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© DAB immunostaining of thin, fixed mouse brain tissue sections using HNA or NCAM to characterize human iPSC-derived cell xenografts

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ABSTRACT

This protocol describes our use of chromogenic 3,3'-diaminobenzidine (DAB) immunohistochemistry to identify human iPSC-derived cells within thin, fixed mouse brain tissue section series'. We apply this workflow for post-mortem assessment of the survival and growth of human iPSC-derived cells which have been transplanted into the living brain of athymic mice.

ATTACHMENTS

it75bj7ap.docx

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PROTOCOL CITATION

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KEYWORDS

NCAM, HNA, Human-to-mouse xenograft, Human iPSC, Immunohistochemistry

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MATERIALS TEXT

Equipment:

- Horizontal rocker
- Vortex
- Microcentrifuge
- Glass petri dish
- Oven

Consumables:

- **20 mL** scintillation vials
- Paint brushes
- Gelatin-Chrom Alum-coating microscope slides
- 1. See related protocol Coating superfrost microscope slides with gelatin-chromium potassium sulfate
- Microscope slide coverslips (no. 1.5, →|<25 mm x →|<75 mm)
- Glass pipettes
- Rubber teats
- Transfer pipettes

Key reagents:

- Optimal Cutting Temperature (OCT) compound
- Bovine Serum Albumin (BSA)



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- Casein
- Sodium citrate
- Tween-20 and Triton X-100
- Ethanol
- Hydrogen peroxide (H₂O₂)
- DEPEX
- 3, 3'-diaminobenzidine (Sigma #D5905)
- Antibodies

⊠ NCAM

- 1 Abcam Catalog #ab75813
- 2. HNA (Novus #NOVNBP-313912)
- 3. Biotinylated anti-rabbit secondary antibody (Vector Labs #BA-1000)

4. Laboratories Catalog #PK-6100

Solutions:

■ 1x PBS, pH 7.4

Α	В			
Antigen retrieval (AR) buffer				
Sodium citrate	2.94 g (10 mM)			
Tween-20	500 μL (0.05%)			
Up to 1L with dH2O, pH 6.0				

- Quenching solution
 10mL (3.3%) 33% H₂O₂, 50mL (50%) ethanol up to 100mL with 1x PBS
- 1x PBST
 500 μL (0.05%) Tween-20 in 1L 1x PBS

A	В		
Blocking solution			
Casein	1 g (1% w/v)		
Triton X-100	250 μL (0.25% v/v)		
Glycine	1.5 g (1.5% w/v)		
BSA	5 g (5% w/v)		
Up to 100mL with 1xPBS			

Material input (animal, cell, tissue, fraction details):

Thin, fixed athymic mouse brain tissue sections prepared from whole mouse brains grafted with human iPSC-derived neural progenitor cells.

Day 1 (~4-6 hrs)

- Pre-heat oven and AR buffer to § 70 °C.
- 2 Label scintillation vials to match labels on section storage plates (mouse and section series IDs, name, date etc.).
- 3

Transfer sections into scintillation vials using a transfer pipette or fine paintbrush.

