Gelatin Zymography Protocol

Key Steps:

1) Transfect MMP producing cell line (C33a) with expression vector containing protein of interest.
2) Collect media conditioned with MMPs
3) If desired concentrate protein in media
4) Run on 10% SDS-PAGE with 0.1% gelatin
5) Incubate gel for 24 hours and then stain

Day 1

1) Plate C33a cells in appropriate size plate so that they will be 80% confluent the following day. (Works best using 10cm plates)

Day 2

1) Transfect C33a cells when 80 percent confluent using Lipofectamine 2000 and incubate for 24 hours.
   * Only use media w/o FBS and Pen/Strep
     - Using Pen/Strep with Lipo 2000 will kill the cells
     - FBS contains Bovine MMPs which will show up on the zymograph

Day 3

1) Make a 10% SDS-PAGE gel containing 0.1% (1mg/ml) gelatin in the resolving gel.
   * Do not use buffers containing EDTA.
   * Make stock gelatin fresh every time at a concentration of 10mg/ml.
   * Use gelatin immediately after making.
2) Collect the media off of the cells and spin at 10,000 rpm for 5mins to remove any cell debris.
3) Concentrate approx. 6mls of media down to 300ul using Centricon 10.
4) Mix 20ul of media with 2x SDS loading buffer w/o 2-mercaptoethanol.
   * Optional: incubate the samples at 37°C for 20 mins.
     Might help activate the MMPs???
5) Run gel at 4°C at 150-200V until the 50kD marker leaves the gel.
6) Cut off marker lanes
7) Wash gels 4 times for 30 mins each at room temperature in washing buffer.
   * The buffer removes the SDS from the gel allowing the protein to refold
8) Wash gels 30mins in ddH_2O
9) Incubate the gel for at least o/n (generally 24-48h) at 37°C in the incubation buffer.

Day 4

1) Quickly rinse the gel with distilled water.
2) Stain the gel with Comassie Brilliant Blue solution for 1 hour.
3) Destain with destain solution for 30mins-1hr or until there is good resolution between the bands and blue background.

4) Image the gel using the Fuji Imager BEFORE drying.

Buffers and Reagents

Stock Gelatin
0.1 g gelatin
ddH_2O to 10ml

5X Resolving Gel Buffer pH 8.8
63.58g Tris
2.5g SDS
ddH_2O to 250ml
Adjust pH to 8.8

5X Stacking Gel Buffer pH 6.8
47.75g Tris
6.25g SDS
ddH_2O to 250ml
Adjust pH to 6.8

SDS-Loading Buffer
1.25ml 1M Tris, pH 6.8
2.0ml 20% SDS
3.75ml ddH_2O
2.0ml Glycerol

Washing Buffer
25ml 1M Tris, pH 7.6
5ml 1M CaCl2
0.2g NaN3
500ul 0.5M ZnCl2
12.5ml Triton X
ddH_2O to 1L

Incubation Buffer
25ml 1M Tris, pH 7.6
5ml 1M CaCl2
0.2g NaN3
75ml 5M MaCl
ddH_2O to 1L

Comassie Brilliant Blue
40% MeOH
10% Acetic Acid
0.1% CCB

Destain Solution
40% MeOH
10% Acetic Acid