Western Blotting

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Blotting buffer (Laemmli - SDS)

2 x Tris-glycine (ph8.5)	500 ml
MeOH	200 ml
add water to 1000 ml	

Procedure

1. remove the gel from the plate
2. immerse it into the blotting buffer
3. sandwitch the nitrocellulose sheet and the gel between filter sheets (Those sheets should be preincubated.)
4. Put the above sheets in the blotting apparatus: red side is nitrocellulose side (red is plus)
5. 200 mA, overnight for drebrin transfer

Staining with amidoblack

staining solution:	45% MeOH	45 ml
	10% AcOH	10 ml
	0.1% Amido Black	0.1g
	add water to	100 ml

Immunostaining

1.	incubation in 10% milk		60 min
2.	brief PBs Wash		
3.	first antibodyb in PBSA (3% albumin)		60 min
4.	PBS wash	5 min	
5.	incubation in 10% milk		5 min
6.	brief PBS wash		
7.	second antibody in in PBSA (3% albumin)		60 min
	(20 µl in 5 ml)		
8.	PBS wash	15 min x 3	
9.	reaction		

HRP reaction

solution A:	0.3% chloronaphthol (12 mg in 4 ml MeOH)
solution B:	0.3% hydrogen peroxide (x 1/100)
solution C:	imidazole 27 mg/ml

Procedure

1. 20 ml of PBS + 4 ml of A + 0.8 ml of B + 0.5 ml of C
2. wash in water