4 🗒 🎢 🔗

Remove anti-freeze solution and perform 3x 7 min washes in 1x PBS at

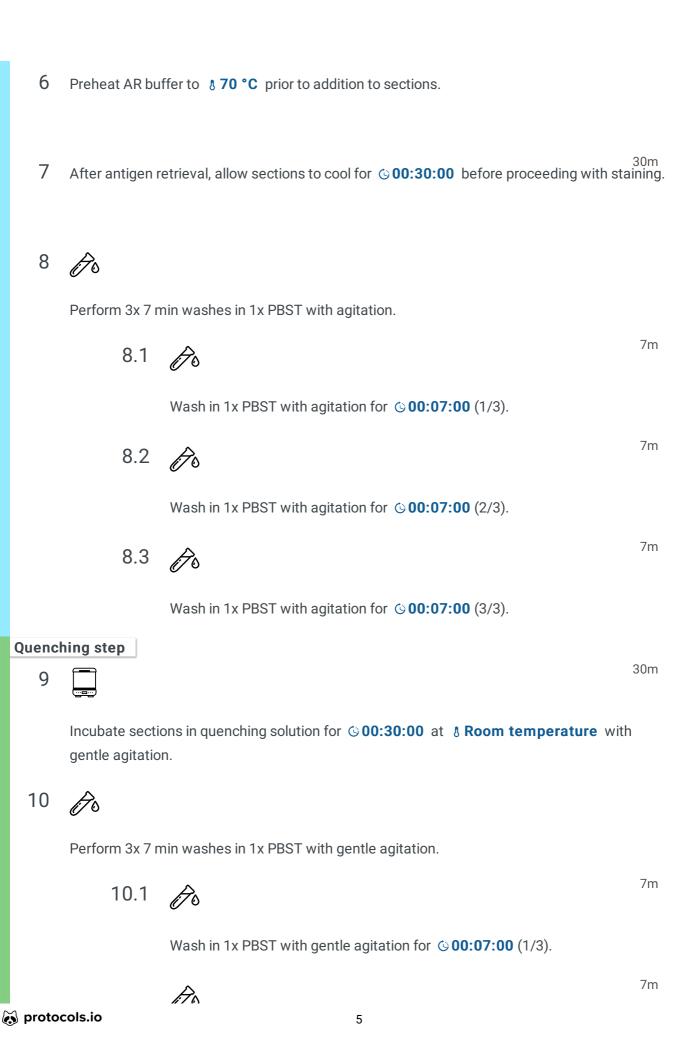
- **8 Room temperature** with gentle agitation.
- Slow shaking on an orbital rocker recommended for washes/incubations to ensure even contact with solutions.
- Use a glass pipette and rubber teat to remove solution during wash changes.
- Anti-freeze solution must be rinsed off prior to immunostaining.
 - 4.1 Remove anti-freeze solution and wash in 1x PBS at & Room temperature for © 00:07:00 (1/3).
 - 4.2 Remove anti-freeze solution and wash in 1x PBS at 8 Room temperature for © 00:07:00 (2/3).
 - 4.3 Remove anti-freeze solution and wash in 1x PBS at 8 Room temperature for © 00:07:00 (3/3).

Antigen retrieval (AR)

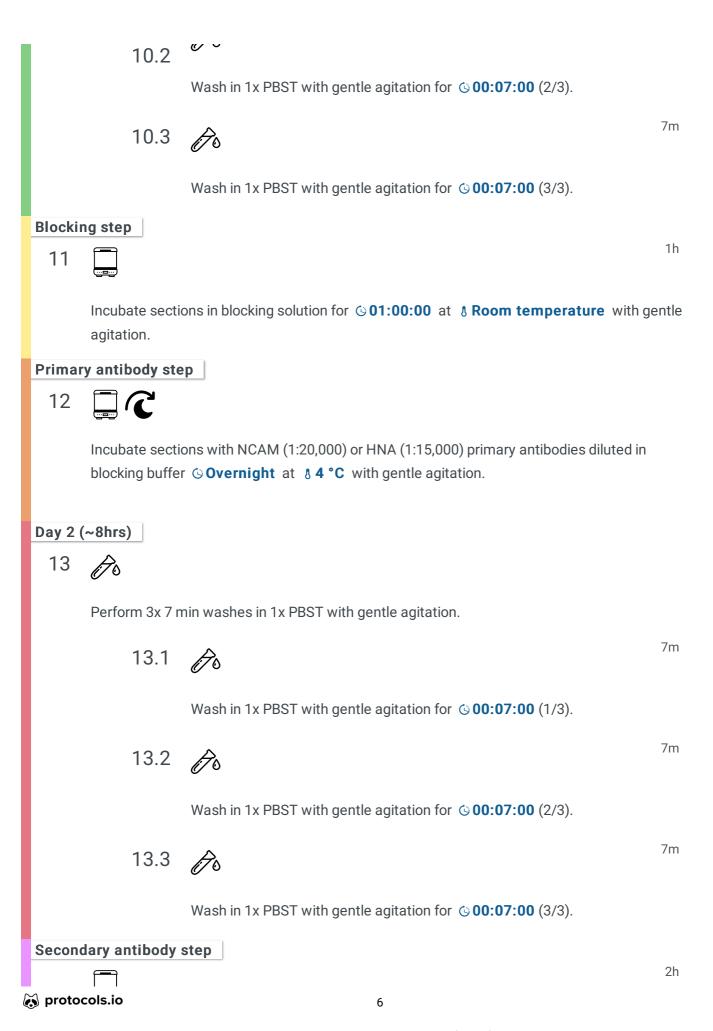
5

30m

Incubate sections in AR buffer for © 00:30:00 at § 70 °C.



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Incubate sections in anti-rabbit biotinylated secondary antibody 1:500 diluted in blocking buffer that has been diluted 2-fold for © 02:00:00 at § Room temperature.

15



Perform 3x 7 min washes in 1x PBST with gentle agitation.

15.1

7m

Wash in 1x PBST with gentle agitation for © 00:07:00 (1/3).

15.2

7m

Wash in 1x PBST with gentle agitation for © 00:07:00 (2/3).

15.3

7m

Wash in 1x PBST with gentle agitation for © 00:07:00 (3/3).

