COSMO BIO CO., LTD.
Inspiration for Life Science

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Catalog No. PMC-AK45-COS

Gelatin-Zymography Kit

Introduction

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 Gelatin-Zymography Kit (Cat.No.AK47) 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.

Components/Storage

Component	Quantity	Storage
Precast gel for 12 samples	5 pieces	4ºC
Electrophoresis Buffer (×10)	100 mL × 2	4ºC
Washing Buffer (×10)	100 mL × 1	4ºC
Reaction Buffer (×10)	25 mL × 1	4ºC
Sample Preparation Buffer	5 mL × 1	4ºC
Staining Solution	100 mL × 1	room temperature
MMP markers (ProMMP-2, MMP-2, ProMMP-9)	0.2 mL	4ºC

The size of the glass plate gel is 120(W)×100(H) and the thickness is 1mm.
One kit contains regents for 60 samples

Materials required but not provided

・acetic acid ・methanol ・distilled water

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.

・De-staining Solution
This solution is not included in this kit. Mix Acetic acid: Methanol: Distilled water = 5 : 30 : 65.
Note: De-staining Solution should be stored at Room Temperature.

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. Mix samples with equivalent volume of sample preparation buffer. Incubate for 15 minutes at Room Temperature. (Do NOT heat the samples.)
5. Apply the samples and MMP markers to gel plate. (Fig.1: 10ul of MMP markers applied and enzymatic reacted for 24 hrs. at 37°C) 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 of the gel plate and peel off the gel carefully with a spatula.
8. Put the gel in the tray with 200 mL of Washing Buffer. Incubate with shaking at Room Temperature.
9. Put the gel in the container with 50 mL of Reaction Buffer and seal up the container. Incubate the gel in the container in incubator at 37°C for 20-40 hrs (Lower enzyme concentration needs longer reaction time.).
10. After enzymatic reaction, put the gel in the container with Staining solution. Incubate for 30 minutes at Room Temperature to stain protein.
11. Put the gel in the container with De-staining solution. And incubate for 30minutes – several hours to de-stain.

Application example

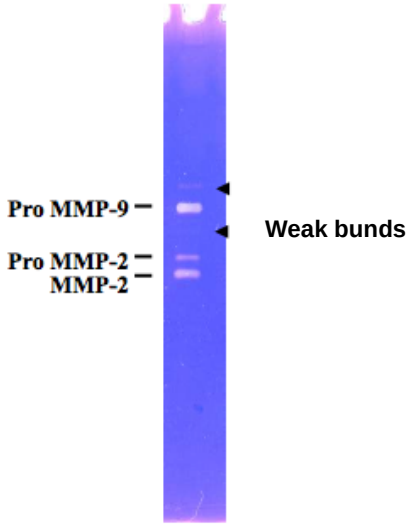

Fig. 1 10ul of MMP markers applied and enzymatic reacted for 24 hrs. at 37°C

References

(1) Miyazaki, K., Ohta, Y., Nagai, M., Morimoto, N., Kurata, T., Takehisa, Y., Ikeda, Y., Matsuura, T., Abe, K. Disruption of Neurovascular Unit Prior to Motor Neuron Degeneration in Amyotrophic Lateral Sclerosis. J. Neurosci. Res. 89, 718-728 (2011)
(2) Fujiwara, M., Kashima, T. G., Kunita, A., Kii, I., Komura, D., Grigoriadis, A. E., Kudo, A., Aburatani, H., Fukayama, M. Stable Knockdown of S100A4 Suppresses Cell Migration and Metastasis of Osteosarcoma. Tumour Biol.
(3) Aoki, T., Kataoka, H., Ishibashi, R., Nozaki, K., Hashimoto, N. Simvastatin Suppresses the Progression of Experimentally Induced Cerebral Aneurysms in Rats. Stroke. 39, 1276-85 (2008)
(4) Kuramochi, D., Unoki, H., Bujo, H., Kubota, Y., Jiang, M., Rikihisa, N., Udagawa, A., Yoshimoto, S., Ichinose, M.,Saito, Y. Matrix Metalloproteinase 2 Improves The Transplanted Adipocyte Survival in Mice. Eur. J. Clin. Invest. 38, 752-759 (2008)
(5) Fujimoto, M., Takagi, Y., Aoki, T., Hayase, M., Marumo, T., Gomi, M., Nishimura, M., Kataoka, H., Hashimoto, N., Nozaki, K. Tissue Inhibitor of Metalloproteinases Protect Blood-brain Barrier Disruption in Focal Cerebral Ischemia. J. Cereb. Blood Flow Metab. 28, 1674-1685 (2008)

Notice to Purchaser

This product has a short expiration date (less than 6 months). Please use it after arrival as soon as possible.

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