

DEC 01, 2023

OPEN ACCESS



DOI:

dx.doi.org/10.17504/protocol s.io.8epv5x3mdg1b/v1

Protocol Citation: Jeffrey Kordower, Yaping Chu 2023. Immunohistochemistry Protocol for Free-floating Fixed Tissue. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8epv5x3mdg1b/v1

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Protocol status: Working We use this protocol and it's working

Created: Oct 19, 2023

• Immunohistochemistry Protocol for Free-floating Fixed Tissue

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ABSTRACT

Immunohistochemistry protocol for staining free-floating fixed tissue in the Kordower Laboratory.

ATTACHMENTS

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GUIDELINES

HISTO-NOTES:

- Primate tissue staining dishes use 🚨 100 mL solution per dish
- Rodent tissue staining dishes Solution per dish
- 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.
- Always include 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: Ideally, use tissue that you know does not contain the targeted antigen.
 If not available, use a section of tissue not incubated in the 1' Ab (primary delete).
- 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.
- Select a secondary antibody directed against the species in which the primary antibody was raised (i.e. if a primary antibody raised in rabbit is used, an antirabbit secondary antibody raised in a species other than rabbit must be used).

Last Modified: Dec 01, 2023

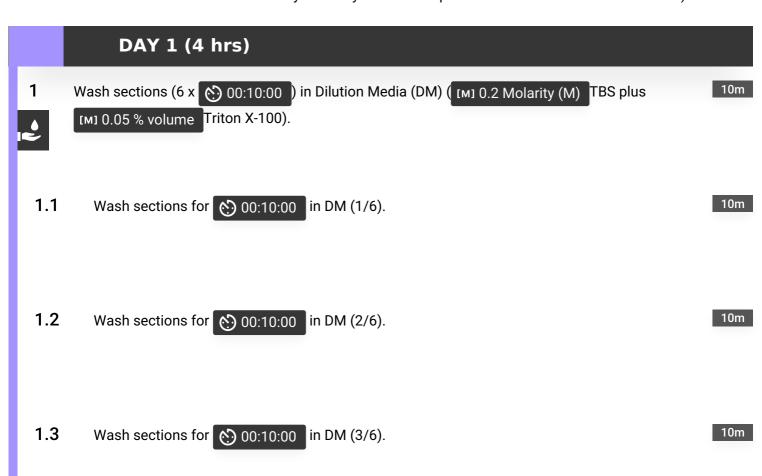
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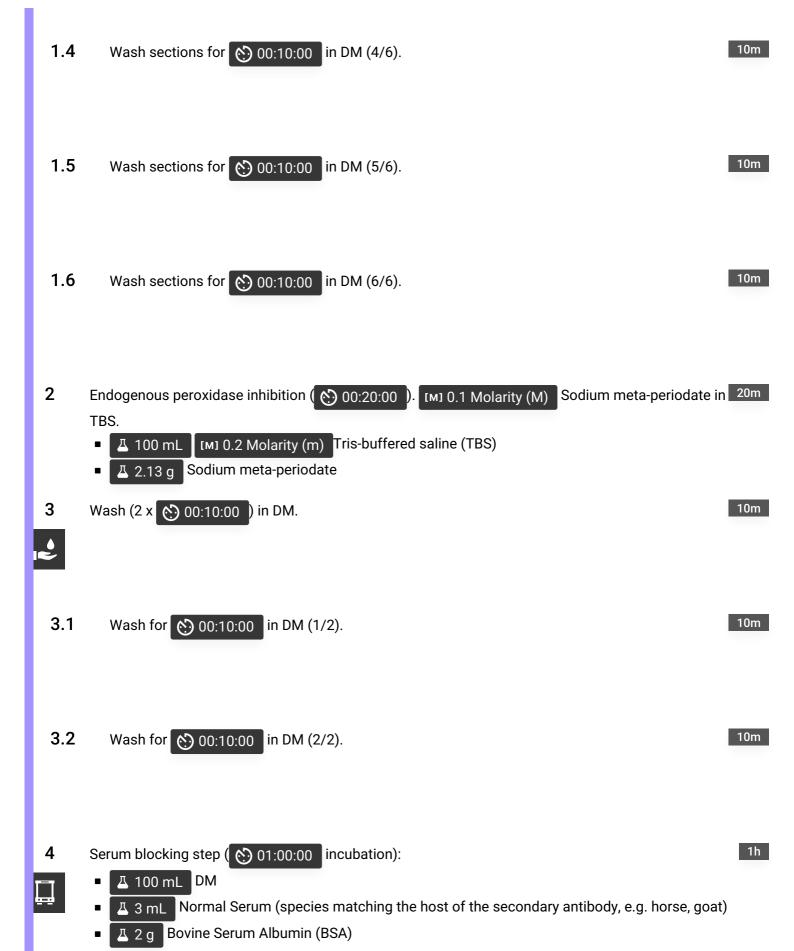
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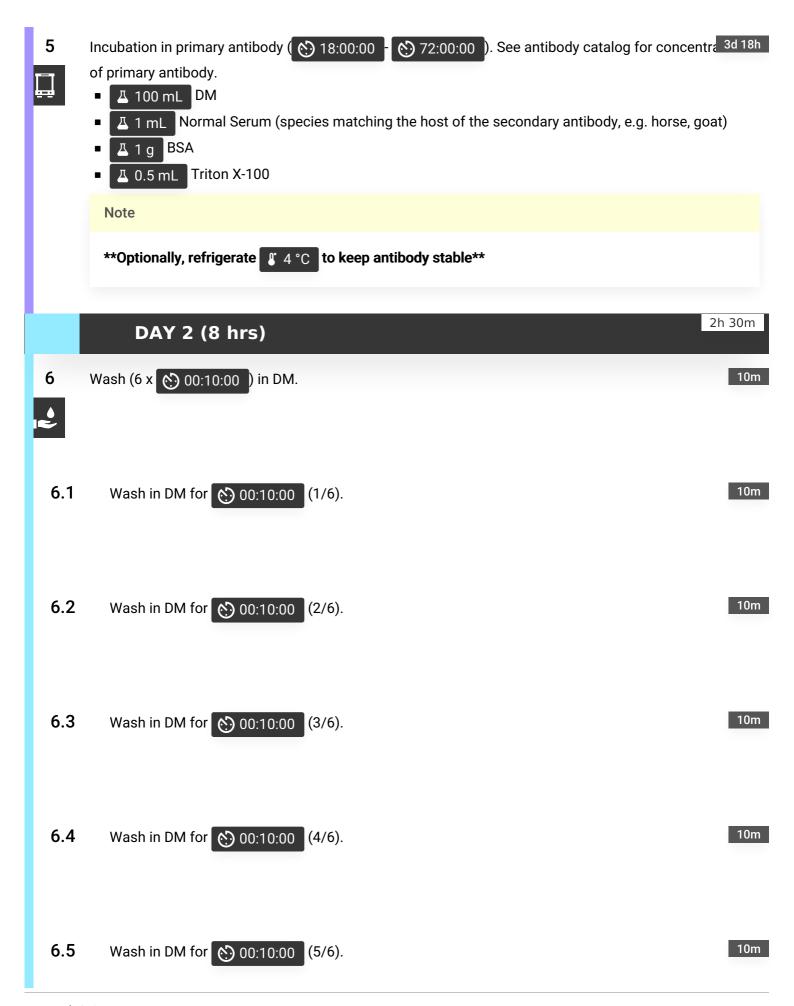
Immunohistochemistry

