Human Dopaminergic Neuron Immunocytochemistry Kit

Catalog no. A29515

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Table 1 Contents and storage

Kit component	Part no.	Conc.	Amount	Storage[1]	Usage notes
Primary antibodies					
anti-FoxA2 (host: mouse)	100034245	2000X	10 µL	–20°C	Dilute with Blocking Solution
anti-Otx2 (host: goat)	100034250	1000X			
anti-Tyrosine Hydroxylase(host: rabbit)	100034251				
Secondary antibodies					
Alexa Fluor™ 488 donkey anti-goat; *for use with anti-Otx2*	100040028	250X	20 µL	–20°C to 4°C; avoid freeze-thaw cycles	Ex/Em[2] 495/519 nm (green); centrifuge before use[3]
Alexa Fluor™ 488 donkey anti-rabbit; *for use with anti-Tyrosine Hydroxylase*	A25535				Ex/Em[2] 495/519 nm (green); centrifuge before use[3]
Alexa Fluor™ 555 donkey anti-mouse; *for use with anti-FOXA2*	100034253				Ex/Em[2] 555/565 nm (orange); centrifuge before use[3]
Alexa Fluor™ 594 donkey anti-mouse; *for use with anti-FOXA2*	100034252				Ex/Em[2] 590/617 nm (red); centrifuge before use[3]
Additional reagents					
NucBlue™ Fixed Cell Stain (DAPI nuclear DNA stain)	R37606	NA	1 vial	–20°C to ambient temperature	Ex/Em[2] 358/461 nm (blue); apply 1–2 drops/mL
Fixative Solution	A24344	1X	10 mL		4% formaldehyde in DPBS
Permeabilization Solution	A24352	NA			0.5% Triton™ X-100 in DPBS
Blocking Solution	A24353	NA	20 mL		3% BSA in DPBS
Wash Buffer	A24348	10X			10X DPBS; dilute to 1X with water[4]

[1] **Handling and shelf life:** Use aseptic technique when handling all reagents. Allow frozen reagents to thaw completely before use. Once thawed, do not re-freeze the kit (aliquots not recommended). Store at 2°C to 8°C for up to 6 months.

[2] Approximate excitation/emission wavelength maxima.

[3] Centrifuge Secondary Antibody solutions (e.g., 2 minutes at 10,000 × *g*) and add only the supernatant to the Blocking Solution. This step minimizes the transfer of any protein aggregates that may have formed during storage, thereby reducing non-specific background staining.

[4] Upon thawing the 10X Wash Buffer, you may observe a precipitate, which will go back into solution when warmed to ambient temperature and mixed well.

Description

The Human Dopaminergic Neuron Immunocytochemistry Kit enables optimal image-based analysis of three key markers of the human dopaminergic neuron lineage: FoxA2 and Otx2 for the intermediary floor plate progenitors and Tyrosine Hydroxylase (TH) for the mature dopaminergic neurons. This high performance immunocytochemistry (ICC) kit includes a complete set of primary and secondary antibodies, a nuclear DNA stain, and pre-made buffers for an optimized staining experiment.

Experimental protocol

See Table 2 (page 3) for the recommended volumes to use based on the culture format of the cells to be stained. See Table 3 (page 3) for multiplex staining options.

CAUTION! Use gentle liquid handling and pipetting techniques when adding or removing liquids to minimize the possibility of dislodging cells and losing them during the handling steps.

1. Prepare the Permeabilization/Blocking Solution (for use in Step 4) by combining the following components:

Component	Volume
Blocking Solution	3.33 mL
Permeabilization Solution	6.0 mL
1X Wash Buffer	670 µL
Total volume	10.0 mL

2. **Two-step fixation:** This method is for samples where cells are fragile (e.g. Neurons) towards air exposure.

    a. Add Fixative solution to to the spent medium so that the final concentration is 0.5X Fixative Solution.

    b. Incubate for 5 minutes.

    c. Aspirate the whole solution and add fresh Fixative Solution to the sample.

    d. Incubate for another 10–15 minutes.

3. Remove Fixative Solution.

    Optional stopping point: After removing the Fixative solution, add Wash Buffer (diluted to 1X with water), wrap the sample in laboratory film to prevent it from drying out, and store at 2°C to 8°C for up to 2 weeks.

4. Add Permeabilization/Blocking Solution and incubate for 30 minutes at room temperature.

5. Add the desired Primary Antibody directly to the Blocking Solution covering the cells to yield a 1X final dilution. Mix gently and incubate for 3 hours at room temperature (or overnight at 2°C to 8°C).

    Note: For co-staining options, see Table 3 (page 3).

6. Remove the solution. Add Wash Buffer (diluted to 1X with water) and incubate for 2–3 minutes. Repeat the wash procedure 2 more times so that the cells are washed a total of 3 times.

7. Add the appropriate Secondary Antibody (diluted to 1X in Blocking Solution; see Table 3 for guidance) and incubate for 1 hour at room temperature.

8. Remove the solution. Add Wash Buffer (diluted to 1X with water) and wait for 2–3 minutes. Repeat the wash procedure 2 more times so that the cells are washed a total of 3 times.

