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Multiplex fluorescent immunostaining of thin, fixed mouse brain tissue sections to characterize human iPSC-derived cell xenografts

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We use this protocol and it's working

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Abstract

This protocol describes our multiplex fluorescent immunohistochemistry protocol used 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, growth and maturation of human iPSC-derived cells which have been transplanted into the living brain of athymic mice.

Attachments

DOCX

519-1074.docx

102KB

Materials

Equipment:

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

Consumables:

- 20mL 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 thickness, 22×50mm)
- glass pipettes
- rubber teats
- transfer pipettes

Key reagents:

- Optimal Cutting Temperature (OCT) compound
- Bovine Serum Albumin (BSA)
- Casein
- Sodium citrate
- Tween-20 and Triton X-100
- Ethanol
- ProLong Diamond Antifade mountant

Primary antibodies:

	A	B	C	D
	Target	**Species**	**Dilution**	**Company, Catalog #**
	alpha-Synuclein	Mouse	1:1000	Santa Cruz, sc-12767
	CTIP	Rat	1:1000	Abcam, ab18465
	FOXG1	Rabbit	1:500	Abcam, ab18259

	A	B	C	D
	HNA	Mouse	1:1000	Novus, #NOVNBP-313912
	NCAM/CD56 (ERIC-1)	Mouse	1:100	Santa Cruz, sc-106
	NeuN	Chicken	1:1000	Merck-Millipore, ABN91
	Pax6	Rabbit	1:1000	ThermoFisher Scientific, #42-660
	SOX2	Mouse	1:500	R&D Systems, AF2018
	Tbr1	Rabbit	1:1000	Abcam, ab31940
	Tyrosine Hydroxylase	Rabbit	1:1500	Pel-freeze, P40101-0

Anti-α-synuclein Antibody (211) **Santa Cruz Biotechnology Catalog #**sc-12767

Anti-Ctip2 antibody [25B6] (ab18465) **Abcam Catalog #**ab18465

Anti-FOXG1 antibody (ab18259) **Abcam Catalog #**ab18259

Anti-NCAM/CD56 Antibody (ERIC 1) **Santa Cruz Biotechnology Catalog #**sc-106

Anti-NeuN Antibody **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**ABN91

PAX6 Polyclonal Antibody **Thermo Fisher Scientific Catalog #**42-6600

Human/Mouse/Rat SOX2 Antibody **R&D Systems Catalog #**AF2018

Anti-TBR1 antibody **Abcam Catalog #**ab31940

Rabbit Tyrosine Hydroxylase **Pel-Freez Catalog #**P40101-0

Secondary antibodies:

	A	B	C	D
	Target	**Species**	**Dilution**	**Company, Catalog #**
	anti-mouse AF647	Donkey	1:500	Life Technologies, A31571
	anti-rabbit AF647	Goat	1:500	Life Technologies, A11008
	anti-chicken CF594	Goat	1:500	Merck, SAB4600094
	anti-goat CF488A	Donkey	1:500	Merck, SAB4600032

Donkey anti-Mouse IgG (H L) Highly Cross-Adsorbed Secondary Antibody Alexa Fluor™ 647 **Thermo Fisher Scientific Catalog #**A-31571

Goat-anti-rabbit-Alexafluor 488 **Thermo Fisher Scientific** **Catalog #**A11008

Anti-Chicken IgG (H L) highly cross-adsorbed CF594 antibody produced in donkey **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**SAB4600094

Anti-Goat IgG (H L) highly cross-adsorbed CF™ 488A antibody produced in donkey **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**SAB4600032

Solutions:

- 1x PBS, pH 7.4
- Antigen retrieval (AR) buffer
  2.94 g ([M] 10 millimolar (mM)) sodium citrate, 500 µL (0.05%) Tween-20, up to 1 L with dH_2O, pH 6
- 1x PBST
  1. 500 µL (0.05%) Tween-20 in 1 L 1x PBS
- Blocking solution
  1. 1 g (1% w/v) casein, 250 µL (0.25% v/v) Triton X-100, 1.5 g (1.5% w/v) glycine, 5 g (5% w/v) BSA up to 100 mL with 1x PBS

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 (~3-4 hrs)

1 Pre-heat oven and Antigen Retrieval (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 3× 7 min washes in 1x PBS at Room temperature with gentle agitation.

Note

- 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 perform 3x 00:07:00 washes in 1x PBS at Room temperature with gentle agitation (1/3). 7m

4.2 Remove anti-freeze solution and perform 3x 00:07:00 washes in 1x PBS at Room temperature with gentle agitation (2/3). 7m

4.3 Remove anti-freeze solution and perform 3x 00:07:00 washes in 1x PBS at Room temperature with gentle agitation (3/3). 7m

Antigen retrieval (AR) - Day 1

5 Incubate sections in pre-heated AR buffer for 00:30:00 at 70 °C . 30m

6 After antigen retrieval, allow sections to cool for 00:30:00 before proceeding with staining. 30m

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

7.1 Perform 3x 00:07:00 washes in 1x PBST with gentle agitation (1/3). 7m

7.2 Perform 3x 00:07:00 washes in 1x PBST with gentle agitation (2/3). 7m

7.3 Perform 3x 00:07:00 washes in 1x PBST with gentle agitation (3/3). 7m

Blocking step - Day 1

8 Incubate sections in blocking solution for 01:00:00 at Room temperature with gentle agitation. 1h

Primary antibody step - Day 1

9 Incubate sections with desired primary antibody combinations (to characterise either cortical or ventral midbrain derived xenografts) diluted in blocking buffer Overnight at 4 °C with gentle agitation. 1h

Day 2 (~4hrs)

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

10.1 Perform 3 x 00:07:00 washes in 1x PBST with gentle agitation (1/3). 7m

10.2 Perform 3 x 00:07:00 washes in 1x PBST with gentle agitation (2/3). 7m

10.3 Perform 3 x 00:07:00 washes in 1x PBST with gentle agitation (3/3). 7m

Secondary antibody step

1d 4h 7m

11 Incubate sections in secondary antibodies, that match the species of chosen antibodies, diluted in blocking buffer for 02:00:00 at Room temperature. Incubate in a dark room or cover the vials with foil to protect the fluorophores from light. 2h

12 Perform 3× 7 min washes in 1x PBST with gentle agitation keeping vials protected from the light.

12.1 Perform 3x 00:07:00 washes in 1x PBST with gentle agitation keeping vials protected from the light (1/3). 7m

12.2 Perform 3x 00:07:00 washes in 1x PBST with gentle agitation keeping vials protected from the light (2/3). 7m

12.3 Perform 3x 00:07:00 washes in 1x PBST with gentle agitation keeping vials protected from the light (3/3). 7m

13 **Mount tissue sections** in a dimly lit room (to protect the fluorophores) onto super-frost slides pre-coated with gelatin-chrome alum and allow to dry in the dark at Room temperature for 01:00:00 - 02:00:00. 3h

14 Remove ProLong Diamond Antifade mountant from storage at 4 °C and allow the reagent to equilibrate to Room temperature before use.

15 **Coverslip slides with ProLong Diamond Antifade mountant** and allow to set at Room temperature for at least 24:00:00 in the dark before proceeding with microscopy. 1d

16 Image sections using fluorescent microscopy for subsequent xenograft characterization.

17 Slides can be stored at 4 °C or -20 °C.