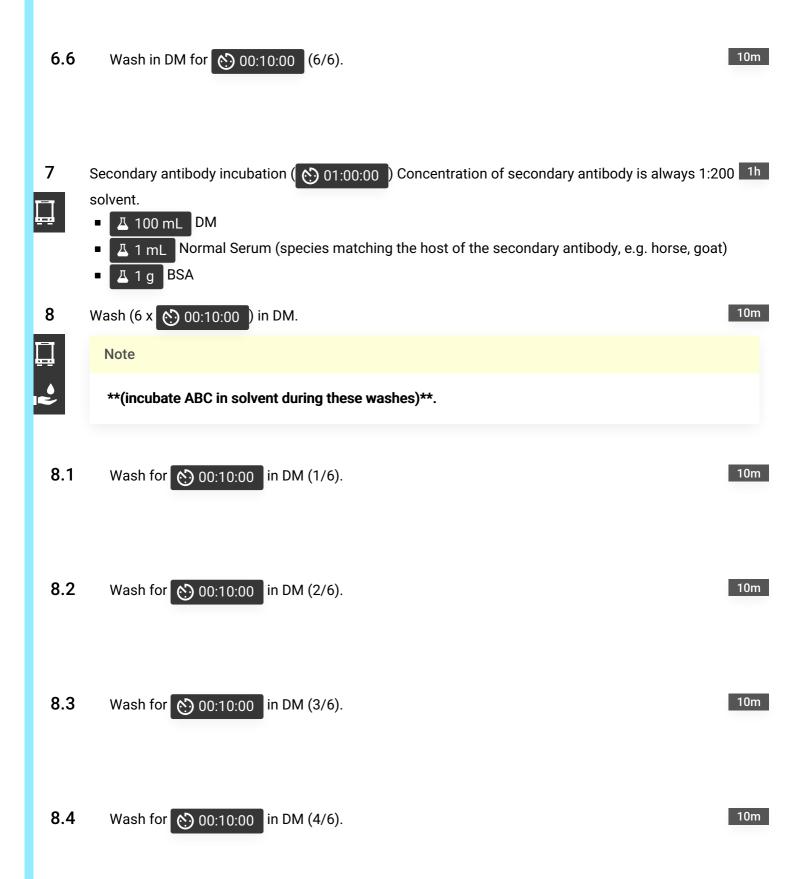
MATERIALS

- Dilution Media (DM) ([M] 0.2 Molarity (M) TBS plus [M] 0.05 % volume Triton X-100)
- [м] 0.2 Molarity (m) Tris-buffered saline (ТВЅ)
- Sodium meta-periodate
- Normal Serum (species matching the host of the secondary antibody, e.g. horse, goat)
- Bovine Serum Albumin (BSA)
- Triton X-100
- Vectastain Elite ABC-HRP Kit (PK-6100)
- Imidazole
- Sodium Acetate
- 3,3-Diaminobenzidine Tetrahydrochloride (DAB)
- [M] 30 % (V/V) hydrogen peroxide
- [м] 0.2 Molarity (m) Phosphate-buffered saline (PBS)
- Household Bleach
- Primary antibody against the target antigen
- Secondary antibody directed against the species in which the primary antibody was raised (i.e. if a primary antibody raised in rabbit is used, an anti-rabbit secondary antibody raised in a species other than rabbit must be used).









8.5 Wash for ৩0:10:00 in DM (5/6).

10m

8.6 Wash for 00:10:00 in DM (6/6).

10m

9 Avidin-Biotin Complex (ABC) Step (01:00:00) - Vectastain Elite ABC-HRP Kit (PK-6100).

- Д 100 mL DM
- A 1 mL Normal Serum (species matching the host of the secondary antibody, e.g. horse, goat)
- ∐ 1 g BSA
- 9.1 Add ABC Reagent A and B to 1/10th of total desired volume of solvent.



