**Kordower Lab: Immunohistochemistry protocol**

**DAY 1 (**4 hrs**):**

1. Wash sections (6 x 10 min) in DM.
2. Endogenous peroxidase inhibition (20 min). 0.1M Sodium meta-periodate in TBS.
	1. 100 mL TBS + 2.13 g sodium meta-periodate
3. Wash (2 x 10 min) in DM
4. Serum blocking step (1 hour incubation):
	1. 100 mL DM
	2. 3 mL normal serum
	3. 2 g BSA
5. Incubation in primary antibody (18 - 72 hrs) \*\*Optionally, refrigerate to keep antibody stable\*\*
	1. 100 mL DM
	2. 1 mL normal serum
	3. 1 g bovine serum albumin (BSA)
	4. 0.5 mL triton X100

**DAY 2 (**8 hrs**):**

1. Wash (6 x 10 min) in DM
2. Secondary antibody incubation (1 hour) Concentration of secondary antibody is 1:200
3. 100 mL DM
4. 1 mL normal serum
5. 1 g BSA
6. Wash (6 x 10 min) in DM **\*\*(incubate ABC in solvent)\*\***
7. Avidin-Biotin Complex (ABC) Step (60 min) - Vectastain ABC Kit
8. 100 mL DM
9. 1 mL normal serum
10. 1 g BSA

Add Reagent A and B to 1/10th of total desired volume of solvent. **Incubate for 30 min** at room temperature. Then dilute 1:10 using the same solvent. This is your working solution. See chart below for example volumes.

|  |  |  |  |
| --- | --- | --- | --- |
| Working Solution | A (drops) | B (drops) | 1/10th Working solution |
| 25 mL | 1 | 1 | 2.5 mL |
| 50 mL | 2 | 2 | 5 mL |
| 100 mL | 4 | 4 | 10 mL |

1. Wash (1 x 10 min) in DM
2. Wash (1 x 10 min) with TBS
3. Wash (3 x 10 min) in 0.2 M Imidazole/1.0 M sodium acetate buffer, pH to 7.2 - 7.4
4. 1000 mL dH2O
5. 0.68 g imidazole
6. 6.8 g sodium acetate.
7. Retain 100 mL of non-pH’d buffer for DAB preparation.
8. DAB step (**develop 4-7 min**, check under microscope)
9. Make DAB solution
	* 1. 100 mL non-pH'd imidazole acetate buffer from above
		2. 50 mg DAB
		3. 2 g nickel sulfate **\*\*(Only with certain primary antibodies)\*\***
10. Make 1% H2O2
	* 1. 3 mL of dH20
		2. 100 µL of 30% hydrogen peroxide
11. Start reaction -- add 500 µL of 1% H2O2 to the above DAB mixture just prior to use.
12. Place tissue in DAB
	* 1. Develop tissue for approximately 4-7 min, check under microscope.
13. To monitor signal place tissue in imidazole buffer and view under microscope. Place back in DAB solution to increase signal intensity.
14. Wash developed tissue in imidazole acetate buffer (3 x 10 min). Store tissue in PBS (refrigerated).