





Sep 18, 2022

# Multiplex fluorescent immunostaining of thin, fixed mouse brain tissue sections to characterize human iPSCderived cell xenografts

Benjamin Trist<sup>1</sup>, Louise Cottle<sup>1</sup> <sup>1</sup>The University of Sydney 1 Works for me dx.doi.org/10.17504/protocols.io.4r3l275mxg1y/v1 Benjamin Trist ABSTRACT This protocol describes our multiplex fluorescent immunohistochemistry protocol used to identify human iPSCderived 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 519-1074.docx DOI dx.doi.org/10.17504/protocols.io.4r3l275mxg1y/v1 PROTOCOL CITATION Benjamin Trist, Louise Cottle 2022. Multiplex fluorescent immunostaining of thin, fixed mouse brain tissue sections to characterize human iPSC-derived cell xenografts. protocols.io https://protocols.io/view/multiplex-fluorescent-immunostaining-of-thin-fixed-cgbxtspn A FUNDERS ACKNOWLEDGEMENT Michael J Fox Foundation Grant ID: ASAP-000497 KEYWORDS Human iPSC, Immunohistochemistry, Fluorescent, Human-to-mouse xenograft LICENSE This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited CREATED Sep 08, 2022 LAST MODIFIED Sep 18, 2022 OWNERSHIP HISTORY Sep 08, 2022 Sep 16, 2022 Benjamin Trist PROTOCOL INTEGER ID 69719 MATERIALS TEXT



1

**Citation:** Benjamin Trist, Louise Cottle Multiplex fluorescent immunostaining of thin, fixed mouse brain tissue sections to characterize human iPSC-derived cell xenografts <a href="https://dx.doi.org/10.17504/protocols.io.4r3l275mxg1y/v1">https://dx.doi.org/10.17504/protocols.io.4r3l275mxg1y/v1</a>

#### **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, 22x50mm)
- 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:

Α	В	С	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
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

(ab18259) Abcam Catalog #ab18259

Biotechnology Catalog #sc-106

■ Anti-NeuN Antibody Sigma

Aldrich Catalog #ABN91

☑ PAX6 Polyclonal Antibody Thermo Fisher

Scientific Catalog #42-6600



2

⋈ Human/Mouse/Rat SOX2 Antibody R&D

Systems Catalog #AF2018

antibody Abcam Catalog #ab31940

Freez Catalog #P40101-0

#### Secondary antibodies:

Α	В	С	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

XAnti-Chicken IgG (H L) highly cross-adsorbed CF594 antibody produced in donkey Sigma

Aldrich Catalog #SAB4600094

Anti-Goat IgG (H L) highly cross-adsorbed CF™ 488A antibody produced in donkey 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<sub>2</sub>O, pH6

1x PBST

1. **□500** µL (0.05%) Tween-20 in **□1** L 1x PBS

Blocking solution

1.  $\square$ 1 g (1% w/v) casein,  $\square$ 250  $\mu$ L (0.25% v/v) Triton X-100,  $\square$ 1.5 g (1.5% w/v) glycine,  $\square$ 5 g (5% w/v) BSA up to  $\square$ 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.

