Synaptic Systems

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MLC-2A

Cat.No. 311 011; Monoclonal mouse antibody, 100 µg purified IgG (lyophilized)

Data Sheet

Reconstitution/ Storage	100 µg purified IgG, lyophilized. Azide was added before lyophilization. For reconstitution add 100 µl H_2O to get a 1mg/ml solution in PBS. Then aliquot and store at -20°C until use.
Applications	**WB**: 1 : 100 up to 1 : 2000 (AP staining) **IP**: not tested yet **ICC**: yes (see remarks) **IHC**: yes (see remarks) **IHC-P/FFPE**: 1 : 200 up to 1 : 1000
Clone	56F5
Subtype	IgG2b (κ light chain)
Immunogen	Recombinant protein corresponding to AA 1 to 175 from human MLC-2A (UniProt Id: Q01449)
Epitop	Epitop: AA 1 to 175 from human MLC-2A (UniProt Id: Q01449)
Reactivity	Reacts with: human (Q01449), rat, mouse (Q9QVP4). No signal: chicken. Other species not tested yet.
Specificity	Specific for MLC-2A, no cross-reactivity to MLC-2V.
matching control	311-0P
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-2A** is only present in the atrium while MLC-2V is exclusively expressed in the ventricle. 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. This monoclonal antibody is a useful tool to distinguish between ventricle and atrium specific cardiomyocytes.

Selected References SYSY Antibodies

Highly enriched cardiomyocytes from human embryonic stem cells.
Xu XQ, Zweigerdt R, Soo SY, Ngoh ZX, Tham SC, Wang ST, Graichen R, Davidson B, Colman A, Sun W
Cytotherapy (2008) 10(4): 376-89. **ICC, IHC**

Simultaneous voltage and calcium mapping of genetically purified human induced pluripotent stem cell-derived cardiac myocyte monolayers.
Lee P, Klos M, Bollensdorff C, Hou L, Ewart P, Kamp TJ, Zhang J, Bizy A, Guerrero-Serna G, Kohl P, Jalife J, et al.
Circulation research (2012) 110(12): 1556-63. **ICC, FACS**

Phosphorylation and translocation of heat shock protein 27 and alphaB-crystallin in human myocardium after cardioplegia and cardiopulmonary bypass.
Clements RT, Sodha NR, Feng J, Mieno S, Boodhwani M, Ramlawi B, Bianchi C, Sellke FW
The Journal of thoracic and cardiovascular surgery (2007) 134(6): 1461-70. **WB, IHC; tested species: human**

Direct nkx2-5 transcriptional repression of isl1 controls cardiomyocyte subtype identity.
Dorn T, Goedel A, Lam JT, Haas J, Tian Q, Herrmann F, Bundschu K, Dobreva G, Schiemann M, Dirschinger R, Guo Y, et al.
Stem cells (Dayton, Ohio) (2015) 33(4): 1113-29. **ICC, IHC**

Rat atrial engineered heart tissue: a new in vitro model to study atrial biology.
Krause J, Löser A, Lemoine MD, Christ T, Scherschel K, Meyer C, Blankenberg S, Zeller T, Eschenhagen T, Stenzig J
Basic research in cardiology (2018) 113(5): 41. **IHC-P; tested species: rat**

Generation of a Urine-Derived Ips Cell Line from a Patient with a Ventricular Septal Defect and Heart Failure and the Robust Differentiation of These Cells to Cardiomyocytes via Small Molecules.
Cao Y, Xu J, Wen J, Ma X, Liu F, Li Y, Chen W, Sun L, Wu Y, Li S, Li J, et al.
Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology (2018) 50(2): 538-551. **ICC; tested species: mouse**

Development of In Vitro Drug-Induced Cardiotoxicity Assay by Using Three-Dimensional Cardiac Tissues Derived from Human Induced Pluripotent Stem Cells.
Takeda M, Miyagawa S, Fukushima S, Saito A, Ito E, Harada A, Matsuura R, Iseoka H, Sougawa N, Mochizuki-Oda N, Matsusaki M, et al.
Tissue engineering. Part C, Methods (2018) 24(1): 56-67. **IHC; tested species: stem cells**

Meticulous optimization of cardiomyocyte yields in a 3-stage continuous integrated agitation bioprocess.
Ting S, Lam A, Tong G, Chen A, Wei H, Wu J, Lam YN, Reuveny S, Oh S
Stem cell research (2018) 31: 161-173. **IHC; tested species: human**

Generation of multipotent induced cardiac progenitor cells from mouse fibroblasts and potency testing in ex vivo mouse embryos.
Lalit PA, Rodriguez AM, Downs KM, Kamp TJ
Nature protocols (2017) 12(5): 1029-1054. **ICC**

Use of Human Pluripotent Stem Cell Derived-Cardiomyocytes to Study Drug-Induced Cardiotoxicity.
Maillet A, Tan KP, Brunham LR
Current protocols in toxicology (2017) 73: 22.5.1-22.5.22. **ICC; tested species: human**

Heparin Promotes Cardiac Differentiation of Human Pluripotent Stem Cells in Chemically Defined Albumin-Free Medium, Enabling Consistent Manufacture of Cardiomyocytes.
Lin Y, Linask KL, Mallon B, Johnson K, Klein M, Beers J, Xie W, Du Y, Liu C, Lai Y, Zou J, et al.
Stem cells translational medicine (2017) 6(2): 527-538. **ICC; tested species: human**

Human Embryonic Stem Cell-Derived Cardiomyocytes Self-Arrange with Areas of Different Subtypes During Differentiation.
Vestergaard ML, Grubb S, Koefoed K, Anderson-Jenkins Z, Grunnet-Lauridsen K, Calloe K, Clausen C, Christensen ST, Møllgård K, Andersen CY
Stem cells and development (2017) 26(21): 1566-1577. **IHC; tested species: human**

Chemical-defined and albumin-free generation of human atrial and ventricular myocytes from human pluripotent stem cells.
Pei F, Jiang J, Bai S, Cao H, Tian L, Zhao Y, Yang C, Dong H, Ma Y
Stem cell research (2017) 19: 94-103. **ICC**

Generation of clinical-grade functional cardiomyocytes from human embryonic stem cells in chemically defined conditions.
Tan Y, Han P, Gu Q, Chen G, Wang L, Ma R, Wu J, Feng C, Zhang Y, Wang L, Hu B, et al.
Journal of tissue engineering and regenerative medicine (2016) : . **ICC; tested species: human**

Differential Expression Levels of Integrin α6 Enable the Selective Identification and Isolation of Atrial and Ventricular Cardiomyocytes.
Wiencierz AM, Kernbach M, Ecklebe J, Monnerat G, Tomiuk S, Raulf A, Christalla P, Malan D, Hesse M, Bosio A, Fleischmann BK, et al.
PloS one (2015) 10(11): e0143538. **FACS**

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Protocol for immunohistochemistry for MLC-2A antibodies

mouse monoclonal antibody (Cl. 56F5); purified IgG; cat. no. 311 011

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.
- Incubate with the secondary antibody for 1h at RT.
- Remove secondary antibody and wash thoroughly with PBS + BSA 3 times for 5min.
- Mount slices and examine microscopically.