Immunocytochemistry Protocol for myrCell Cardiomyocytes

For research use only

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

bit.bio
THE CELL CODING COMPANY

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

1. Introduction

This immunocytochemistry (ICC) / immunofluorescence (IF) protocol, developed and optimised at Myriamed GmbH, has been designed for myrCell Atrial Cardiomyocytes and myrCell Ventricular Cardiomyocytes and derivative products.

Immunocytochemistry staining is a powerful tool that enables specific proteins or other molecules to be quantitatively visualised in a cellular context. As such, ICC can provide insights into the distribution, localisation, and abundance of specific proteins or other molecules. While ICC staining of iPSC-derived cells is a relatively straightforward process, it does require careful consideration of antibody pairings, delicate treatment of cells, and optimisation to reduce non-specific signals.

The protocol outlines recommended reagents and concentrations for the fixation, permeabilisation, and antibody staining of key markers relevant to atrial and ventricular cardiomyocytes. While these specific antibodies and dilutions are recommended for reliable outcomes, the core protocol can be adapted by researchers for use with other primary antibodies of interest.

This document focuses on the ICC procedure itself. It assumes that the thawing and culturing of the cells have been performed according to the instructions in the latest version of the myrCells Cardiomyocytes Handling Instructions.

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

2. Reagents

2.1. Cells

- myrCell Cardiomyocytes and derivatives

2.2. Reagents

Table 1. Reagents for stock solutions.

Reagent	Supplier	Cat. number	Storage
DPBS, no calcium, no magnesium	Thermo Fisher	14190169	4°C or RT
ROTI® Histofix 4% Formaldehyde	Carl Roth	P087.3	RT
Fetal bovine serum (FBS)	Thermo Fisher	A5256701	4°C
Bovine serum albumin (BSA)	Sigma	A3294	4°C
Triton-X-100	Sigma	T8787)	RT

Primary and secondary antibodies and recommended dilutions, see section 4.

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

3. Protocol

The following protocol recommends general guidelines. We encourage users to optimise the critical steps according to their experimental conditions.

Step 1: Cell fixation
Step 2: Immunostaining

Throughout this protocol, use a micropipette to remove liquids from each well, making sure not to disturb the cell layer.

Do not allow the cell layer to dry out; leave behind approximately 50 µL in the well after removing media.

Before starting

Refer to the latest version of the handling instructions for complete details on cell coating, cell thawing, and cell culture.

myrCell Atrial Cardiomyocytes Handling Instructions

myrCell Ventricular Cardiomyocytes Handling Instructions

Table 2. Culture dish surface area and suggested volumes for washing, storage or incubation of cells in DPBS or Blocking Buffer.

Culture vessel	Surface area (cm^2)	Washing volume/well (mL)	Volume/area (mL/cm^2)
T-75 flask	75	10	0.13
T-25 flask	25	3	0.13
6-well plate*	9.6	1.2	0.13
12-well plate*	4	0.6	0.15
24-well plate*	2	0.3	0.15
48-well plate*	1	0.15	0.15
96-well plate*	0.32	0.08	0.23
384-well plate*	0.084	0.02	0.22

* Tested by myriamed

3.1. Cell fixation

3.1.1. Aspirate the DPBS from the cell culture dishes and add a sufficient volume of **Histofix** to coat the surface area (0.15–0.2 mL/cm^2); see Table 2 for recommended volumes.
3.1.2. Incubate the plate at RT for 15 minutes or at 4°C overnight.
3.1.3. Aspirate the Histofix solution and wash the plate twice with DPBS (0.15-0.2 mL/cm^2). Dispose of formaldehyde containing solutions or Histofix appropriately.
3.1.4. After the second DPBS wash, add sufficient volume of DPBS to ensure the plate does not dry out before staining.

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

3.2. Immunostaining

3.2.1. Aspirate the DPBS from the cell culture dishes and add a sufficient volume of **Blocking buffer** to coat the surface area (0.15–0.2 mL/cm^2); see Table 2 for recommended volumes.

3.2.2. Incubate the plate at RT for 10 minutes; optional, the plate can be placed on a shaker.

3.2.3. Prepare the **primary** antibody solution in **Blocking buffer**; antibodies with different host species can be combined. Prepare 10% higher volume of immunostaining solution than recommended for the vessel size to ensure sufficient volume for all samples.

3.2.4. Following step 3.2.2, aspirate the Blocking buffer and replace it with the **primary** antibody immunostaining solution. Apply sufficient volume of the primary antibody staining solution, at least 0.1–0.15 mL/cm^2. The lower range of the volume may be used if the plates are placed on a shaker during staining, ensuring that the surface never dries out.

3.2.5. Incubate at RT for at least 1 hour or at 4°C overnight.

3.2.6. Aspirate the primary antibody staining solution and wash the cells twice in DPBS (0.15–0.2 mL/cm^2).

3.2.7. Dilute the fluorescently conjugated **secondary** antibodies in **Blocking buffer**. Protect the solution and samples from light at all times to prevent photobleaching. Apply 0.1–0.15 mL/cm² of the staining solution to fully cover the sample. The lower volume limit (0.1 mL/cm²) is sufficient if incubating on a shaker, provided the sample remains continuously submerged to prevent drying out.

