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Protocol for Zymogram

Reagents:

1% Gelatin in H2O (Fisher Blood 275)
1% Casein (Sigma)
SDS-PAGE gel stock w/o urea
Wash buffer: 2.5% Triton X-100 in H2O (+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 37ºC for 24h to 48h
9. Fix and stain with fresh Coomassie Blue solution
10. Destain with MeOH:AcOH:H2O(5:1:5)
11. Replace with 10% AcOH and continue destaining
12. Photograph and dry gel for storage