Store at -20°C

#48243

Cardiogenesis Marker Antibody Sampler Kit

1 Kit (7 x 20 µl)

Support: +1-978-867-2388 (U.S.)
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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
α-Actinin (D6F6) XP® Rabbit mAb	6487	20 µl	100 kDa	Rabbit IgG
GATA-6 (D61E4) XP® Rabbit mAb	5851	20 µl	55 kDa	Rabbit IgG
MEF2C (D80C1) XP® Rabbit mAb	5030	20 µl	50-60 kDa	Rabbit IgG
NKX2.5 (E1Y8H) Rabbit mAb	8792	20 µl	30-42 kDa	Rabbit IgG
Troponin I (D6F8) Rabbit mAb	13083	20 µl	28 kDa	Rabbit IgG
Connexin 43 Antibody	3512	20 µl	39, 41, 43, 44 kDa	Rabbit
Troponin T (Cardiac) Antibody	5593	20 µl	40 kDa	Rabbit
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

Recommended Antibody Dilutions:

Western blotting	1:1000

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Description: The Cardiogenesis Marker Antibody Sampler Kit provides an economical means of evaluating proteins involved in heart development. This kit contains enough antibody to perform two western blot experiments per primary antibody.

Background: Cardiogenesis is a complex developmental event involving numerous transcription factors. NKX2.5 is a member of the NKX homeobox transcription factor family, which plays an essential role in heart development and is among the earliest factors expressed in the cardiac lineage in developing embryos. Mutations in NKX2.5 are associated with several congenital heart conditions, such as atrial defect with atrioventricular conduction defects (ASD-AVCD) and Tetralogy of Fallot (TOF) (1,2). GATA proteins comprise a group of transcription factors that are related by the presence of conserved zinc finger DNA binding domains, which bind directly to the nucleotide sequence core element GATA (3-5). GATA-6 plays a critical role in endoderm development and knock out of GATA-6 is embryonic lethal due to defects in formation of the heart tube and a failure to develop extraembryonic endoderm (6). MEF2C is a member of the MEF2 (myocyte enhancer factor 2) family of transcription factors. The MEF2 family members were originally described as muscle-specific DNA binding proteins that recognize MEF2 motifs found within the promoters of many muscle-specific genes (7,8). α-Actinin was first recognized as an actin cross-linking protein. The α-actinin protein interacts with a large number of proteins involved in signaling to the cytoskeleton, including those involved in cellular adhesion, migration, and immune cell targeting (9). The muscle isoforms 2 and 3 (ACTN2, ACTN3) localize to the Z-discs of striated muscle and to dense bodies and plaques in smooth muscle (9). Troponin, working in conjunction with tropomyosin, functions as a molecular switch that regulates muscle contraction in response to changes in the intracellular Ca^{2+} concentration. Troponin consists of three subunits: the Ca^{2+}-binding subunit troponin C (TnC), the tropomyosin-binding subunit troponin T (TnT), and the inhibitory subunit troponin I (TnI) (10). Assays for measuring serum concentrations of cardiac muscle TnT (cTNT), as well as cTnI, have been reported for analyzing cardiac injury. Connexin 43 (Cx43) is a member of the large family of gap junction proteins, which assemble as a hexamer and are transported to the plasma membrane to create a hemichannel that can associate with hemichannels on nearby cells to create cell-to-cell channels. Gap junction communication is important in development and regulation of cell growth. Phosphorylation of Cx43 is important in regulating assembly and function of gap junctions (11,12).

Specificity/Sensitivity: NKX2.5 (E1Y8H) Rabbit mAb recognizes endogenous levels of total NKX2.5 protein. GATA-6 (D61E4) XP® Rabbit mAb recognizes endogenous levels of total GATA-6 protein. MEF2C (D80C1) XP® Rabbit mAb detects endogenous levels of total MEF2C protein. α-Actinin (D6F6) XP® Rabbit mAb recognizes endogenous levels of total α-actinin protein. Troponin I (D6F8) Rabbit mAb recognizes endogenous levels of total troponin I protein. Troponin T (Cardiac) Antibody detects endogenous levels of total cardiac Troponin T protein. Connexin 43 Antibody detects endogenous levels of total connexin 43. This antibody does not cross-react with other connexins.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Pro67 of human NKX2.5 protein, residues near the amino terminus of human GATA-6 protein, a region surrounding Met182 of human MEF2C protein, residues surrounding Phe316 of human α-actinin-1 protein, or residues near the amino terminus of human troponin I protein. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to a region surrounding Pro69 of human cardiac troponin T protein or residues of human connexin 43. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Benson, D.W. et al. (1999) *J Clin Invest* 104, 1567-73.

(2) Reamon-Buettner, S.M. and Borlak, J. (2010) *Hum Mutat* 31, 1185-94.

(3) Ko, L.J. and Engel, J.D. (1993) *Mol Cell Biol* 13, 4011-22.

(4) Merika, M. and Orkin, S.H. (1993) *Mol Cell Biol* 13, 3999-4010.

(5) Lowry, J.A. and Atchley, W.R. (2000) *J Mol Evol* 50, 103-15.

(6) Cai, K.Q. et al. (2008) *Dev Dyn* 237, 2820-9.

(7) Martin, J.F. et al. (1994) *Mol Cell Biol* 14, 1647-56.

(8) Yu, Y.T. et al. (1992) *Genes Dev* 6, 1783-98.

(9) Otey, C.A. and Carpen, O. (2004) *Cell Motil Cytoskeleton* 58, 104-11.

(10) Ward, D.G. et al. (2002) *J Biol Chem* 277, 41795-801.

(11) Musil, L.S. et al. (1990) *J Cell Biol* 111, 2077-88.

(12) Musil, L.S. and Goodenough, D.A. (1991) *J Cell Biol* 115, 1357-74.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.

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