Synaptic Systems

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MLC-2V

Cat.No. 310 111AT1; Monoclonal mouse antibody, 100 µg purified IgG (lyophilized)

Data Sheet

Reconstitution/ Storage	100 µg purified IgG, lyophilized, fluorescence-labeled with ATTO® 647N. For reconstitution add 100 µl H_2O to get a 1mg/ml solution in PBS. Either add 1:1 (v/v) glycerol, then aliquot and store at -20°C until use, or store aliquots at -80°C without additives. Reconstitute immediately upon receipt! Avoid bright light when working with the antibody to minimize photo bleeching of the fluorescent dye.
Applications	**WB**: N/A **IP**: N/A **ICC**: yes (see remarks) **IHC**: yes (see remarks) **IHC-P/FFPE**: 1 : 200
Label	ATTO 647N
Clone	330G5
Subtype	IgG2a (κ light chain)
Immunogen	Recombinant protein corresponding to AA 1 to 165 from human MLC-2V (UniProt Id: P10916)
Epitop	Epitop: AA 105 to 111 from human MLC-2V (UniProt Id: P10916)
Reactivity	Reacts with: human (P10916), rat (P08733), mouse (P51667), pig, chicken. Other species not tested yet.
Specificity	Specific for MLC-2V, no cross-reactivity to MLC-2A.
Remarks	**ICC**: Only methanol fixation, recommended protocol. **IHC**: Protocol for immunohistochemistry of this antibody will be provided with the goods.

TO BE USED IN VITRO / FOR RESEARCH ONLY
NOT TOXIC, NOT HAZARDOUS, NOT INFECTIOUS, NOT CONTAGIOUS

During cardiogenesis two major isoforms of **m**yosin **l**ight **c**hain **2** are co-expressed in a tightly regulated manner. **MLC-2V** is only present in the ventricle while MLC-2A is exclusively expressed in the atrium. Knock out studies revealed that the 2A isoform cannot substitute for the 2V variant in the ventricular chamber.
Recently it has been demonstrated that embryonic and adult stem cells can be differentiated into cardiomyocytes which may generate suitable replacements for damaged heart tissue in the future. These antibodies are useful tools to distinguish between ventricle and atrium specific cardiomyocytes.

Selected References SYSY Antibodies

Defining human cardiac transcription factor hierarchies using integrated single-cell heterogeneity analysis.
Churko JM, Garg P, Treutlein B, Venkatasubramanian M, Wu H, Lee J, Wessells QN, Chen SY, Chen WY, Chetal K, Mantalas G, et al.
Nature communications (2018) 9(1): 4906. **ICC; tested species: human**

Selected General References

Mechanism of spontaneous excitability in human embryonic stem cell derived cardiomyocytes.
Satin J, Kehat I, Caspi O, Huber I, Arbel G, Itzhaki I, Magyar J, Schroder EA, Perlman I, Gepstein L
The Journal of physiology (2004) 559(Pt 2): 479-96.

Selection of ventricular-like cardiomyocytes from ES cells in vitro.
Müller M, Fleischmann BK, Selbert S, Ji GJ, Endl E, Middeler G, Müller OJ, Schlenke P, Frese S, Wobus AM, Hescheler J, et al.
FASEB journal : official publication of the Federation of American Societies for Experimental Biology (2000) 14(15): 2540-8.

Transgenic remodeling of the contractile apparatus in the mammalian heart.
Palermo J, Gulick J, Colbert M, Fewell J, Robbins J
Circulation research (1996) 78(3): 504-9.

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Protocol for immunohistochemistry for MLC-2V antibodies

mouse monoclonal antibody (Cl. 330G5); purified IgG; cat. no. 310 111AT1 and 310 111AT2

Deparaffinization and rehydration:

- Incubate sections with xylene twice for 5-10min.
- Incubate section with acetone once for 10min.
- Incubate section with acetone diluted 1:2 with TBS pH 7.6 once for 10min.
- Incubate section with TBS once for 10min.
- Rinse slides in dest. H2O.

Antigen retrieval: is <u>necessary</u>

- Microwave sections for 15 min in freshly mixed 10 mM citrate buffer, pH 6.0 to retrieve the antigen.
- Cool slides slowly to RT (20-30min).
- Rinse slides in PBS:

Citrate buffer: *A*: 21.01g citric acid in 1000ml dest. H_2O

B: 29.41g sodium citrate in 1000ml dest. H_2O

Mix 9ml *A* with 41ml *B* and 450ml dest. H_2O. Adjust the pH to 6.0.

Immunofluorescence:

- Block sections in 10% normal goat serum in PBS (30min at RT).
- Remove blocking solution and incubate sections overnight at 4°C with the primary antibody (anti-MLC-2V) in PBS / 2% BSA at a dilution of 1:200 (5µg/ml).
- Remove primary antibody and wash thoroughly with PBS +BSA 3 times for 5min.
- Mount slices and examine microscopically.