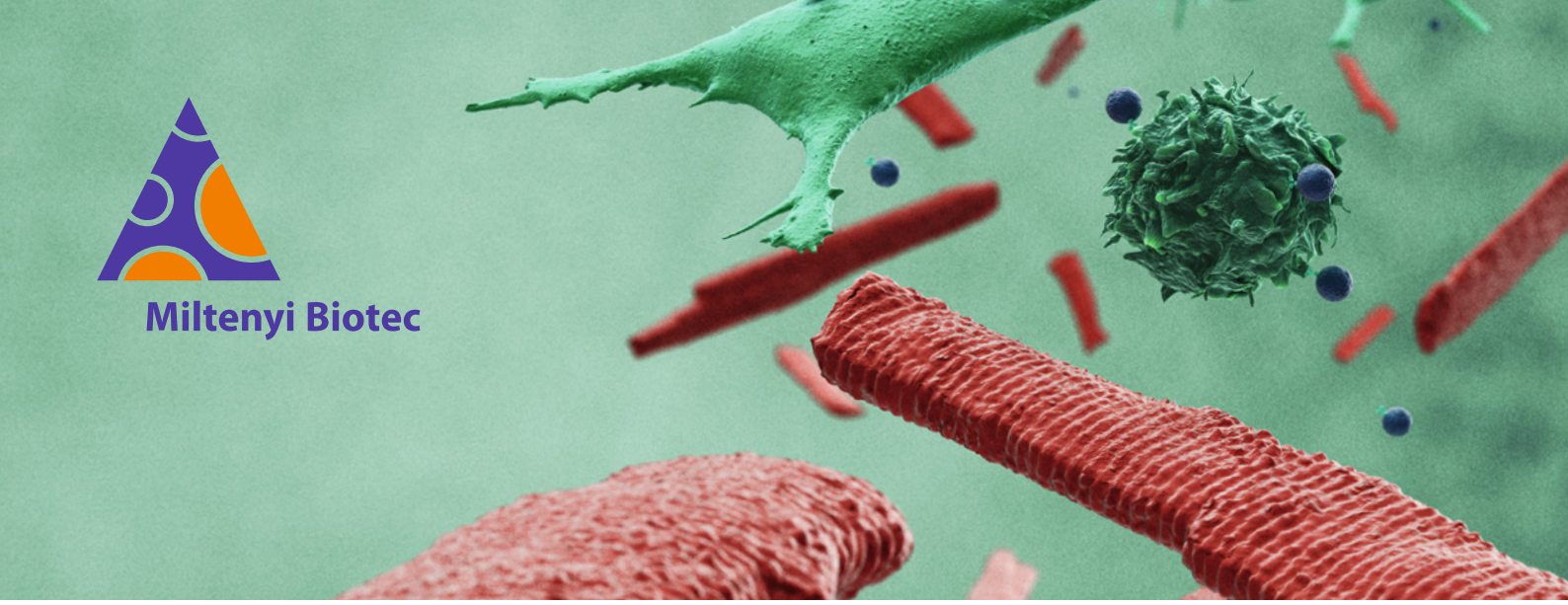


Miltenyi Biotec

Neonatal cardiomyocytes

Complete workflow for purification, cultivation, and analysis

Background

Neonatal cardiomyocytes (CMs) have long been used to study biochemical, physiological, pharmacological, or morphological characteristics. Cultured CMs allow for an easy investigation and manipulation of biochemical pathways, which helps to elucidate how individual pathways affect the biomechanical properties of spontaneously beating CMs.

Recent reports on the regeneration of neonatal hearts and the progress being made in the development of processes for the direct conversion of fibroblasts into CMs have boosted research on cardiac regeneration[1]. The efforts to analyze the cellular composition of neonatal hearts in detail were intensified and a strong need for functional characterization of the individual cardiac cell subtypes emerged. However, current protocols to dissociate primary heart tissue and to isolate and analyze cardiac cells are labor intensive and difficult to standardize[2].

In order to better analyze CMs and to gain a better understanding of CM subtypes, such as atrial CMs and ventricular CMs, new techniques and protocols are needed. CM subtypes are characterized by differential expression of MLC2a (in atrial CMs) and MLC2v (in ventricular CMs)[3,4].

This note describes a complete workflow from dissociation of neonatal mouse or rat hearts and the separation of CMs through to flow cytometry. Recombinantly engineered REAfinity® Recombinant Antibodies against MLC2a and MLC2v enable the unambiguous analysis of atrial and ventricular CMs, respectively.

