

GELATIN/CASEIN ZYMOGRAPHY

- **For gelatinase KO phenotyping:** collect a droplet of blood right after cutting the tail, resuspend in 20 ul water and run 5 -10ul with loading buffer.

Loading buffer:	1X	4X
- Tris	62.5	250 mM pH 6.8
- SDS	2%	8%
- Glycerol	10 %	40%
- bromophenol blue	0.1%	0.4%

- **Migration :** Acrylamid gel with 1 mg/ml gelatin;
 For casein zymogram use 1 mg casein per ml of gel,
 For fibronectin zymogram, use 0.33 mg fibronectin per ml of gel.

Gel	3%	4%	5%	6%	7%	8%	10%	12%	14%
Gelatin or casein solution					2 mg/ml or 40mg/20ml	2.15 mg/ml or 43mg/20ml	2.65 mg/ml or 53mg/20ml		
Gelatin solution or water	13.21	12.51	11.81	11.11	10.41	9.71	8.31	6.91	5.51
1.5M Tris pH 8.8	5.25	-	-	-	-	-	-	-	-
30% Acrylamide	2.1	2.8	3.5	4.2	4.9	5.6	7	8.4	9.8
50% Glycerol	0.165	-	-	-	-	-	-	-	-
10% SDS	0.165	-	-	-	-	-	-	-	-
Temed	0.010	-	-	-	-	-	-	-	-
10% APS	0.100	-	-	-	-	-	-	-	-

All volumes in ml

Stacking gel:

	1	1/2	1/2	1
H2O	4.75 ml	2.375 ml	2.375 ml	4.75 ml
30% Acrylamid-bis	0.75 ml	0.375 ml	0.375 ml	0.75 ml
0.5 M Tris pH 6.8	1.9 ml	0.95 ml	0.95 ml	1.9 ml
10% SDS	75 ul	37.5 ul	37.5 ul	75 ul
10% APS	75 ul	37.5 ul	37.5 ul	75 ul
Temed	10 ul	5 ul	5 ul	10 ul
		% Glycerol	0.075	0.165