

PrP Immunohistochemistry Worksheet

IHC Run reference & Date	PRIMARY ANTIBODY Mouse monoclonal DILUTION in TBST	BIOTINYLATED ANTIBODY Vector Goat anti mouse IgG: 1 drop, 10ml TBST, 3 drops normal goat serum	CONJUGATE Vector ABC Elite 5 ml TBST 2 drops A 2 drops B mix and stand for 30 mins. (Do not vortex mix)
SLIDES HEATED to 60°C for 30 mins to melt wax to aid adhesion	EPI TOPE DEMASKING FORMIC ACID 30min RUNNING TAP WATER 15 min CITRATE BUFFER pH6.1 30 min at 121°C in autoclave	WASH BUFFER TBST	CHROMOGEN 1 DAB tablet 20 ml TBST 12ul H ₂ O ₂ Filter before use
BLOCKING SERUM Vector Normal goat serum 3 drops to 10ml TBST			

	<i>Sign and date at each stage</i>	<i>Time</i>	<i>Initials</i>	<i>Comments & Variation</i>
1	Dewax Xylene <input type="checkbox"/> Xylene <input type="checkbox"/> Alcohol <input type="checkbox"/> Alcohol <input type="checkbox"/>	5 mins each		<i>(check that temperature is above 18C)</i>
2	Endogenous peroxidase block 3% Hydrogen Peroxide (30%v/v) in Methanol	10 mins		Fume cupboard
3	Running water	5mins		
4	Formic acid epitope unmasking <i>(Undiluted)</i>	30 mins		Fume cupboard
5	Running water <i>(Tap water or purified water)</i>	15 mins		
6	Citrated Autoclaving pH 6.1 at 121°C With glass beads & leave dish uncovered	30 mins		Total time from heating up to cooling down
7	Slowly add water to reduce temperature	5 mins		
8	Clip slides into Sequenza (purified water) Or lay out in humid chamber			Hold slides overnight at room temperature if required
9	TBST Buffer rinse	5 mins		
10	Normal Goat serum Do NOT wash	20 mins		Room temperature °C
11	Primary Antibody: Dilution:	60 mins		Room temperature °C
12	TBST Buffer wash <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	3 x 5 mins		
13	Biotinylated antibody (goat anti mouse)	30 mins		
14	TBST Buffer wash <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	3 x 5 mins		
15	Conjugate - Vector ABC	30 mins		Prepare 30 mins before use
16	TBST Buffer wash <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	3 x 5 mins		
17	Chromogen DAB * check <input type="checkbox"/> apply <input type="checkbox"/>	10 mins		Filter before use
18	Purified water rinse + un-clip slides			
19	Mayer's Haematoxylin counterstain			Establish time required for each batch of Mayer's
20	Tap water wash and to blue haematoxylin	10 mins		If water pH is too acidic to blue the haematoxylin, use alkaline Scott's tapwater, or ammoniated water.
21	Dehydrate Alc <input type="checkbox"/> Alc <input type="checkbox"/> Clear Xyl <input type="checkbox"/> Xyl <input type="checkbox"/>			
22	Coverslip with resin mountant			
23	Dry & label slides			

Apply 200µl immunological reagent & 400µl of chromogen per slide

* Note if alcohol soluble chromogen is used, then slides must not be dehydrated at step 21 and must be mounted using aqueous mountant.

