】 Features

- Precast gel and reagents for zymography are included in this kit.
- You can check the enzyme reaction continuously.

【 2-1 】 Preparation of reagents

- Electrophoresis Buffer
Dilute Electrophoresis Buffer (× 10) with distilled water to a 1x concentrated solution.
Note: Diluted buffer should be stored at 4° C.
- Washing Buffer
Dilute Washing Buffer (× 10) with distilled water to a 1x concentrated solution.
Note: Diluted buffer should be stored at 4° C.
- Reaction Buffer
Dilute Reaction Buffer (× 10) with distilled water to a 1x concentrated solution.
Note: Diluted buffer should be stored at 4° C.

【 2-2 】 Preparation of samples

Mix Sample Preparation Buffer and a samples in the ratio of 1 to 1 and leave it in room temperature for 15 minutes.

Note: Do not heat the samples.

【 3 】 Protocol

1. Load 100-150 mL of Electrophoresis Buffer to the lower chamber (anode). Refer to the instruction manual of your electrophoresis tank/chamber to know appropriate volume of the buffer.
2. Take out the comb on precast gel carefully and set the gel to electrophoresis chamber. The side of sample holes should be set on the upper side. If the sample holes are disturbed, fix them up with needle etc.
3. Load around 100 mL of Electrophoresis Buffer to the upper chamber (cathode).
4. Apply samples and MMP markers to gel plate. (Fig.1: 10 μ L of MMP markers applied and enzymatic reacted for 24 hrs at 37°C).
5. Run electrophoresis at 15 mA constant current. (If you use 2 gels, set the current at 30 mA)
*MMP markers can be used without sample preparation buffer.
6. After the run is completed, turn off the electrophoresis chamber and take out the gel plate from the chamber.
7. Remove the upper glass plate and peel off the gel from the lower glass plate (the biggest glass plate) carefully with a spatula.
8. Put the gel in a tray with 200 mL of Washing Buffer. Incubate with shaking at Room Temperature.
9. Put the gel in a container with 50 mL of Reaction Buffer and seal up the container. Incubate the gel at 37°C for 20-40 hrs. (Lower enzyme concentration needs longer reaction time.)
10. After enzymatic reaction, remove the gel and take a photo by gel imaging system. (fluorescent wavelength: 535 nm)

【 4 】 Electrophoresis patterns

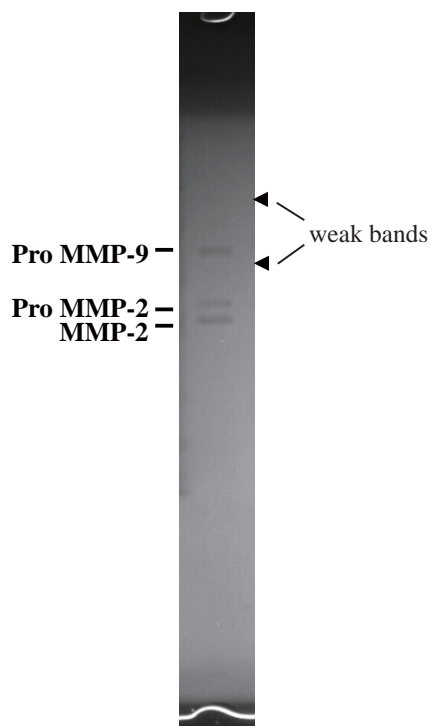


Fig. 1 Electrophoresis pattern of MMP marker

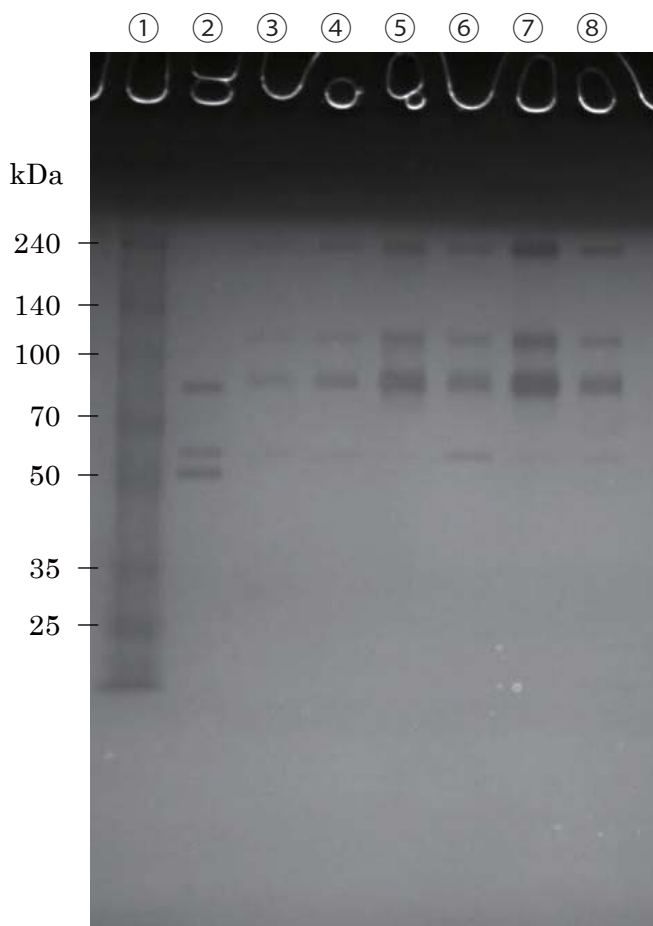


Fig. 2 Electrophoresis pattern for human tear samples

- ① Molecular-weight marker
- ② MMP marker
- ③~⑧ human tear samples (6 lanes)

【 5 】 Related Products

Cassette Electrophoresis Units for this kit are sold.

Cat No.	Description	Components
DCB-303111	Cassette Electrophoresis Unit Model DPE-1020 for Mini Gel	1 Lid with power cables 1 Anode Buffer Chamber 1 Cathode Buffer Chamber Frame with electrodes 2 Gaskets 2 Wedges 2 Acrylic Plates 1 Instruction manual
DCB-500000	P-BEAT Electrophoresis Cell	Dimension: 145 W × 115 D × 150 H mm Buffer Volume: About 700 mL Maximum Sheets: 2 Sheets Setting Voltage: 300V-500V Electrophoresis Time: about 40min-25min



Precast gels and MMP markers are sold separately.

Cat No.	Description	Components
PMC-AK84-COS	FITC-labeled Gelatin-zymography Precast gel (Cosmo Bio Type)	5 pieces of Precast gel for 12 samples
PMC-AK85-COS	FITC-labeled Gelatin-zymography Precast gel (Cosmo Bio Type)	10 pieces of Precast gel for 12 samples
PMC-AK38-COS	MMP markers	200 μL ((ProMMP-2, MMP-2, ProMMP-9)



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