	For final concentration of gel (% T):					
	Separating gel (10 ml)				Stack gel (10 ml)	stack gel 5 ml
	7%	**10%**	**12,5 %**	**15%**	**5%**	
30% Acrylamide-bis solution 29:1 (A)	2.33	3.33	4.17	5	1.67	0.835
4x Separation buffer **1.5 M Tris/HCl pH 8.8**	2.5	2.5	2.5	2.5		0
4x Stacking buffer **0.5 M Tris/HCl pH 6.8 + phenol red**					2.5	1.25 0
aqua dest.	5	4	3.2	2.4	5.7	2.85
SDS (10 %)	0.1	0.1	0.1	0.1	0.1	0.05
TEMED	0.015	0.015	0.015	0.015	0.015	0.0075
APS (10%)	0.03	0.03	0.03	0.03	0.03	0.015
total ml	9.98	9.98	10.02	10.05	10.02	
Acryl in (A)	0.699	0.999	1.251	1.5	0.501	
Acryl %	7.01	10.02	12.49	14.93	5.00	

SDS-sample buffer

	final conc	6x
Tris/HCl pH6.8	0.125	0.75
Glycerol	10%	60%
SDS	2%	12%
DTT		739 mg
Bromophenol blue		4.8 mg

6X Sample Buffer (with DTT)

1 M Tris-Cl (pH 6.8)	2.4 ml
SDS	0.96 g
Glycerol	4.8 ml
DTT	739 mg
Bromophenol Blue	4.8 mg

> ca. 600 m > final conc. 100mM

Do not adjust the volume.

Reduction: either by 2% β-Mercaptoethanol
(final conc., equals about 255 mM SH-groups, would mean about 125 mM DTT with 2 SH groups

STRIPPING BUFFER (2% SDS, 62.5mM TRIS pH6.8, 100mM Beta-mercaptoethanol (bME)

Reagent	ml for 500ml	ml for 100 ml
20% SDS	50	10
1M TRIS pH6.8	31.25	6.25
H2O	418.75	83.75

****ADD THE bME FRESH BEFORE**

beta-Mercapto	3.55	0.71

Incubate the membrane for 15 - 30 min at 50ºc submerged in stripping buffer.