**IHC: Tyramine Signal Amplification(TSA)** (Kordower Lab IHC)

**DAY 1 (**3.5hrs**):**

1. Wash sections (6 x 5 min) in DM.
2. Endogenous peroxidase inhibition (20min). ~0.1M Sodium meta-periodate in TBS.
	1. 2.13g sodium meta-periodate + 100mL TBS (20min)
3. Wash (2 x 10 minutes) 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 \*(18h-72h)\* refrigerate to keep antibody stable.
	1. 100 mL DM
	2. 1 mL normal serum
	3. 1 g bovine serum albumin (BSA)
	4. 0.5mL triton X100

**DAY 2 (**4hrs**):**

1. Wash (6 x 5 minutes) in DM
2. Secondary antibody incubation (1 hour) Concentration of secondary antibody is 1:500.
3. 100 mL DM
4. 1 mL normal serum
5. 1 g BSA
6. Wash (6 x 5 minutes) in DM **\*\*(incubate ABC in solvent)\*\***
7. Actin Biotin Complex Step (60 min)-Vectastain ABC Kit**.**

**\*\*Re-use for step 10. Can be stored overnight in fridge.\*\***

1. 100 ml DM
2. 1 mL normal serum
3. 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 | 2.5mL |
| 50 mL | 2 | 2 | 5mL |
| 100mL | 4 | 4 | 10mL |

1. Wash (1 x 10 minutes) in DM.
2. Wash (1 x 10 minutes) in TBS.
3. Wash (2 x 10 minutes) 0.05M Borate buffer pH 8.5.
4. 1000mL dH2O
5. 4.77g sodium tetraborate decahydrate
6. 2.32g Boric Acid
7. Incubate sections with biotin tyramide solution (30 min) DO NOT USE IF >6 MONTHS OLD.
8. 100mL borate buffer
9. 2ul of 50mg/mL biotin tyramid stock
10. 10ul 30% H202
11. Wash (3 x 10 min) with TBS (Antigen is now labeled with biotin)

**DAY 3 (**4hrs**):**

10. Repeat Step 4 then wash (1 x 10 min) with TBS.

11. Wash (3 x 10 minutes) in 0.2 M Imidazole/1.0 M sodium acetate buffer, pH to 7.2-7.4

1. 1000 mL dH2O
2. 0.68 g imidazole
3. 6.8 g sodium acetate.
4. Retain 100mL of non-pH’d buffer for DAB preparation.

12. DAB step (Neutralize DAB with bleach when done)

1. Make DAB solution
	* 1. 100 mL non-pH'd imidazole acetate buffer from above
		2. 50 mg DAB
		3. 2 g nickel sulfate \*(For specific Primary Ab)\*
2. Make 1% H202
	* 1. 100uL of 30% hydrogen peroxide
		2. 3mL of dH20
3. Start reaction -- add 500ul of 1%H202 to the above DAB mixture just prior to use.
	* 1. OR add 16.7uL of 30% H2O2, per 100mL
4. Place tissue in DAB
	* 1. Develop tissue for approx. ~5 minutes.
5. To monitor signal place tissue in imidazole buffer and view under microscope. Place back in dab solution to increase signal intensity.

13. Wash developed tissue in imidazole acetate buffer (3 x 10 minutes). Store tissue in PBS (refrigerated).

**\*\*Neutralize DAB with BLEACH!!!\*\***

**HISTO- NOTES:**

* Primate tissue staining dishes **≈ 100mL**
* Rodent tissue staining dishes **≈ 50mL**
* If staining a large number of primate cases, incubate 1° & 2° Ab in individual cups to conserve volume of Ab used.
* Prepare bleach neutralizing solution prior to **Step 12.**
* Be conscious of tissue saturation while washing and incubating.\*\*
	+ i.e. Check that tissue is fully submerged in solution & not clumping. This will ensure proper penetration of antibodies & other reagents.
* **Positive & Negative Controls**
	+ Positive: Use relevant control tissue to confirm specific antibody detection.
		- i.e. pS129; control tissue should consist of nigral sections previously, successfully stained for pS129.
	+ Negative: Use tissue that does not contain the targeted antigen.
* When incubating 1° Ab overnight, leave on shaker in refrigerator.
	+ Can incubate in fridge on a shaker, covered in parafilm, over the weekend or **up to 3 days.**
* ABC working solution can be stored & re-used up to 24hrs after preparation (must be refrigerated).