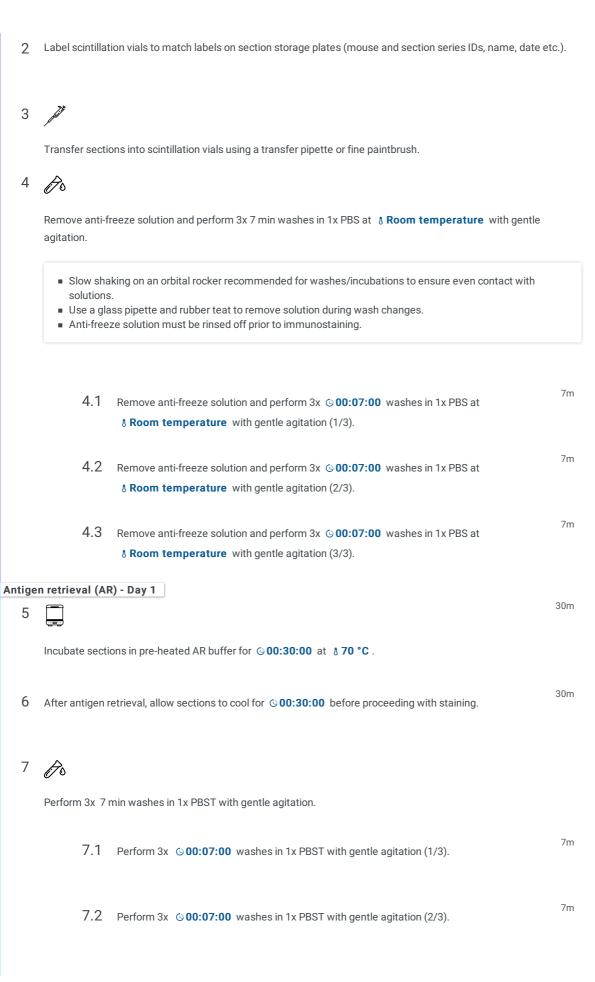
### Day 1 (~3-4 hrs)

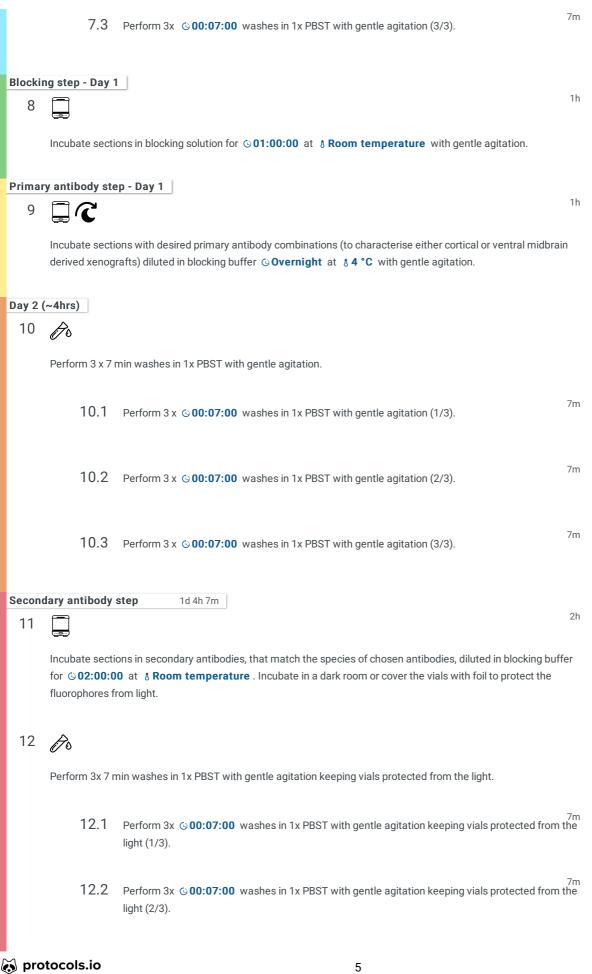
1 Pre-heat oven and Antigen Retrieval (AR) buffer to  $\,\,\it \&\,\, 70\,\,^{\circ}C$  .

## protocols.io

3

**Citation**: Benjamin Trist, Louise Cottle Multiplex fluorescent immunostaining of thin, fixed mouse brain tissue sections to characterize human iPSC-derived cell xenografts <a href="https://dx.doi.org/10.17504/protocols.io.4r3l275mxg1y/v1">https://dx.doi.org/10.17504/protocols.io.4r3l275mxg1y/v1</a>





- 12.3 Perform  $3x \otimes 00:07:00$  washes in 1x PBST with gentle agitation keeping vials protected from the light (3/3).
- 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.
- 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.
- 16 🗞

 $Image\ sections\ using\ fluorescent\ microscopy\ for\ subsequent\ xenograft\ characterization.$ 

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