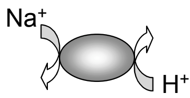
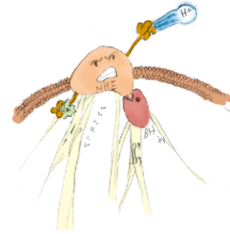
MMP Zymogen Gel Assay

Protocol



Wallert and Provost Lab

Excel with hard work and inquiry



INTRODUCTION: *Proteolytic degradation and remodeling of the extra cellular matrix (ECM) is largely controlled by a superfamily of Zn(2+)-dependent extracellular enzymes called matrix metalloproteinases (MMPs). So far, 15 MMPs have been cloned and characterized from humans. Gelatinases A and B, also known as 72 kDa and 92 kDa type IV collagenases (MMP2 and **MMP9**) digest denatured collagen (gelatin) and other ECM components including basement membranes. Processing of the precursor yields different active forms of 64, 67 and 82 kDa. Sequentially processing by MMP3 yields the 82 kDa matrix metalloproteinase-9.*

One method to assay MMP activity is to concentrated conditioned (serum free media – serum contains protease inhibitors) by ultrafiltration or lyophilization and running the protein on a native SDS PAGE gel saturated with gelatin (B-Casein). After electrophoresis, the gel is incubated in a buffer which includes triton X-100. The detergent and buffer allows the protein to renature and then the protease will hydrolyze the gelatin. After staining and destaining, clear bands indicate a protease is present. Molecular weight standards are required to learn the molecular weight of the active band.

PROTOCOL:

Preparing MMP Sample

1) Culture 35mm dish to >90% confluence of cells.
2) 12-18 hour prior to assay rinse cells and incubate in 0.5% serum media
3) 1 hour prior to stimulation, remove media, rinse with PBS and replace with 1 ml of serum free media
4) In 0% serum media, add agonist treatment.
5) Allow cells to incubate 24 hrs at 37 °C.
6) Collect 1.00 ml media (for fairly active sample). If low MMP activity, collect 2.0mL media.
7) Centrifuge cells in microfuge (max RPM for 10 min) to remove cell debris. Transfer media to new tubes.
8) Lyophilize media to dryness. *(May take 5-8 hrs)* **Freeze sample if not using right away.**
9) Rehydrate in designated volume Zymogen Loading Dye. If low activity, use 40uL and load 25uL. If higher MMP activity, add up to 100uL. DO NOT HEAT SAMPLES BEFORE RUNNING GEL.

Zymogen Gel

1) Load samples onto zymogen using SDS-PAGE Running Buffer. Run until the blue Zymogen Loading Dye has reached the bottom of the gel (~90 min).
2) After electrophoresis, incubate gel at room temperature in 0.01% Triton X-100 for 1 hr with gentle rocking.
3) Decant solution and incubate gel in Zymogen Buffer 24 hours at 37°C.
4) Incubate 1 hour in Coomassee Blue Stain. *A stain with 0.5%% (w/v) Coomassie Blue-R 250 may give better contrast.*
5) Incubate 1 hour in Destain or until the contrast of bands is obtained. Then remove destain and incubate in ddH_2O.

Zymogen Buffer (1L):
0.1M Tris-HCl pH 8.0 (12.1g)
1 mM $CaCl_2$ (.11g)
50 mM NaCl
2% Triton X-100
(Note – some use 0.02% Brij 35 buffer and or 1 μM Zn+)

Zymogen Loading Dye (5mL):
0.0625 mL 0.5M Tris pH 6.8
2mL 20%SDS
2g sucrose
5mg bromophenol blue dye
QS with ddH_2O