



Characterization by flow cytometry

PSC-derived cardiomyocyte subtypes

Background

In recent years, various protocols to differentiate pluripotent stem cells (PSCs) into cardiovascular cells such as cardiomyocytes (CMs) have been published^{1,2}. However, cardiovascular cells differentiated from human PSC cultures are not homogeneous cell populations, but rather composed of a variety of CMs, including different CM subtypes or subpopulations, and non-CM cell types^{3,4}. These CM subpopulations contain atrial-like, ventricular-like, and pacemaker-like cells as shown by electrophysiological characterization of the respective cell cultures⁵. Currently, mainly the patch-clamp technique is used to characterize cardiac cells and their subtypes based on their electrophysiological properties such as specific action potentials. This technique can also be used to analyze the effects of specific drugs on cardiac function, to understand the impact of certain ion channel mutations on cell physiology, or to determine the maturity status of CMs. However, since this is a rather complex and time-consuming method requiring specially trained personnel, it can be barely used as quality control or for fast characterization of CMs. Moreover, the quantification of subtype populations remains difficult, since a statistically relevant number of cells needs to be examined by the patch-clamp technique. Additionally, the usage of genetically modified reporter cell lines is widely spread in the field.^{4,6} This enables not only the purification of the desired population by flow sorting or antibiotic selection but also the live observation of marker expression over a certain time frame. This method however is limited by the efficiency of gene delivery and therefore the number of reporter genes that can be introduced. Moreover, the introduction of genes always bears the risk of off-target effects, which might lead to erroneous results. Lastly, the use of fluorescent reporter cell lines limits the number of markers that can be analyzed by flow cytometry or immunofluorescence, since some detection channels will already be occupied for the analysis of the fluorescent reporter.

Flow cytometry represents a fast and efficient method to analyze and quantify different cell types within a given cell population, without the need of genetic engineering of

the starting material. Therefore, this method is extremely useful for quality control of initial and differentiated cell populations or as a readout tool in toxicity screens, cell therapy, and cell manufacturing processes. Moreover, a detailed analysis of cardiac marker expression during differentiation might help to develop more robust or even subtype-specific differentiation protocols.

Here we describe a simple and time-saving method for the characterization of PSC-derived CM populations by flow cytometry using novel recombinantly engineered CM-specific REAfinity™ Recombinant Antibodies.

Materials and methods

Instrument

MACSQuant® Analyzer 10

Antibodies and kits

- Anti-Cardiac Troponin T-FITC, human, mouse, rat; clone REA400
- Anti- α -Actinin (Sarcomeric)-VioBlue®, human, mouse, rat; clone REA402
- Anti-Myosin Heavy Chain-APC, human, mouse, rat; clone REA399
- Anti-MLC2a-APC, human, mouse, rat; clone REA398
- Anti-MLC2v-PE, human, mouse, rat; clone REA401
- Multi Tissue Dissociation Kit 3
- (optionally) PSC-Derived Cardiomyocyte Isolation Kit, human
- Inside Stain Kit

Buffers

- autoMACS® Running Buffer – MACS® Separation Buffer
- MACS BSA Stock Solution
- DPBS

For a complete list of antibodies for flow cytometry, please visit www.miltenyibiotec.com/antibodies

Procedure

1. CMs were differentiated from human PSCs according to a modified version of the method described by Lian *et al.*¹
2. On day 14, CMs were harvested using the Multi Tissue Dissociation Kit 3 according to the [data sheet](#). To download the PDF simply click on the hyperlink in boldface.
3. Cells were counted by flow cytometry using the MACSQuant[®] Analyzer 10.
4. 5×10^5 cells per staining were transferred into a 1.5 mL microtube.
5. Cells were centrifuged at $300 \times g$.
6. The supernatant was removed and the pellet was resuspended in InsideFix (a component of the Inside Stain Kit, 1:1 diluted in DPBS).
7. Cells were incubated for 10 min at RT.
8. Cells were washed with 1 mL MACS[®] Separation Buffer and centrifuged at $300 \times g$ for 5 min.
9. The supernatant was removed.
10. Cells were resuspended in Inside Perm (a component of the Inside Stain Kit). Following antibodies were added according to the manufacturer's recommendations:
 - a. Anti-Cardiac Troponin T-FITC and Anti- α -Actinin (Sarcomeric)-VioBlue[®]
 - b. Anti-Cardiac Troponin T-FITC and Anti-Myosin Heavy Chain-APC
 - c. Anti-Cardiac Troponin T-TnT-FITC and Anti-MLC2a-APC
 - d. Anti-Cardiac Troponin T-TnT-FITC and Anti-MLC2v-PE
 - e. Anti-MLC2a-APC and Anti-MLC2v-PE
11. Cells were incubated for 10 min at RT in the dark.
12. Cells were washed with 1 mL Inside Perm and centrifuged at $300 \times g$ for 5 min.
13. The supernatant was removed and the cell pellet resuspended in 250 μ L MACS Separation Buffer.
14. Stained cells were analyzed on the MACSQuant Analyzer 10.