3.2.8. For nuclei staining, add Hoechst to the secondary antibody immunostaining solution.

3.2.9. Incubate at RT for at least 1 hour or at 4°C overnight.

3.2.10. Wash the cells at least twice in DPBS (0.15–0.2 mL/cm^2).

3.2.11. Store the cells in DBPS (0.15–0.3 mL/cm^2) protected from light and sealed with Parafilm at 4° C until imaging.

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

4. Reagents and solutions preparation

4.1. Preparation of stock solutions

Table 3.
Note: To avoid freeze-thaw cycles, aliquot the stock solutions as appropriate for future use.

Reagent	Stock solution	Storage
Blocking buffer	DPBS, no calcium, no magnesium 5% v/v Fetal bovine serum (FBS) 1% w/v BSA 0.5% v/v Triton X-100	4°C or -20°C

4.2. Primary antibodies

Table 4.

Antibody	Species	Supplier	Cat no	Dilution	Storage
Anti-Sarcomeric Alpha Actinin antibody	Rabbit	abcam	ab68167	1:1000	-20°C
Anti-ACTN2 Antibody	Mouse	Merck	A7811	1:1000	-20°C
Anti-Cardiac Troponin T antibody	Rabbit	abcam	ab45932	1:1000	-20°C
Mouse IgG1 Isotype Control	Mouse	R&D Systems	MAB002	1:1000	4°C
Rabbit IgG Isotype Control	Rabbit	R&D Systems	NBP2-24891	1:1000	4°C

4.3. Secondary antibodies

Table 5. Ensure the secondary antibody is raised against the host species of the primary antibody. When multiplexing, use highly cross-adsorbed secondary antibodies to prevent cross-reactivity between targets.

Antibody	Species	Supplier	Cat no	Dilution	Storage
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488	Goat	Thermo Fisher	A-11001	1:1000	-20°C
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488	Goat	Thermo Fisher	A-11008	1:1000	-20°C
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 546	Goat	Thermo Fisher	A-11010	1:1000	-20°C
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 546	Goat	Thermo Fisher	A-11003	1:1000	-20°C
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 568	Goat	Thermo Fisher	A-11004	1:1000	-20°C
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647	Goat	Thermo Fisher	A-21244	1:1000	-20°C
Phalloidin Alexa Fluor™ 633 (F-actin stain)		Thermo Fisher	A22284	1:400	-20°C
Hoechst 33342		Thermo Fisher	H3570	1:1000	4°C

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio

5. Troubleshooting

Problem: Cells lifting or detaching during washes or antibody incubations

Likely causes

- Too vigorous aspiration/dispensing (serological pipettes, high suction)
- Over-permeabilisation (Triton-X-100 concentration too high or incubation too long)
- Inadequate coating of the plate surface

Fix

- Always leave ~50 µL when aspirating and use low-retention micropipette tips held at the side wall
- Always adhere to recommended Triton-X-100 concentration and incubation time
- Consider using dPBS +/+ for washes

Problem: Very weak or no specific fluorescence signal

Likely causes

- Antibody concentration too low or antibody degraded (freeze-thaw damage)
- Insufficient incubation time or poor antibody penetration
- Fluorophore bleached by light exposure

Fix

- Prepare fresh primary antibody or dilute at the upper end of recommended range (e.g. 1:200 instead of 1:500)
- Primary antibody incubation: A 1-hour incubation at RT is generally sufficient for standard staining. If signal intensity is low or non-specific background staining is observed, the incubation may be extended to ~24 h at 4°C, preferably on a very gentle shaker. Prolonged incubation at 4°C can enhance antibody–target binding and may improve the signal-to-background ratio by reducing non-specific interactions
- Keep samples in the dark; wrap plates in foil during all secondary antibody incubation steps

Problem: Uneven staining (edge-brightening or centre-dullness)

Likely causes

- Poor reagent distribution (static plate)
- Evaporation at plate edges
- Incomplete incubation during washing steps

Fix

- Make sure antibodies distribute evenly
- Avoid using only the outer wells for critical samples; include perimeter wells as ‘buffer’
- Ensure to incubate for full 5 minutes during every washing step

Problem: Autofluorescence or bleed-through between channels

Likely causes

- Over-fixation (excessive time)
- Imaging settings (filters or gain too broad/high)

Fix

- Ensure fixation time is adhered to
- Confirm that the installed filter sets match the specific requirements for Alexa Fluor and Hoechst dyes
- Reduce the exposure and/or gain to a lower level to establish a baseline and avoid overexposure

Problem: Nuclei signal uneven or weak

Likely causes

- Hoechst concentration too low or degraded

Fix

- Prepare fresh solution

Our technical support team at bit.bio is available to answer any questions you may have about either the protocol or the cells, contact us at technical@bit.bio.

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
www.bit.bio

Customer support:
technical@bit.bio
orders@bit.bio