Protocol for Zymogram

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Reagents:

1% Gelatin in H_2O (Fisher Blood 275)
1% Casein (Sigma)
SDS-PAGE gel stock w/o urea
Wash buffer: 2.5% Triton X-100 in H_2O (+0.02% NaN3)
Incubation Buffer: 50mM Tris-HCl (PH8.0), 5mM CaCl2, 0.02% NaN3

Gels:

Regular separating gel containing 10-12% substrate
Regular stacking gel

Protocol:

1- Collect media from cells
(if desired, inactivate non-metalloproteases with PMSF and/or NEM)
2- Centrifuge to remove cellular debris
(if necessary concentrate with centricon units or dialyze and lyophilize)
3- Add Laemmli loading buffer **(OMIT UREA AND REDUCING AGENTS, DO NOT HEAT)**
4- Load samples directly onto gel
5- Run gel
6- Wash 2X 20min in wash buffer
7- Wash 10min in incubation buffer
8- Place gel in sealable container with fresh incubation buffer and incubate at 37°C for 24h to 48h
9- Fix and stain with fresh Coomassie Blue solution
10-Destain with MeOH:AcOH:H_2O(5:1:5)
11-Replace with 10% AcOH and continue destaining
12-Photpgraph and dry gel for storage