9.2 Incubate for 00:30:00 at Room temperature. Then dilute 1:10 using the same solvent.



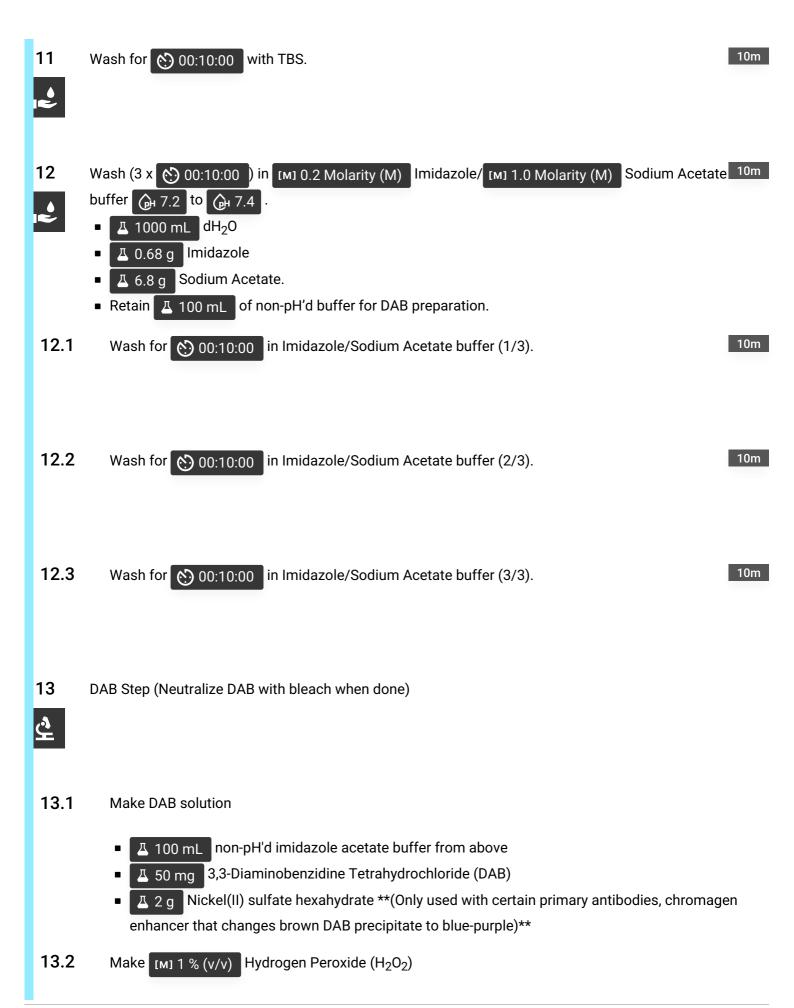
Note

This is your working solution. See chart below for example volumes.

A	В	c	D
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

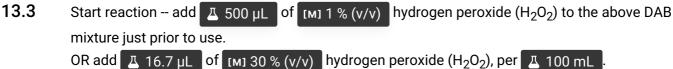
10 Wash for 00:10:00 in DM. 10m







 \perp 100 μ L of [M] 30 % (V/V) hydrogen peroxide (H₂O₂)



13.4 Place tissue in DAB solution. 11m

- Develop tissue for approximately () 00:04:00 to () 00:07:00
- Timing is critical, ensure all tissue spends the same amount of time in DAB solution.
- 13.5 To monitor signal, move all tissue to imidazole buffer, remove one section and mount on an UNSUBBED slide and view under microscope. Place all tissue back in DAB solution to increase signal intensity, if needed.
- 14 Wash developed tissue in imidazole acetate buffer (3 x 👏 00:10:00).

10m



Note

Neutralize DAB with BLEACH!!!

14.1 Wash developed tissue in imidazole acetate buffer for 00:10:00

10m

14.2 Wash developed tissue in imidazole acetate buffer for 00:10:00

10m

14.3 Wash developed tissue in imidazole acetate buffer for (5) 00:10:00



10m

Store tissue in MI 0.2 Molarity (m) Phosphate-buffered saline (PBS) in refrigerator 4 °C until mounted on slides.