BIOMEDICAL RESEARCH SERVICE CENTER
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MMP2/MMP9 Gel Assay Kit (Cat#: E-118GA)

COMPONENTS: 5x MMP Reaction Buffer- 180 ml (for 10 gels)
50x $CaCl_2$- 20 ml
TX-100- 15 ml
5x SDS-PAGE Sample Buffer- 5 ml
1000x $ZnSO_4$- 1 ml
Gelatin- 0.15 g

PRODUCT DESCRIPTION: The assay kit is for detecting MMP2/9 present in tissue extracts, serum, urine, and spent culture medium. The kit does not include glass plates, spacers or reagents for performing SDS-PAGE. Gel staining/destaining solutions are also not included. Handcasting by the end user is required. All reagents are stored at room temperature.

Sample preparation: Four parts of cell/tissue extract, plasma/serum and cell culture medium are mixed with one part of 5x SDS-PAGE Sample Buffer immediately prior to electrophoresis. Typically 10 − 30 µg tissue proteins, 2 − 5 µl plasma/serum and 5 − 20 µl cell culture medium are sufficient for detecting MMP2/9. Do NOT heat or treat sample with β-mercaptoethanol.

Gelatin solution: Weigh 5 mg gelatin and mix with 0.5 ml dH_2O in a microtube. Secure cap and place tube in a 95°C heat block for ~10 min with intermittent vortexing. Spin tube briefly to deposit liquid. Add 0.5 ml gelatin solution to the resolving gel solution immediately. Follow the order of addition as indicated below.

Resolving gel solution (5 ml 10% gel containing 1 mg/ml gelatin): Mix 1.25 ml dH_2O, 1.88 ml 1M Tris pH8.8, 1.25 ml acrylamide/bis-acrylamide solution (40% acrylamide + 1.05% bis-acrylamide), 50 µl 10% SDS, 0.5 ml heated gelatin solution, 75 µl 3% ammonium persulfate, and 5 µl TEMED in a small beaker. Cast resolving gel in a 10x10 cm glass assembly. See this web page for general gel casting instructions.
https://openwetware.org/wiki/Lidstrom:_SDS-PAGE

Stacking gel solution (2.5 ml 5% gel): Mix 1.8 ml dH_2O, 0.31 ml 1M Tris pH6.8, 0.31 ml acrylamide/bis-acrylamide solution, 25 µl 10% SDS, 38 µl 3% ammonium persulfate, and 2.5 µl TEMED in a small beaker.

Sample loading and electrophoresis: The amount of sample required for detecting MMP2/9 should be determined empirically. Perform gel electrophoresis with cooling water circulation at ~300 volts until the blue dye reaches the bottom of the gel. Trim off and discard the stacking gel. Process the resolving gel as described below.

MMP Wash Solution: Each gel assay requires 60 ml Wash Solution prepared FRESH by mixing 46 ml dH_2O, 12 ml 5x MMP Reaction Buffer, 1.2 ml 50x $CaCl_2$, 60 µl 1000x $ZnSO_4$, and 1.5 ml TX-100 in a beaker with constant stirring. Note: TX-100 is sticky. Avoid using pipette tip to transfer TX-100.

MMP Activation Solution: Each gel assay requires 25 ml Activation Solution prepared FRESH by mixing 20 ml dH_2O, 5 ml 5x MMP Reaction Buffer, 0.5 ml 50x $CaCl_2$ and 25 µl 1000x $ZnSO_4$.

Gel washing: Gently agitate the resolving gel in ~15 ml MMP Wash Solution for 15 min at room temperature. Discard solution and repeat the washing step three times (a total of 1 hour required for gel washing).

Gel activation: Remove wash solution completely. Gently agitate gel in 25 ml MMP Activation Solution at 37°C for 20 − 40 hours. Note that increased sample volume and/or increased incubation time may be necessary to detect sufficient MMP9 activity.

Gel staining:

1. Rinse gel in a 10% methanol/10% acetic acid solution for ~5 min. Discard solution.
2. Stain gel in a filtered 3% Coomassie Brilliant Blue G250 solution containing 50% methanol and 10% acetic acid for 20 − 30 min with gentle agitation at room temperature. Remove staining solution (can be reused many times).
3. Destain gel in a 10% methanol/10% acetic acid solution for 10 − 30 min or until the MMP bands fully develop against the blue background with a good contrast. Rinse gel with water prior to imaging. MMP2 and MMP9 yield major bands in the vicinity of ~65 kDa and 85 kDa, respectively. Additional proteolytic bands may also be visible. Use densitometry to quantify MMP band intensity.

General information: Please refer to *Molecular Cloning- A Laboratory Manual (Sambrook, Fritsch & Maniatis)* for SDS-PAGE and gel staining protocols as well as safety information. Refer to the product page of our website for MSDS information on acrylamide, TEMED, ammonium persulfate, SDS and TX-100.