Materials and methods

Materials

- gentleMACS™ Octo Dissociator with Heaters
- gentleMACS C Tubes
- Centrifuge
- Neonatal Heart Dissociation Kit, mouse and rat
- MACS® SmartStrainers (70 µm) or Pre-Separation Filters (70 µm)
- Neonatal Cardiomyocyte Isolation Kit, mouse or Neonatal Cardiomyocyte Isolation Kit, rat
- Phosphate-buffered saline (PBS)
- PEB buffer: Prepare a solution containing PBS (pH 7.2), 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution at a ratio of 1:20 with autoMACS® Rinsing Solution. Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.
- Inside Stain Kit
- Cardiac Troponin T Antibody, anti-human/mouse/rat, FITC, REAfinity
- MLC2a Antibody, anti-human/mouse/rat, VioBlue®, REAfinity
- MLC2v Antibody, anti-human/mouse/rat, APC, REAfinity
- MACSQuant® Analyzer 10

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For a complete list of antibodies for flow cytometry, please visit

▸ **miltenyibiotec.com/antibodies**

Methods

1. Extract neonatal mouse or rat heart (P0–P3).
2. (Optional) Cut atria from ventricle if separated dissociation is needed.
3. Dissociate the tissue according to the protocol of the Neonatal Heart Dissociation Kit, mouse and rat, including post-dissociation wash steps.
4. Determine cell number.
5. Isolate mouse or rat CMs according to the protocol of the respective Neonatal Cardiomyocyte Isolation Kit.
6. Centrifuge up to 10^6 cells at 300×g for 5 minutes. Aspirate supernatant carefully.

7. Resuspend cells in 100 µL of PBS.
8. Add 100 µL of Inside Fix. Mix well and incubate for 10 minutes in the dark at room temperature.
9. Wash cells by adding 1 mL of PEB buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
10. Resuspend cells in Inside Perm and add the following staining antibodies according to the manufacturer's recommendations: Cardiac Troponin T Antibody, anti-human/mouse/rat, FITC, REAfinity; MLC2a Antibody, anti-human/mouse/rat, VioBlue, REAfinity; and MLC2v Antibody, anti-human/mouse/rat, APC, REAfinity.
11. Mix well and incubate for 10 minutes in the dark at room temperature.
12. Wash cells by adding 1 mL of PEB buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
13. Resuspend cell pellet in a suitable amount of PEB buffer for flow cytometry analysis. Store cells at 2–8 °C in the dark until analysis. Mix well before flow cytometric acquisition.

Results

Neonatal hearts from mouse or rat were dissociated into single-cell suspensions and CMs were isolated as described in the methods section. Using the recombinantly engineered antibodies against cardiac troponin T, MLC2a, and MLC2v, distinct populations of atrial and ventricular CMs could be identified by flow cytometry (figs. 1 and 2). Isolation of CMs with the Neonatal Cardiomyocyte Isolation Kit resulted in the efficient enrichment of both atrial and ventricular CMs from whole heart single-cell suspensions. Additionally, selective isolation of atrial or ventricular cardiomyocytes was possible if heart chambers (atria and ventricles) were processed separately.

Conclusion

The Neonatal Heart Dissociation Kit in combination with the gentleMACS Octo Dissociator with Heaters, the Neonatal Cardiomyocyte Isolation Kit, and the tools for flow cytometry including REAfinity Antibodies against cardiac troponin T, MLC2a, and MLC2v provide a complete workflow for the effective enrichment and detailed analysis of CM subpopulations from mouse and rat hearts. As shown below, the mentioned antibodies are useful tools to differentiate between CM subpopulations by flow cytometry before and after enrichment of neonatal mouse (fig. 1) and rat (fig. 2) hearts.

References

1. O'Meara, C.C. *et al.* (2015) Transcriptional reversion of cardiac myocyte fate during mammalian cardiac regeneration. Circ. Res. 116 (5): 804–815.
2. Ehler, E. *et al.* (2013) Isolation and culture of neonatal mouse cardiomyocytes. J. Vis. Exp. 79. doi: 10.3791/50154.
3. Eckardt, D. *et al.* (2014) P363: Antibody-based selective isolation of atrial and ventricular cardiomyocytes from neonatal mouse heart. Cardiovasc. Res. (Suppl. 1) 103: S57–S94.
4. Chuva de Sousa Lopes, S. M. *et al.* (2006) Patterning the heart, a template for human cardiomyocyte development. Dev. Dyn. 235: 1994–2002.

Product table

Product	Order no.
gentleMACS Octo Dissociator with Heaters	130-134-029
gentleMACS C Tubes	130-093-237, 130-096-334
Neonatal Heart Dissociation Kit, mouse and rat	130-098-373
MACS SmartStrainers (70 µm)	130-098-462
Pre-Separation Filters (70 µm)	130-095-823
Neonatal Cardiomyocyte Isolation Kit, mouse	130-100-825
Neonatal Cardiomyocyte Isolation Kit, rat	130-105-420
autoMACS Rinsing Solution	130-091-222
Inside Stain Kit	130-090-477
Cardiac Troponin T Antibody, anti-human/mouse/rat, FITC, REAfinity	130-119-575
MLC2a Antibody, anti-human/mouse/rat, VioBlue, REAfinity	130-125-510
MLC2v Antibody, anti-human/mouse/rat, APC, REAfinity	130-123-847
MACSQuant Analyzer 10	130-096-343

