

BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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

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 Assay 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 initially in 0.5-ml microtubes. Set up a control tube by adding items in the following order: 22 µl dH₂O, 8 µl 5x MMP Buffer, and finally 10 µl Blue Gelatin. Mix contents well.
2. Set up sample tube by adding items in the following order: 22 µl (MMP2/MMP9 + dH₂O), 8 µl 5x MMP Buffer, and 10 µl Blue Gelatin. Mix contents well.
3. Close tubes and incubate 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 10 min.
6. Centrifuge tubes in a microfuge at ~14,000 rpm for 5 min.
7. Transfer 100 µl of the supernatant from each tube to a plain (uncoated) 96-well plate. Immediately measure O.D._{595nm} using a plate reader.
8. Subtract 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 webpage or contact us for SDS information on acetone.