Immunofluorescent Labeling

Introduction

Immunofluorescent labeling is a straight-forward technique for assessing the presence and the 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 protocol presented here has demonstrated utility in detecting the presence of microtubule-associated protein 2 (MAP2B), γ-aminobutyric acid (GABA), vesicular GABA transporter (vGAT), vesicular glutamate transporter 2 (vGLUT2), and β-tubulin, class III (tuj-1). This protocol has also shown usefulness in assessing the lack of nestin or glial fibrillary acid protein (GFAP) expression on iCell® GABANeurons (formerly known as iCell Neurons). 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 GABANeurons User's Guide.

Item	Vendor	Catalog Number(s)
Equipment		
Fluorescent Microscope with Digital Camera	Multiple Vendors	
Consumables		
iCell GABANeurons Kit, 01279[1]	Cellular Dynamics International (CDI)	R1011, R1084, R1118
iCell GABANeurons Kit, 01434[1, 2]	Cellular Dynamics International (CDI)	R1013, R1053
Donkey Serum	MilliporeSigma	D9663-10ML
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)[3]	STEMCELL Technologies	37350
Formaldehyde (37%)	MilliporeSigma	252549, F8775
Hoechst 33342	Thermo Fisher Scientific	H1399
Triton X-100	MilliporeSigma	X100-5ML

1 Order the kit whose iCell GABANeurons were derived from the desired donor. iCell GABANeurons, 01279 and iCell GABANeurons, 01434 were derived from apparently healthy, normal donors. iCell GABANeurons, 01434 are exclusive to CDI.
Note: *This Application Protocol was optimized using iCell GABANeurons Kit, 01434 (Cat. No. R1013). CDI anticipates you will achieve similar results using iCell GABANeurons derived from other apparently healthy, normal donors.*

2 Formerly known as iCell Neurons (Cat. No. NRC-100-010-001).

3 Similar products are available from multiple vendors.

Recommended Antibodies

The following table of primary and secondary antibodies provides the dilution factor to use for labeling iCell GABANeurons. 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-GABA	MilliporeSigma	A0310	1:500 - 1:1,000
Mouse Anti-MAP2B, AlexaFluor 488 Conjugated	BD Biosciences	560399	1:20
Mouse Anti-nestin, AlexaFluor 647 Conjugated	BD Biosciences	560393	1:20
Mouse Anti-vGAT	Synaptic Systems	131 011	1:1,000
Rabbit Anti-GFAP	Thermo Fisher Scientific	18-0063	1:100
Rabbit Anti-vGLUT2	Synaptic Systems	135 403	1:1,000
Secondary Antibodies			
Donkey Anti-mouse, AlexaFluor 488	Thermo Fisher Scientific	A-21202	1:1,000
Donkey Anti-rabbit, AlexaFluor 594	Thermo Fisher Scientific	A-21207	1:1,000

Methods

Culturing iCell GABANeurons

1. Prepare the Complete Maintenance Medium according to the iCell GABANeurons 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 GABANeurons

The following procedure details labeling the 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.

1. Dilute 37% formaldehyde solution in D-PBS to a final concentration of 4%.

   Note: *Alternatively, use a paraformaldehyde solution diluted in D-PBS to a final concentration of 4%, pH 7.2 - 7.4.*

2. Aspirate the spent medium from the culture. Do not allow the cells to dry.
3. Fix the neurons with 90 µl/well of 4% formaldehyde solution at room temperature for 15 minutes.

Notes

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4. Carefully rinse the neurons 3 times with 90 µl/well of D-PBS for 5 minutes each rinse. Do not allow the cells to dry.

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

5. Prepare the blocking buffer by diluting donkey serum to 10% (v/v) and Triton X-100 to 0.1% (v/v) in D-PBS.

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

7. Incubate the neurons with 90 µl/well of diluted primary antibody at room temperature for 2 hours.

   Note: *Alternatively, incubate the neurons in diluted primary antibody at 4°C overnight.*

8. Carefully rinse the neurons 3 times with 90 µl/well of D-PBS for 5 minutes each rinse. Do not allow the cells to dry.

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

9. Combine the appropriate secondary antibody diluted 1:1000 and Hoechst 33342 diluted 1:2,000 - 1:5,000 in blocking buffer.

10. Incubate the neurons with 90 µl/well of diluted secondary antibody and Hoechst 33342 solution at room temperature for 30 minutes.

11. Carefully rinse the neurons 3 times with 90 µl/well of D-PBS for 5 minutes each rinse. Do not allow the cells to dry.

12. 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.*

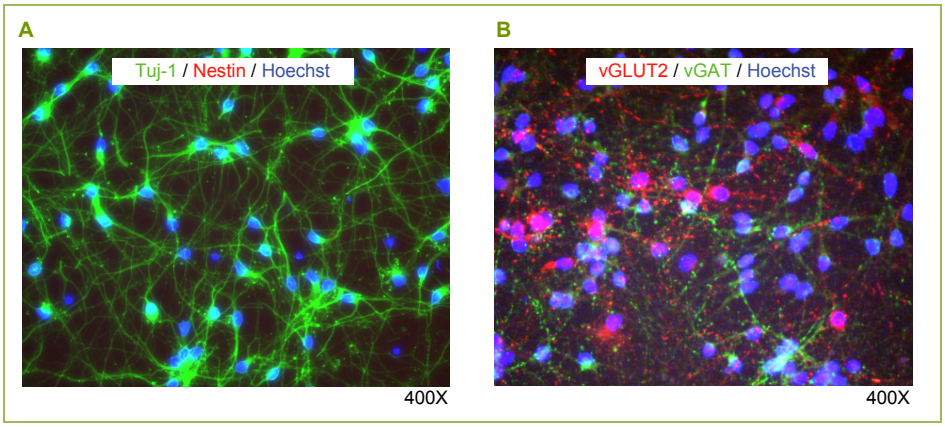

Figure 1: Immunofluorescent-labeled iCell GABANeurons
Panel A shows presence of β-tubulin, class III (tuj-1, neuronal marker) and low levels of nestin (neuronal progenitor marker), 7 days post-plating of iCell GABANeurons, 01434. Panel B shows punctate labeling pattern for vesicular glutamate transporter 2 (vGLUT2) and vesicular GABA transporter (vGAT), 14 days post-plating of iCell GABANeurons, 01434. Nuclei were stained with Hoechst 33342.

Notes

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Revision History
Version 1.0: April 2018
AP-GABAIMM180418