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

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 and sensitive analysis of MMP activity. The assay format is compatible with large-scale screening of compounds possessing MMP inhibitory activity. MMP activity is determined by optical measurement of 595nm after acetone precipitation of undigested Blue Gelatin. Kit components are stable for at least one year if stored and handled properly.

MMP assay protocol:

1. Set up MMP assay in a set of 0.5-ml microtubes. Include a control tube, which contains 22 µl dH_2O, 8 µl 5x MMP Buffer, and 10 µl 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 dH_2O required for each assay tube so the final assay volume is maintained at 40 µl. Add items in the following order: dH_2O, 8 µl 5x MMP Buffer, 10 µl 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 µl ice-cold acetone (not included in the kit) to each tube. Vortex tubes thoroughly to mix contents.

5. Keep tubes on ice for protein precipitation 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 µl 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 (from the control tube set up at step 1) 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.