abcam

Gelatin zymography protocol

Detect MMP activity in conditioned media.

abcam

Gelatin zymography protocol

Gelatin zymography is a method to detect activity of gelatinase enzymes, such as the matrix metalloproteinases (MMPs) MMP-2 and MMP-9. Active gelatinases digest gelatin embedded in a polyacrylamide gel. After Coomassie staining, areas of degradation are visible as clear bands against a darkly stained background.

This protocol is optimized for detecting secreted MMP-9 and MMP-2 activity in conditioned media.

Preparation of conditioned media

1. Plate cells cultured in fetal bovine serum (FBS) in a six-well plate (2 mL/well) or in a 75 cm^2 flask (10 mL)
2. At 70–80% confluency, remove FBS media, wash cells twice with RBS-free media and continue to grow cells in FBS-free media.

   The duration of growth in FBS-free media must be optimized for the cell line: for example, 231G and 468 breast cancer cells require a 40–44 h growth period before collection of conditioned media.

3. Collect conditioned media and centrifuge or filter to eliminate dead cells
4. Concentrate conditioned media 10X

Gelatin zymography

Running the gel

1. Adjust conditioned media in all samples to the same protein concentration.

   For each sample, test one aliquot at a low protein concentration (5 µg/mL) and one at a high protein concentration (15 µg/mL).

2. Add 5x non-reducing sample buffer to your samples
3. Prepare a 7.5% acrylamide gel containing gelatin. Use a 1 mm thickness plate.

Separating gel (7.5 % acrylamide)		Stacking gel	
1.5 M Tris pH 8.8	2 mL	0.5 M Tris pH 6.8	1.25 mL
30% acrylamide	2 mL	30% acrylamide	0.670 mL
H2O	2 mL	H2O	3.075 mL
Gelatin (4 mg/mL)	2 mL	10 % SDS	50 µL
10% SDS	80 µL	10 % APS	50 µL
10% APS	80 µL	TEMED	10 µL
TEMED	10 µL		

4. Load sample to each well; typically, 10 µL protein per well is suitable. Include a protein molecular weight marker in one well. Run the gel at 150 V until good band separation is achieved.

Gel washing and staining

1. Wash the gel 2 x 30 min with washing buffer.

   Soaking the gel in washing buffer removes SDS from the gel.

2. Rinse for 5–10 min in incubation buffer at 37°C with agitation.
3. Replace with fresh incubation buffer and incubate for 24 h at 37°C.

   The incubation buffer contains cofactors necessary for the gelatinase reaction to occur.

4. Stain the gel with staining solution for 30 min to 1 h. Rinse with H_2O.
5. Incubate with destaining solution until bands can clearly be seen.

   Areas of enzyme activity appear as white bands against a dark blue background.

Buffers

5X non-reducing sample buffer

Final concentration	For 250 mL
4% SDS	10 g
20% glycerol	50 mL of 100%
0.01% bromophenol blue	0.025 g
125 mM Tris-HCl, pH 6.8	4.91 g

Washing buffer

Final concentration	**For 250 mL**
2.5% Triton X-100	6.25 mL of 100%
50 mM Tris-HCl, pH 7.5	12.5 mL of 1 M stock
5 mM $CaCl_2$	625 µL of 2 M stock
1 µM $ZnCl_2$	2.5 µL of 0.1 M stock

Incubation buffer

	For 250 mL
Triton x 100 1 %	2.5 mL of 100 %
Tris HCl 50 mM pH 7.5	12.5 mL of 1 M
5mM $CaCl_2$	625 µl of 2 M
1 µM $ZnCl_2$	2.5 µl of 0.1 M

Staining solution

	For 100 mL
Methanol	40 mL
Acetic acid	10 mL
H_2O	50 mL
Coomassie Blue	0.5 g

Destaining solution

	For 1 L
Methanol	400 mL
Acetic acid	100 mL
H_2O	500 mL