Results

In this study CMs were purified based on MACS Technology and also characterized and quantified by flow cytometry using novel recombinantly engineered REAfinity[™] Antibodies. To that end, the cardiac cells were stained for the CM-specific markers cardiac Troponin T (cTnT), α -actinin, and myosin heavy chain (MHC), and the CM subtype-specific markers myosin light chain 2a and 2v (MLC2a, MLC2v) and analyzed on a MACSQuant Analyzer 10. These measurements revealed a high purity of cTnT- and α -actinin-positive CMs after magnetic enrichment by MACS Technology (fig. 1A). Furthermore, a strong co-expression

of cTnT and MHC in the enriched PSC-derived CMs could be observed, demonstrating the effectiveness of the PSC-Derived Cardiomyocyte Isolation Kit and the specificity of the different antibodies (fig. 1B).

Even though the CMs analyzed represented a rather young developmental stage, we could also detect a minor population of MLC2v-positive cells (i.e. ventricular CMs; fig. 1C and E) within the total cell population. It was expected that ventricular CMs make up only a minor proportion since full development of these cells occurs at later stages and expression of MLC2v is associated with more mature CMs. Finally, the majority of the enriched CMs expressed both cTnT and MLC2a (fig. 1D).

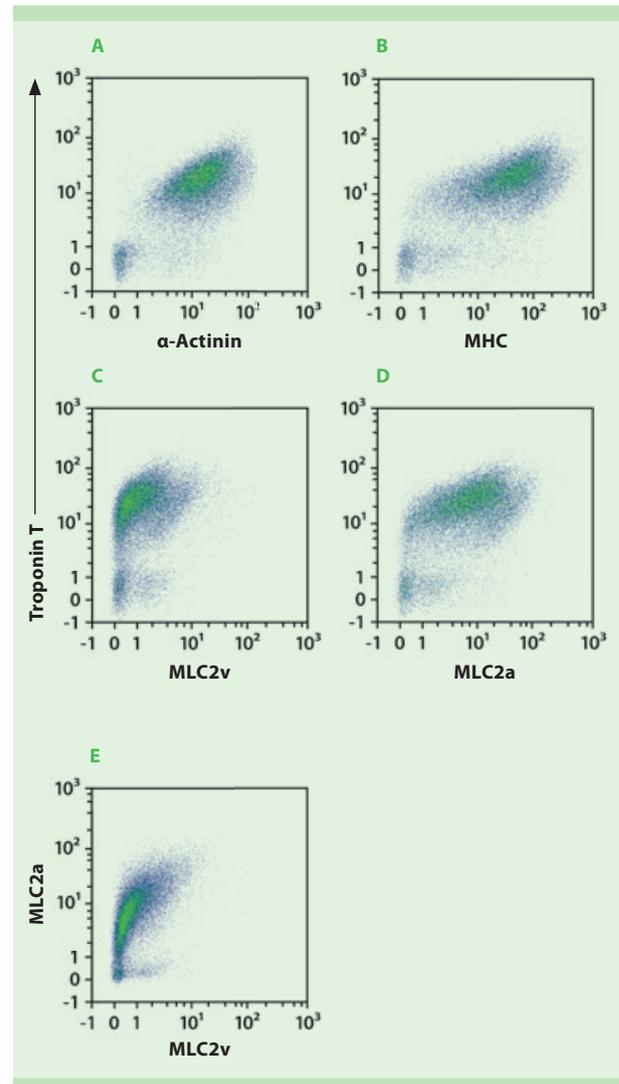


Figure 1: Flow cytometry analysis of CMs isolated with the PSC-Derived Cardiomyocyte Isolation Kit, human. Enriched cells were stained with REAfinity[™] Recombinant Antibodies and analyzed on a MACSQuant Analyzer 10.

Conclusion

The novel recombinantly engineered REAfinity™ Recombinant Antibodies against cTnT, MHC, α -actinin, MLC2a, and MLC2v are powerful tools for a detailed analysis of CM subpopulations derived from human PSCs. Flow cytometry allows reliable quantification of different subpopulations through the analysis of a large number of CM-specific markers in a single measurement. This enables the determination of correlations between different markers during CM development. Flow cytometry in combination with the highly specific REAfinity Antibodies provides reproducible results in the analysis of PSC-derived CMs and therefore is a compelling tool for quality control during CM development.

Product table

Product	Order no.
MACSQuant® Analyzer 10	130-096-343
Multi Tissue Dissociation Kit 3	130-110-204
PSC-Derived Cardiomyocyte Isolation Kit, human	130-090-477
Inside Stain Kit	130-090-477
autoMACS® Running Buffer – MACS® Separation Buffer	130-091-221
MACS BSA Stock Solution	130-091-376

References

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5. Burridge, P.W. (2014) Chemically defined generation of human cardiomyocytes. *Nat. Methods* 11: 855–860.
6. Weng, Z. *et al.* (2014) A simple, cost-effective but highly efficient system for deriving ventricular cardiomyocytes from human pluripotent stem cells. *Stem Cells Dev.* 23: 1704–1716.



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