Tertiary complex step

16

2h

Incubate sections in Avidin-Biotin Complex (ABC) kit solution (Vector Laboratories) for © 02:00:00 at & Room temperature.

Prepare tertiary complex **© 00:30:00** prior to use according to the manufacturer's instructions.

30m

■ 100 μL A + 100 μL B + 9800 μL 1x PBS (1:100 A + 1:100 B in 1x PBS).

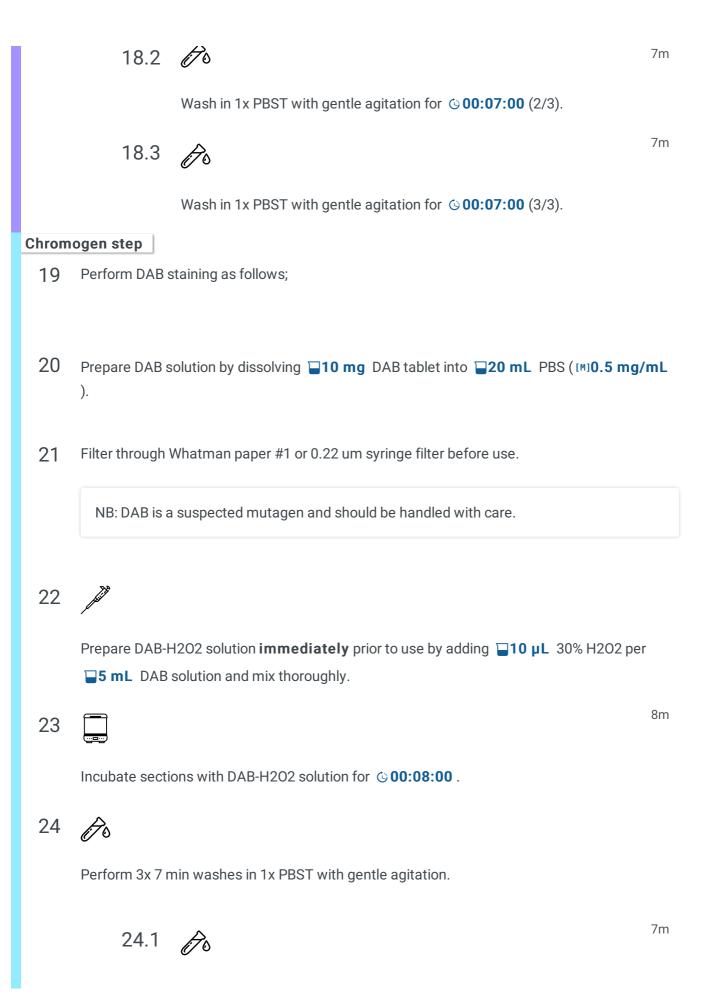
18

Perform 3x 7 min washes in 1x PBST with gentle agitation.

18.1

7m

Wash in 1x PBST with gentle agitation for © 00:07:00 (1/3).



Wash in 1x PBST with gentle agitation for © 00:07:00 (1/3).

24.2

7m

Wash in 1x PBST with gentle agitation for © 00:07:00 (2/3).

24.3

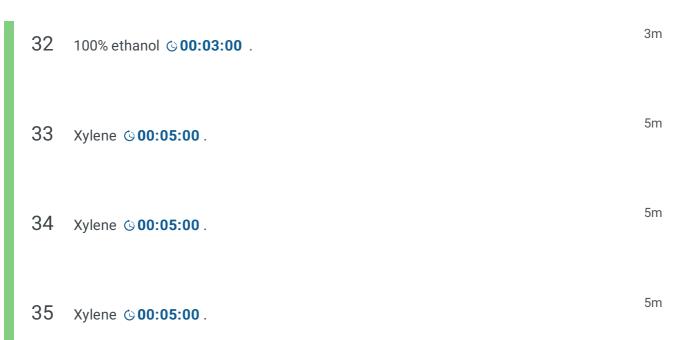
7m

Wash in 1x PBST with gentle agitation for **© 00:07:00** (3/3).

25

Mount tissue sections onto super-frost slides pre-coated with gelatin-chrome alum and allow to dry at \$ Room temperature O Overnight.

Day 3	(2 days later) 33m	
26	Process slide-mounted tissue sections through the following solutions;	
27	dH2O © 00:03:00 .	3m
28	50% ethanol © 00:03:00 .	3m
29	70% ethanol © 00:03:00 .	3m
30	95% ethanol © 00:03:00 .	3m
31	100% ethanol © 00:03:00 .	3m



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Coverslip slides with DEPEX mounting media and allow to dry in the fume hood **Overnight** before proceeding with microscopy.

37 Image sections using bright field microscopy for subsequent xenograft characterization.