Immunofluorescent Labeling

Introduction

Immunofluorescent labeling is a straight-forward technique for assessing the presence and subcellular localization of an antigen or a protein. Several labeling methods are available depending on the biological sample, cell preparation, and availability of antibodies against the target. The method presented here has demonstrated utility in detecting the presence of ß-III tubulin (tuj-1), microtubule-associated protein 2 (MAP2), forkhead box protein A2 (FoxA2), and tyrosine hydroxylase (TH) in iCell® DopaNeurons. Additionally, this protocol has been used to illustrate the absence of the neural progenitor marker, nestin. This protocol should serve as a guide for immunofluorescent labeling other neuronal proteins.

Required Equipment and Consumables

The following equipment and consumables are required in addition to the materials specified in the iCell DopaNeurons User's Guide.

Item	Vendor	Catalog Number
Equipment		
Fluorescent Microscope with Digital Camera	Multiple Vendors	
Consumables		
iCell DopaNeurons Kit, 01279*	Cellular Dynamics International (CDI)	R1032
Donkey Serum	Sigma	D9663-10ML
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)	Life Technologies	14040
Fetal Bovine Serum	HyClone	SH30396.03
Formaldehyde (36.5%)	Sigma	F8775
Hoechst 33342	Life Technologies	H3570
PES Filter Unit, 0.2 µm, 500 ml	Multiple Vendors	
Triton X-100	Sigma	X100-5ML

* Formerly known as iCell DopaNeurons Kit (Cat. No. DNC-301-030-001).

Recommended Antibodies

The following table of primary and secondary antibodies provides the dilution factor to use for labeling iCell DopaNeurons. Select the appropriate combination of primary and secondary antibodies.

Item	Vendor	Catalog Number	Dilution Factor
Primary Antibodies			
Mouse Anti-β-tubulin, Class III, AlexaFluor 488 Conjugated	BD Biosciences	560381	1:20
Mouse Anti-MAP2, AlexaFluor 488 Conjugated	Millipore	MAB3418X	1:1,000
Mouse Anti-nestin, AlexaFluor 647 Conjugated	BD Biosciences	560393	1:20
Mouse Anti-TH	Sigma	T2928	1:8,000 - 1:10,000 (1 µg/ml)*
Rabbit Anti-FoxA2	Cell Signaling Technologies	8186	1:500
Secondary Antibodies			
Donkey Anti-mouse IgG, AlexaFluor 488	Life Technologies	A-21202	1:1,000
Donkey Anti-rabbit IgG, AlexaFluor 594	Life Technologies	A-21207	1:1,000

* The dilution factor varies based upon the lot-specific concentration. It is recommended to dilute the antibody to a final concentration of 1 µg/ml.

Methods

Culturing iCell DopaNeurons

1. Prepare the Complete Maintenance Medium according to the iCell DopaNeurons User's Guide.
2. Thaw the neurons according to their User's Guide.
3. Culture the neurons in a cell culture incubator at 37°C, 5% CO_2.
4. Maintain the neurons according to their User's Guide until ready to perform immunofluorescent labeling.

Labeling iCell DopaNeurons

The following procedure details labeling the dopaminergic neurons cultured in 96-well cell culture plates. Scale volumes appropriately for other vessel formats. If using a plastic or glass coverslip, transfer the coverslip to a glass slide with mounting solution after step 11, below.

1. Dilute 36.5% formaldehyde solution in D-PBS to a final concentration of 4%.
2. Aspirate the spent medium from the culture. Do not allow the cells to dry.
3. Fix the neurons with 100 µl/well of the 4% formaldehyde solution at room temperature for 15 minutes.

Notes

Notes

4. Carefully rinse the cells twice with 150 µl/well of D-PBS. Do not allow the cells to dry.

   Note: *Adding D-PBS too forcefully during this step can easily dislodge the neurons from the plate.*

5. Prepare the blocking buffer by diluting fetal bovine serum to 2% (v/v), donkey serum to 2% (v/v), and Triton X-100 to 0.2% (v/v) in D-PBS. Filter the blocking buffer through a 0.2 µm PES filter unit.

6. Dilute the primary antibody in blocking buffer. Use the dilution factor specified in the table above.

7. Incubate the neurons with 100 µl/well of diluted primary antibody solution overnight at 4°C.

8. Prepare the washing buffer by diluting fetal bovine serum to 2% (v/v) in D-PBS. Filter the washing buffer through a 0.2 µm PES filter unit.

9. Carefully rinse the cells twice with 150 µl/well washing buffer. Do not allow the cells to dry.

   Note: *Adding washing buffer too forcefully during this step can easily dislodge neurons from the plate.*

10. Prepare the secondary antibody solution by combining the appropriate secondary antibody diluted 1:1,000 and Hoechst 33342 diluted 1:10,000 in washing buffer. See the table above to determine the appropriate secondary antibody.

11. Incubate the neurons with 100 µl/well of the secondary antibody solution at room temperature for 1 hour.

12. Carefully rinse the cells twice with 150 µl/well of washing buffer. Do not allow the cells to dry.

13. Add 100 µl/well of washing buffer and take images using the fluorescent microscope.

    Note: *If necessary, store plates or slides with labeled neurons at 4°C, for up to 1 month, protecting from light and properly sealing to prevent evaporation.*

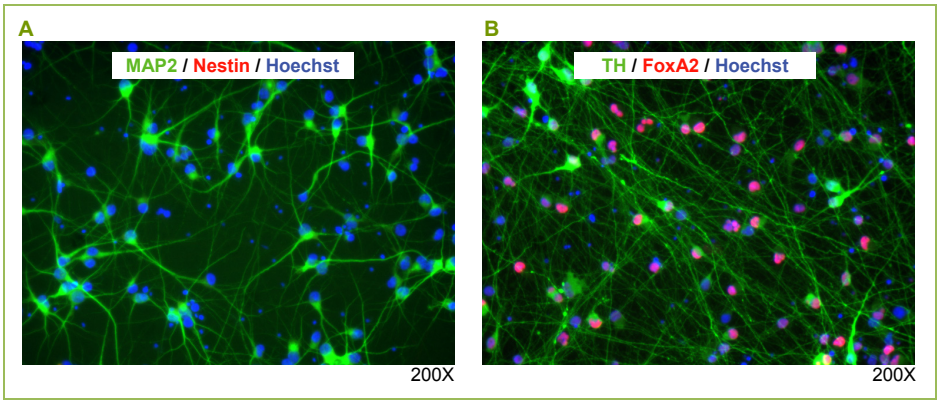

Figure 1: Fluorescently Labeled iCell DopaNeurons
Panel A shows the presence of MAP2 (neuronal marker) and absence of nestin (neural progenitor marker), 14 days post-plating. Panel B shows the majority of the cells labeled with FoxA2 and TH, characteristic midbrain dopaminergic neuron markers, 14 days post-plating. Nuclei were stained with Hoechst 33342.

Notes

Customer's Responsibilities
CDI does not guarantee that you will obtain equivalent results from using iCell or MyCell products as described herein or that such use will not infringe any intellectual property right(s) of any third party(ies). You are solely responsible for obtaining any licenses you may require for your specific research use(s) of the iCell or MyCell products not expressly conveyed under CDI's terms and conditions of sale or other transfer of the iCell or MyCell products to you.

Conditions of Use
For life science research use only.

Trademarks
iCell and MyCell are registered trademarks, and Cellular Dynamics and the  logo are trademarks of Cellular Dynamics International, Inc.

All other brands, product names, company names, trademarks, and service marks are the properties of their respective owners.

Revision History
Version 1.1: September 2017
AP-DNCIMM170901