

OCT 19, 2023

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DOI:

dx.doi.org/10.17504/protocol s.io.rm7vzx725gx1/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.rm7vzx725gx1/v1

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

Created: Oct 18, 2023

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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
- 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: Oct 19, 2023 MATERIALS

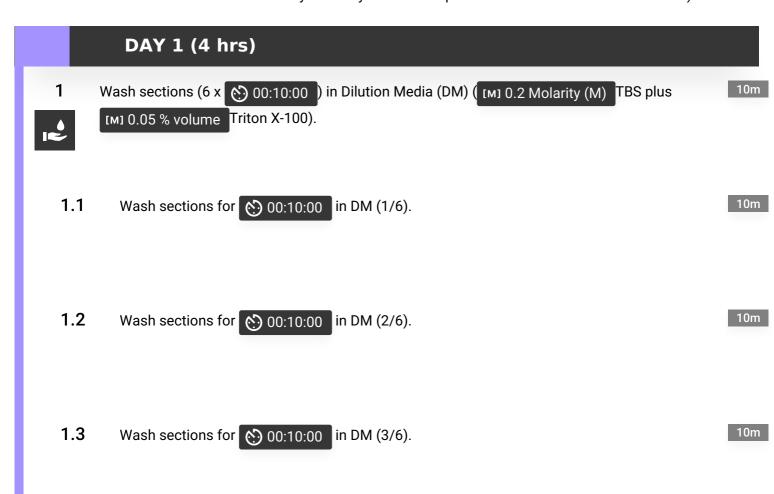
PROTOCOL integer ID:

89626

Keywords:

Immunohistochemistry

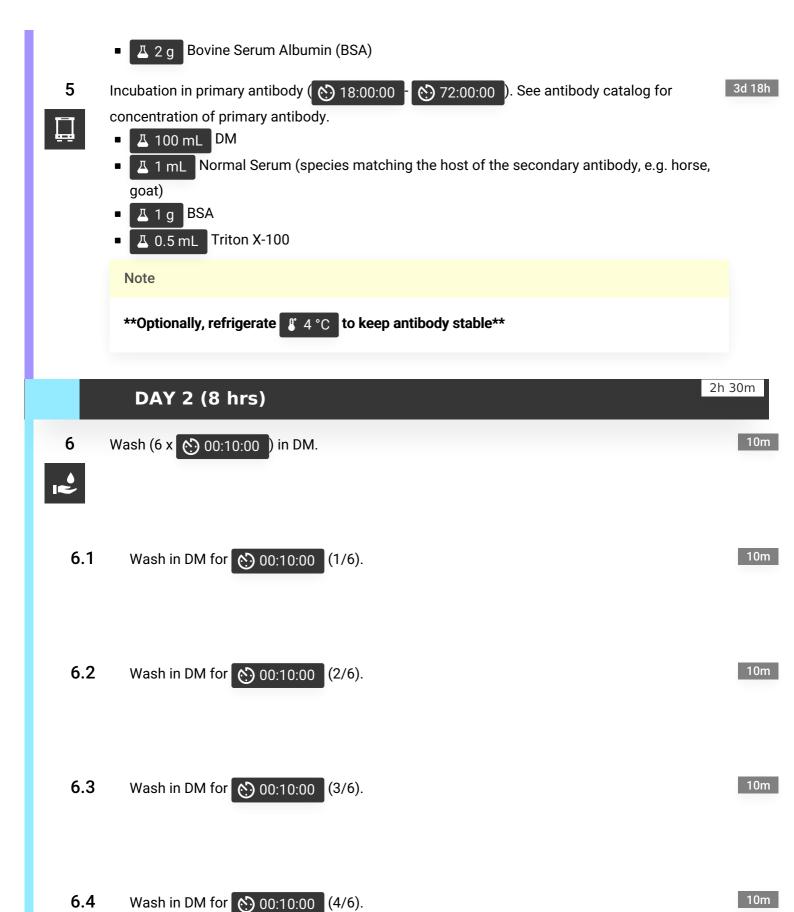
- Dilution Media (DM) ([M] 0.2 Molarity (M) TBS plus [M] 0.05 % volume Triton X-100)
- [м] 0.2 Molarity (m) Tris-buffered saline (TBS)
- Sodium meta-periodate
- Normal Serum (species matching the host of the secondary antibody, e.g. horse, goat)
- Bovine Serum Albumin (BSA)
- Triton X-100
- Vectastain 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).

