#8655 Store at -20°C

Wnt/β-Catenin Activated Targets Antibody Sampler Kit

☑ 1 Kit
(8 x 20 µl)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
CD44 (156-3C11) Mouse mAb	3570	20 µl	80 kDa	Mouse IgG2a
Cyclin D1 (92G2) Rabbit mAb	2978	20 µl	36 kDa	Rabbit IgG
c-Jun (60A8) Rabbit mAb	9165	20 µl	43, 48 kDa	Rabbit IgG
LEF1 (C12A5) Rabbit mAb	2230	20 µl	25-58 kDa	Rabbit IgG
Met (D1C2) XP® Rabbit mAb	8198	20 µl	140, 170 kDa	Rabbit IgG
MMP-7 (D4H5) XP® Rabbit mAb	3801	20 µl	28, 20-22 kDa	Rabbit IgG
c-Myc (D84C12) Rabbit mAb	5605	20 µl	57-65 kDa	Rabbit IgG
TCF1/TCF7 (C63D9) Rabbit mAb	2203	20 µl	48, 50 kDa	Rabbit IgG
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

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 antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

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

Description: The Wnt/β-Catenin Activated Targets Antibody Sampler Kit provides an economical means to investigate target proteins of the Wnt/β-Catenin signaling pathway. This kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: The Wnt family includes several secreted glycoproteins that play important roles in animal development. β-catenin is a key downstream effector of the Wnt signaling pathway that research studies have shown is implicated in early embryonic development and tumorigenesis in vertebrates (1-3). Following binding of Wnt family proteins to the Frizzled receptor, β-catenin translocates to the nucleus where it interacts with LEF1 and and TCF1/TCF7 to activate canonical targets (4). Accepted canonical targets include CD44, cyclin D1, c-Jun, c-Myc, Met, and MMP-7 (5-11).

Specificity/Sensitivity: Each antibody in the Wnt/ß-Catenin Activated Targets Antibody Sampler Kit detects its respective target at endogenous levels. LEF1 (C12A5) Rabbit mAb does not recognize the dominant negative forms of LEF1 generated by an alternative promoter. c-Myc (D84C12) Rabbit mAb is not recommended for detection of Myc-tagged fusion proteins. TCF1/TCF7 (C63D9) Rabbit mAb does not recognize the dominant negative isoforms of TCF1/TCF7 lacking the amino-terminal ß-catenin binding domain and does not cross-react with LEF1.

Source/Purification: Rabbit monoclonal antibodies are produced by immunizing animals with a GST-c-Jun protein corresponding to the amino-terminal sequence of human c-Jun protein or synthetic peptides corresponding to residues near the amino terminus of c-Myc protein, residues surrounding Pro82 of human LEF1 protein, residues surrounding Ile264 of mouse MMP-7 protein, residues surrounding Pro95 of human TCF1/TCF7 protein, residues near the carboxy terminus of human Met protein, or residues near the carboxy-terminus of human cyclin D1 protein.

The mouse monoclonal antibody is produced by immunizing BALB/c mice with stimulated human leukocytes for CD44.

Background References:

(1) Cadigan, K.M. and Nusse, R. (1997) *Genes Dev* 11, 3286-305.
(2) Wodarz, A. and Nusse, R. (1998) *Annu Rev Cell Dev Biol* 14, 59-88.
(3) Polakis, P. (1999) *Curr Opin Genet Dev* 9, 15-21.
(4) Brantjes, H. et al. (2002) *Biol Chem* 383, 255-61.
(5) Zeilstra, J. et al. (2008) *Cancer Res* 68, 3655-61.
(6) Shtutman, M. et al. (1999) *Proc Natl Acad Sci USA* 96, 5522-7.
(7) Mann, B. et al. (1999) *Proc Natl Acad Sci USA* 96, 1603-8.
(8) Wilkins, J.A. and Sansom, O.J. (2008) *Cancer Res* 68, 4963-6.
(9) Boon, E.M. et al. (2002) *Cancer Res* 62, 5126-8.
(10) Brabletz, T. et al. (1999) *Am J Pathol* 155, 1033-8.
(11) Clevers, H. and Nusse, R. (2012) *Cell* 149, 1192-205.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **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 **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#8655

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.