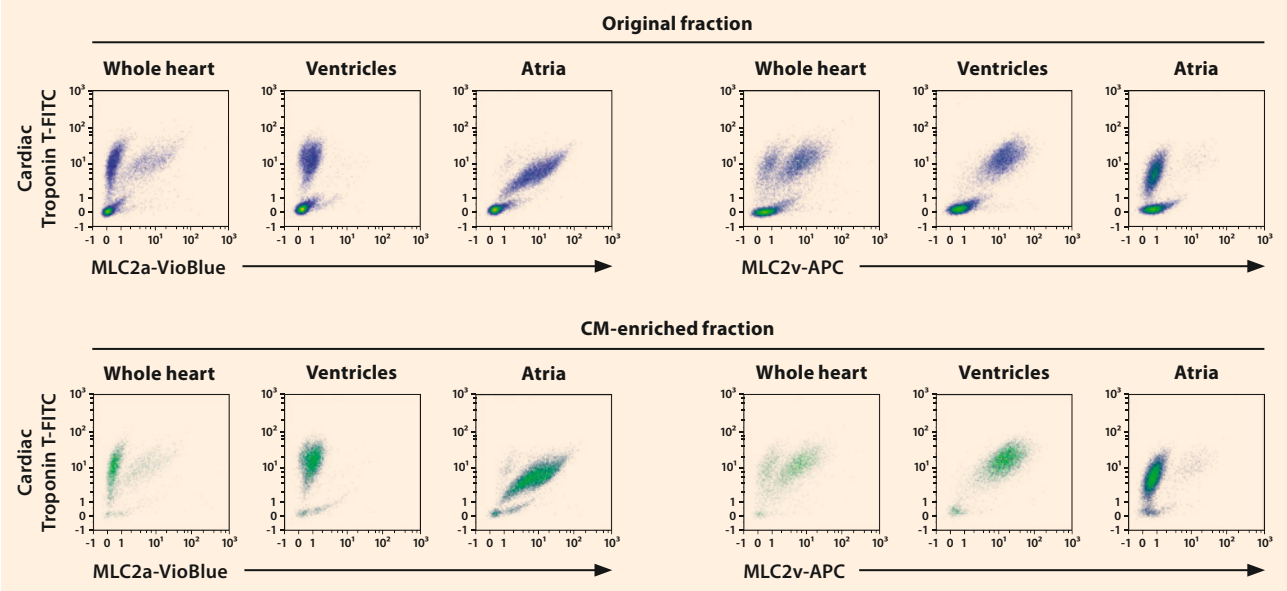


Figure 1: Flow cytometry analysis of enriched neonatal mouse CMs. Neonatal mouse CMs were isolated from dissociated whole hearts or individually dissociated chambers using the Neonatal Cardiomyocyte Isolation Kit, mouse. The cells were labeled with indicated antibodies before and after enrichment, and analyzed by flow cytometry using the MACSQuant Analyzer 10.

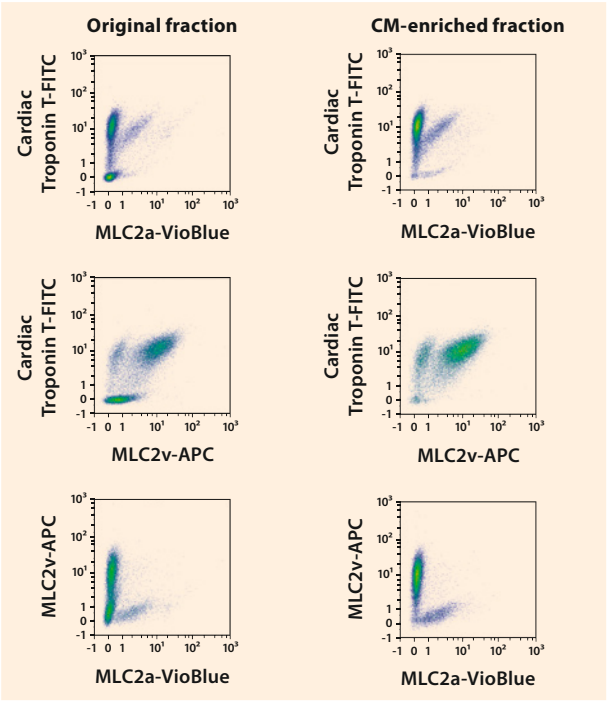


Figure 2: Flow cytometry analysis of enriched neonatal rat CMs. Neonatal rat CMs were isolated from dissociated whole hearts of rat using the Neonatal Cardiomyocyte Isolation Kit, rat. The cells were labeled with indicated antibodies before and after enrichment, and analyzed by flow cytometry using the MACSQuant Analyzer 10.



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