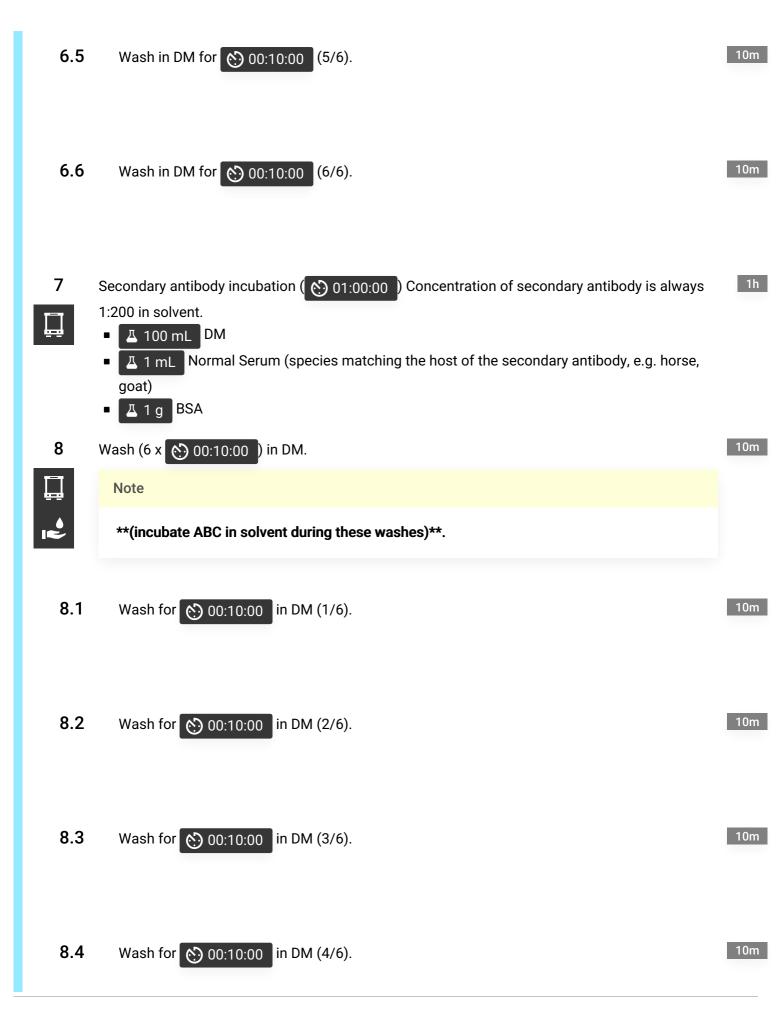


- 10m 1.4 Wash sections for 00:10:00 in DM (4/6). 1.5 10m Wash sections for (5) 00:10:00 in DM (5/6). 10m 1.6 Wash sections for (5) 00:10:00 in DM (6/6). 2 20m Endogenous peroxidase inhibition () 00:20:00). [M] 0.1 Molarity (M) Sodium metaperiodate in TBS. ттіз-buffered saline (ТВS) Д 100 mL ∆ 2.13 g Sodium meta-periodate Wash (2 x 6) 00:10:00) in DM. 3 10m 3.1 10m Wash for 00:10:00 in DM (1/2). 3.2 10m Wash for 00:10:00 in DM (2/2).
- 🗸 100 mL DM

Serum blocking step () 01:00:00 incubation):

■ A 3 mL Normal Serum (species matching the host of the secondary antibody, e.g. horse, goat)





8.5 Wash for 00:10:00 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).

1h

- <u>I</u> 100 mL DM
- I mL Normal Serum (species matching the host of the secondary antibody, e.g. horse, goat)
- <u>I</u> 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.

30m



Note

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

A	В	С	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



- 🗸 50 mg 3,3-Diaminobenzidine Tetrahydrochloride (DAB)
- <u>A 2 g</u> Nickel(II) sulfate hexahydrate **(Only used with certain primary antibodies, chromagen enhancer that changes brown DAB precipitate to blue-purple)**
- 13.2 Make Make [M] 1 % (V/V) Hydrogen Peroxide (H_2O_2)
 - \bot 3 mL of dH₂O
 - $\boxed{\bot}$ 100 μ L of \boxed{M} 30 % (v/v) hydrogen peroxide (H₂O₂)
- Start reaction add \bot 500 μ L of [M] 1 % (V/V) hydrogen peroxide (H₂O₂) to the above DAB mixture just prior to use.

 OR add \bot 16.7 μ L of [M] 30 % (V/V) hydrogen peroxide (H₂O₂), per \bot 100 mL.
- **13.4** Place tissue in DAB solution.
 - 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.
- 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 (1/3)
- Wash developed tissue in imidazole acetate buffer for 00:10:00 (2/3).

10m

10m

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