Gelatin Zymography

A 10% acrylamide gelatin gel recipe:

Gelatin solution: 2.65 mg/ml in water (heat at 65 degrees to dissolve), sterile filter (can store this for 6 months at 4 degrees)

8.3 ml of gelatin solution
5.25 ml 1.5 M Tris pH8.8
7 ml 30% acrylamide-bisacrylamide
0.165 ml 50%Glycerol
0.165 ml 10% SDS
0.010 ml TEMED
0.100 ml 10% APS

Use whatever stacking gel recipe you use.

Sample prep/electrophoresis:

Add SDS-loading buffer WITHOUT a reducing agent (i.e. DTT)

DO NOT boil

Add 10 microg or LESS total protein per lane (detection limit for MMP2/9 is on the order of a couple hundred picograms). Adding more total protein just obscures the result as abundant proteins will stain with the coomassie

Run the gel (20 mA/gel: 0.75 mm thick minigel)

Developing the zymogram:

Wash (incubate 1 hr at room temp on a rotating shaker to remove the SDS and renature your proteinases) with 2.5% TritonX100 in 50 mM tris pH 7.4, 5 mM CaCl2, 1 microM ZnCl2.

Rinse briefly with deionized water.

Incubate overnight at 37 degrees C with 50 mM Tris pH 7.4, 5 mM CaCl, 1 microM ZnCl2, (optional 0.01% sodium Azide).

Stain with 0.5% Coomassie G250 in 30% Ethanol, 10% acetic acid for 30 minutes
(entire gel should be dark blue)

Destain in 30% Ethanol/10% acetic acid until you see clear bands-- this can happen fast (a couple of minutes).

Change to 2% acetic acid to stop staining.

Rehydrate in 2% acetic acid overnight