

## Microwave Pre embed antibody Protocol for Electron Microscopy

1. Anesthetize the mouse/rat and perfuse transcardially with 3-6 ml 0.1M phosphate buffer (PB, pH 7.3) with 1000 units/ml of heparin, respectively, followed by 1% glutaraldehyde/ 0.5 % paraformaldehyde/0.1% picric acid in 0.1M PB. Fix brain tissue overnight in the cold then wash with PB.
2. Cut 60-70µm sections on a vibratome and place into cold 0.1M PB.
3. Place sections into well plate and continue with microwave pre-embed immune protocol below

\*note: for the procedure below in the microwave, use just enough liquid to cover tissue, approx. 100ul/well.

STEPS#	Description	time (hr:min:sec)	Watts	Temp	Repetitions	Vacuum
1	ANTIGEN RETREIVAL	0:05:00	550	35	1	CYCLE
2	PBS RINSE	0:01:00	150	35	1	OFF
3	0.3% HYDROGEN PERIOXIDE	0:01:00	150	35	1	OFF
4	PBS RINSE	0:01:00	150	35	3	OFF
5	0.5%TRITON X 100	0:05:00	550	35	1	CYCLE
6	PRIMARY Ab	0:12:20*	200	35	1	CONT
7	DILUTE BLOCKING RINSE	0:01:00	150	35	2	OFF
8	SECONDARY Ab	0:15:20 <sup>2</sup>	200	35	1	CONT
9	IMIDAZOLE RINSE	0:01:00	150	35	2	OFF
10	ABC IN IMIDAZOLE	0:15:20 <sup>2</sup>	150	35	1	CONT
11	IMIDAZOLE BUFFER RINSE	0:01:00	150	35	2	OFF
12	DAB	0:05:20 <sup>3</sup>	200	35	1	CONT
13	IMIDAZOLE BUFFER RINSE	0:01:00	150	35	2	OFF
14	PBS RINSE	0:01:00	150	35	2	OFF

\* =10SEC PREVAC(CYCLE)/ 2 MIN WATTS ON(CONT VAC)/2 MIN WATTS OFF(CONT VAC)/2 MIN WATTS ON(CONT VAC)/5 MIN WATTS OFF(CONT VAC)/10 SEC FINISH(NO VAC)

<sup>2</sup> = 10SEC PREVAC(CYCLE)/ 4 MIN WATTS ON(CONT VAC)/3 MIN WATTS OFF(CONT VAC)/4 MIN WATTS ON(CONT VAC)/5 MIN WATTS OFF(CONT VAC) /10 SEC FINISH(NO VAC)

<sup>3</sup> = 10SEC PREVAC(CYCLE)/5 MIN WATTS ON /10 SEC FINISH(NO VAC)

DAB Can be done on the countertop while watching reaction.

### Solutions:

Antigen retrieval:10mM sodium citrate 6.0 or 10mM Tris-1mM EDTA 9.0, which is antibody dependent. This will need to be customized for each antibody.

Dilute Blocking solution: 1%Normal goat serum; 1% BSA;0.3% TWEEN 20

Secondary: biotinylated anti-mouse, -rabbit, -rat, -goat, etc: 1:50 dilution

ABC: ABC Elite Kit from Vector; 1ul/ml of each solution A and B in PBS

Imidazole Buffer: 75.5mls of Millipore filtered deionized water; 17.5mls of 1M NaAc pH 7.2; 5mls of 0.2M imidazole

DAB: 0.5ug/ml+1.5% hydrogen peroxide