

BIOMEDICAL RESEARCH SERVICE CENTER

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MMP2/MMP9 Solution Assay Kit (Cat#: E-118SA)

COMPONENTS: 5x MMP Buffer: 1 ml; store at 4°C

Blue Gelatin: 1 ml (100 reactions); store at 4°C (short term) or -80°C (long term)

PRODUCT DESCRIPTION: The MMP2/MMP9 Solution Assay (SA) kit is based on MMP2/9-mediated hydrolysis of blue gelatin in solution, which allows for convenient analysis of MMP activity. MMP activity is determined by optical measurement of 595nm after acetone precipitation of undigested Blue Gelatin. Kit components are stable for ~1 year if stored and handled properly.

MMP assay protocol:

1. Perform MMP assay in a set of 0.5-ml microtubes. Include a control tube, which contains 22 μ l dH₂O, 8 μ l 5x MMP Buffer, and 10 μ l Blue Gelatin.

Note- The control tube serves to provide a baseline O.D._{595nm} for undigested Blue Gelatin after acetone precipitation. Do not add MMP to the control tube.

2. Calculate the volume of dH₂O required for each assay tube so the final assay volume is maintained at 40 μ l. Add items in the following order: dH₂O, 8 μ l 5x MMP Buffer, 10 μ l Blue Gelatin, MMP2/9 enzyme, and test compound (where applicable). Vortex tubes thoroughly to mix contents.

Note- Since drug vehicle such as dimethyl sulfoxide (DMSO) or other solvent may affect the activity of MMP, it is advised to include an additional vehicle control for the assay.

3. Cap tubes well and incubate tubes at 37°C overnight.
4. Stop reaction by adding 80 μ l ice-cold acetone (not included in the kit) to each tube. Vortex tubes thoroughly to mix contents.
5. Keep tubes on ice for 10 min.
6. Spin tubes in a microfuge at maximum speed (~13,000 rpm) for 5 – 10 min.

Note- Cold acetone will efficiently precipitate undigested Blue Gelatin. Small digested gelatin fragments will remain in the supernatant after centrifugation.

7. Transfer 100 μ l of the supernatant from each tube to a 96-well plate. Immediately measure O.D._{595nm} using a plate reader.
8. Subtract the control well reading (no MMP) from each sample well reading. The subtracted O.D._{595nm} represents the relative activity of MMP2/MMP9.

Note- Using the assay format, 2.5 nM Prinomastat, a potent MMP2/MMP9 inhibitor, is found to inhibit the activity of a recombinant MMP9 by ~75%.

Additional information:

- Avoid using chelating agents such as EDTA/EGTA and reducing agents such as β -Mercaptoethanol/DTT for sample preparation. These compounds may inhibit the activity of MMP.
- 4-Aminophenylmercuric acetate (APMA) induces processing of proMMP to mature MMP through autocatalysis. Enzyme samples may be pretreated with 1mM APMA at 37°C for 1 hour prior to MMP assay.
- Keep a small bottle of acetone at -20°C. Use the ice-cold acetone for gelatin precipitation as described in the protocol.
- Avoid skin contact and inhalation of acetone. Please refer to the product page of our website or contact us for MSDS information on acetone.