   Optional: Add 1–2 drops/mL of NucBlue™ Fixed Cell Stain (DAPI) into the last wash step and incubate for 5 minutes.

9. Image the cells immediately or store cells at 2°C to 8°C in the dark, wrapped with laboratory film to prevent the samples from drying out, for up to 1 month.

   Alternatively, for prolonged storage, apply a suitable antifade mounting medium, such as ProLong™ Diamond Antifade Mountant, to the sample.

Table 2 Recommended final volumes of primary and secondary antibodies.

Culture format	No. of tests[1]	Staining volume	Amount of each 1000X primary antibody[2]	Amount of each 2000X primary antibody[2]	Amount of each 250X secondary antibody
96-well plate	80	50 µL/well	0.05 µL	0.025 µL	0.2 µL
48-well plate	40	100 µL/well	0.1 µL	0.05 µL	0.4 µL
24-well plate	20	200 µL/well	0.2 µL	0.1 µL	0.8 µL
12-well plate	10	400 µL/well	0.4 µL	0.2 µL	1.6 µL
6-well plate	4	1000 µL/well	1 µL	0.5 µL	4 µL
35-mm dish	4	1000 µL/dish	1 µL	0.5 µL	4 µL
4-well chamber slide	10	400 µL/well	0.4 µL	0.2 µL	1.6 µL
8-well chamber slide	20	200 µL/well	0.2 µL	0.1 µL	0.8 µL

[1] When using the suggested staining volume, this kit contains sufficient reagents for the indicated number of tests per primary antibody.

[2] To avoid working with very small volumes, first prepare a 10X working dilution (e.g., add 1 µL of each 1000X primary antibody to 100 µL of Blocking Solution) and then dispense a 1/10 volume (e.g., add 5 µL to 45 µL in the well) to dilute to a 1X final concentration.

Table 3 Dual antibody staining options. Note that the NucBlue™ Fixed Cell Stain (a DAPI nuclear DNA stain) provided in this kit is also compatible with these antibody combinations. See Figure 1 for example pictures (page 4).

Color options	**Green**[1] (e.g., FITC filter)	**Orange**[1] (e.g., Cy™3 / TRITC filter) or **Red**[1] (e.g., Texas Red™ filter)
Antibody combination 1: FoxA2 + Otx2		
Primary antibody	anti-**Otx2** (host: goat)	anti-**FoxA2** (host: mouse)
Secondary antibody	Alexa Fluor™ 488 donkey anti-goat	Alexa Fluor™ 555 donkey anti-mouse or Alexa Fluor™ 594 donkey anti-mouse
Antibody combination 2: Tyrosine Hydroxylase		
Primary antibody	anti-Tyrosine Hydroxylase (host: rabbit)	anti-**FoxA2** (host: mouse)
Secondary antibody	Alexa Fluor™ 488 donkey anti-rabbit	Alexa Fluor™ 555 donkey anti-mouse or Alexa Fluor™ 594 donkey anti-mouse

[1] See Table 1 (page 1) for the approximate excitation/emission wavelengths.

Figure 1 hPSC treated with Floor Plate Specification medium from the PSC Dopaminergic Neuron Differentiation Kit (Prototype) (Cat. no. A30416SA) differentiate into floorplate progenitor cells. These floorplate progenitors cells can be detected as early as 7 days after addition of specification medium. **(A)** anti-Otx2, **(B)** anti-FoxA2, **(C)** NucBlue™, **(D)** Merged image.

