Immunofluorescent Labeling

Introduction

Immunofluorescent labeling is a straightforward technique for assessing the presence and the subcellular localization of an antigen. Several labeling methods are available based 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 albumin (ALB), alpha 1-antitrypsin (A1AT), asialoglycoprotein receptor (ASGPR), and occludin on iCell® Hepatocytes. This protocol should serve as a guide for immunofluorescent labeling other hepatocyte targets.

Required Equipment and Consumables

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

Item	Vendor	Catalog Number
Equipment		
Fluorescent Microscope with Digital Camera	Multiple Vendors	
Consumables		
iCell Hepatocytes	Cellular Dynamics International (CDI)	HCC-100-010-001
Bovine Serum Albumin (BSA)	Fisher Scientific	BP1605
Donkey Serum	Sigma	D9663-10ML
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)	Invitrogen	14040
Hoechst 33342	Invitrogen	H3570
Paraformaldehyde	Sigma	F8775
Triton X-100	Sigma	X100-5ML

Recommended Antibodies

The following table of primary and secondary antibodies provides the dilution factor to use for labeling iCell Hepatocytes with validated antibodies. Select the appropriate combination of primary and secondary antibodies for specific applications. An empirical determination of appropriate dilutions may be required for other antibody clones obtained from other sources than those specified here.

Item	Vendor	Catalog Number	Dilution Factor
Primary Antibodies			
Mouse Anti-alpha 1 Antitrypsin	Abcam	ab20830	1:500 - 1:1000
Mouse Anti-ASGPR1	Santa Cruz	Sc-52623	1:500
Mouse Anti-human Albumin	Cedarlane Laboratories	CL21513A	1:5000 – 1:10000
Mouse Anti-occludin, AlexaFluor 488 Conjugated	Life Technologies	331588	1:250
Secondary Antibody			
Donkey Anti-mouse, AlexaFluor 488	Invitrogen	A-21202	1:500

Notes

Methods

Culturing iCell Hepatocytes

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

Labeling iCell Hepatocytes: Day 1 - Fixation, Permeabilization, and Primary Antibody Incubation

The following procedure details labeling iCell Hepatocytes cultured in 96-well cell culture plates. Scale volumes appropriately for other cell culture vessel formats.

1. Dilute the paraformaldehyde solution 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. Carefully wash the hepatocytes twice with 200 µl/well of D-PBS.
4. Fix the hepatocytes with 100 µl/well of 4% paraformaldehyde solution at room temperature for 20 - 30 minutes.
5. Carefully wash the hepatocytes twice with 200 µl/well of D-PBS.

   Note: *If necessary, store plates in D-PBS at 4°C for up to 1 week.*
6. Prepare the Permeabilization Buffer by diluting BSA to 1% (w/v) and Triton X-100 to 0.2% (v/v) in D-PBS.
7. Incubate hepatocytes with 100 µl/well of Permeabilization Buffer.
8. Wash the hepatocytes once with 200 µl/well of D-PBS.

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9. Prepare the Blocking Buffer by diluting donkey serum to 10% (v/v) in D-PBS.
10. Dilute the primary antibody in Blocking Buffer. Use the dilution factor specified in the table above.
11. Incubate the hepatocytes with 100 µl/well of diluted primary antibody at 4°C overnight.

    Note: *Alternatively, incubate the hepatocytes in diluted primary antibody at room temperature for 2 hours.*

Labeling iCell Hepatocytes: Day 2 – Secondary Antibody Incubation and Nuclei Staining

1. Wash the hepatocytes twice with 200 µl/well of D-PBS for 5 minutes each wash.
2. Dilute the appropriate secondary antibody in D-PBS. Use the dilution factor specified in the table above.
3. Incubate the hepatocytes with 100 µl/well of diluted secondary antibody at room temperature, protected from light, for 1 hour.
4. Wash the hepatocytes twice with 200 µl/well of D-PBS for 5 minutes each rinse.
5. Dilute the Hoechst 33342 in D-PBS to 1:1000.
6. Incubate the hepatocytes with 100 µl/well of diluted Hoechst 33342 at room temperature, protected from light, for 3 - 5 minutes.
7. Wash the hepatocytes once with 200 µl/well of D-PBS for 5 minutes.
8. Take images using a fluorescence microscope.

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

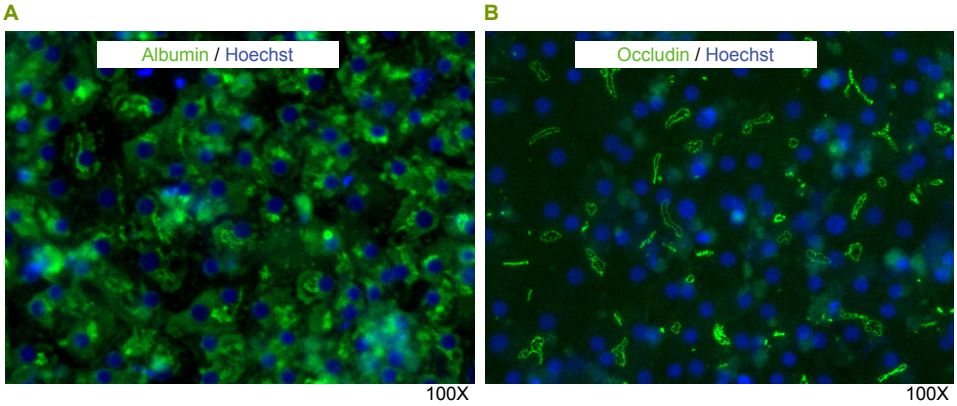

Figure 1: Immunofluorescent-labeled iCell Hepatocytes
These images show the presence of (A) intracellular albumin and (B) occludin at tight junctions, 3 days post-plating. Nuclei were stained with Hoechst 33342.

Notes

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