

Western blotting protocol summary

RPN2106/8/9

STAGE	1 Electrophoresis and blotting	2 Block	3 Wash	4 Primary antibody	5 Wash
REAGENT		5% blocking reagent in TBS-T or PBS-T	TBS-T or PBS-T	Diluted in TBS-T or PBS-T	TBS-T or PBS-T
VOLUME USED		10 ml	10 ml	10 ml	10 ml
TIME	Usual electrophoresis and blotting times	1 hour	1 × 15 min 2 × 5 min	1 hour	1 × 15 min 2 × 5 min <i>see over</i>

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.



6 Biotinylated antibody or HRP labelled antibody	7 Wash - if using HRP labelled antibody omit steps 7 and 8	8 Streptavidin - HRP	9 Wash	10 Detection	11 Exposure
Diluted in TBS-T or PBS-T	TBS-T or PBS-T	Diluted in TBS-T or PBS-T	TBS-T or PBS-T	Mix the two reagents 1:1	Drain excess reagent, cover with Saran Wrap™ Immediately
10 ml	10 ml	10 ml	10 ml	0.125 ml/cm ²	expose to film
20 min	1 × 15 min	20 min -	1 × 15 min	1 min	for 30 seconds
1 hour	2 × 5 min	1 hour	4 × 5 min		- 10 min

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