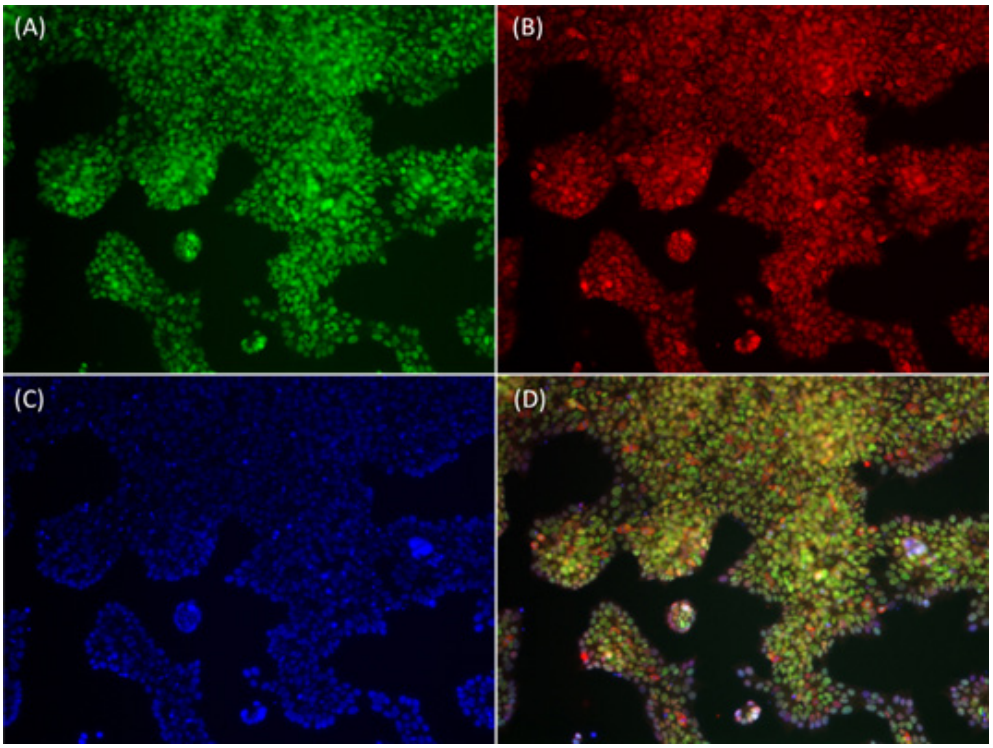
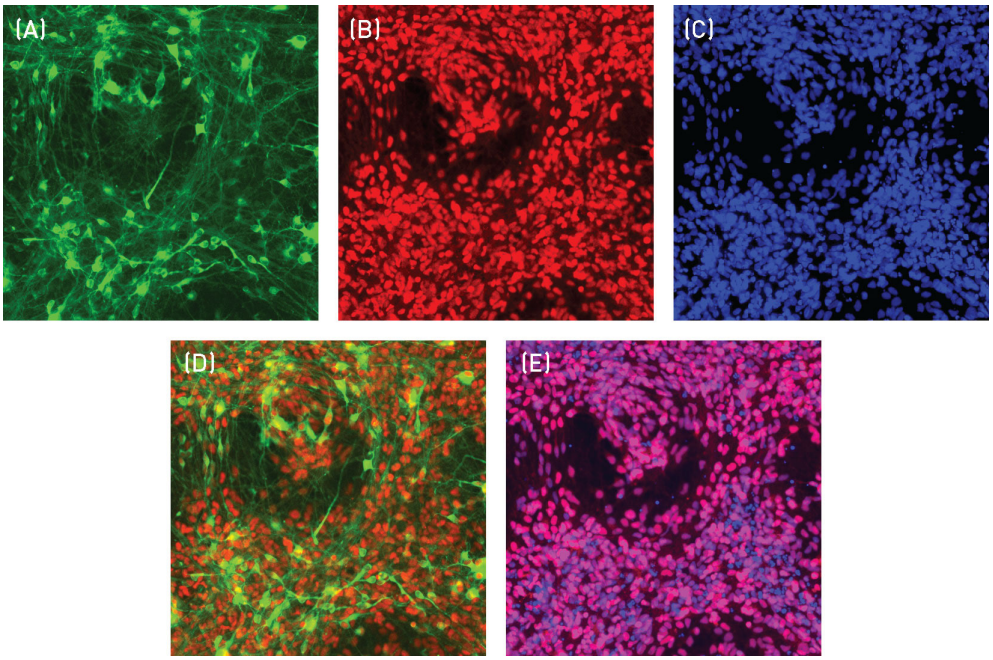


Figure 2 Mature Dopaminergic Neurons can be visualized as early as 10 days after the addition of maturation medium from the PSC Dopaminergic Neuron Differentiation Kit (Prototype) (Cat. no. A30416SA). **(A)** anti-Tyrosine Hydroxylase (green), **(B)** anti-FoxA2 (red), **(C)** NucBlue™ (blue), **(D)** merged image with anti-Tyrosine Hydroxylase and anti-FoxA2 (green and red), **(E)** merged image with anti-FoxA2 and NucBlue™ (red and blue).



Product list Current prices may be obtained from our website or from our Customer Service Department.

Cat. No.	Product name	Unit size
A29515	Human Dopaminergic Neuron Immunocytochemistry Kit	1 kit
Related products		
A30416SA	PSC Dopaminergic Neuron Differentiation Kit (Prototype)	1 kit
A30412SA	Floor Plate Cell Expansion Kit (Prototype)	1 kit
A28895SA	Dopaminergic Neuron Maturation Supplement (50X) (Prototype)	1 kit
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A25526	PSC (OCT4, SSEA4) Immunocytochemistry Kit	1 kit
A25538	3-Germ Layer Immunocytochemistry Kit	1 kit
A25973	Human Cardiomyocyte Immunocytochemistry Kit	1 kit
P36965	ProLong™ Diamond Antifade Mountant	5 × 2 mL
A15871	TaqMan™ hPSC Scorecard™ Kit, FAST 96 well	2 plates
A14353	Alkaline Phosphatase Live Stain	50 µL
A18945	Gibco™ Human Episomal iPSC Line	1 vial
A16517	CytoTune™-iPS 2.0 Sendai Reprogramming Kit	1 pack
A14703	Episomal iPSC Reprogramming Vectors	1 kit
A15960	Epi5™ Episomal iPSC Reprogramming Kit	1 kit
A1517001	Essential 8™ Medium	500 mL
A2858501	Essential 8™ Flex Medium	500 mL
A14700	Vitronectin (VTN-N) Recombinant Human Protein, Truncated	1 mL
A1647801	PSC Neural Induction Medium	500 mL
A25042SA	PSC Cardiomyocyte Differentiation Kit (Prototype)	1 kit

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