Store at -20°C

#13236

Adherens Junction Antibody Sampler Kit

1 Kit (5 x 40 µl)

www.cellsignal.com

Support: 877-678-TECH (8324)
www.cellsignal.com/support

Orders: 877-616-CELL (2355)
orders@cellsignal.com

rev. 07/31/14

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Afadin (D1Y3Z) Rabbit mAb	13531	40 µl	205 kDa	Rabbit IgG
α-E-Catenin (23B2) Rabbit mAb	3240	40 µl	100 kDa	Rabbit IgG
β-Catenin (D10A8) XP® Rabbit mAb	8480	40 µl	92 kDa	Rabbit IgG
γ-Catenin Antibody	2309	40 µl	83 kDa	Rabbit IgG
Catenin δ-1 Antibody	4989	40 µl	100 kDa	Rabbit IgG
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 antibodies.*

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 Adherens Junction Antibody Sampler Kit provides an economical means of detecting the protein components of adherens junctions. The kit includes enough antibody to perform four western blot experiments per primary antibody.

Background: Adherens junctions are dynamic structures that form cell-cell contacts and are important in development, differentiation, tissue integrity, morphology and cell polarity. They are composed of the transmembrane proteins, cadherins, which bind cadherins on adjacent cells in a calcium-dependent manner. On the cytoplasmic side of adherens junctions, the classic model states that cadherins are linked to the cytoskeleton through β- and α-catenin. α-E-catenin is ubiquitously expressed, α-N-catenin is expressed in neuronal tissue, and α-T-catenin is primarily expressed in heart tissue. Research studies have demonstrated that loss of E-cadherin and α-E-catenin occurs during the progression of several human cancers, indicating that the breakdown of adherens junctions is important in cancer progression (reviewed in 1).

Research studies also suggest that, rather than acting as a static link between cadherins and actin, α-catenin regulates actin dynamics directly, possibly by competing with the actin nucleating arp2/3 complex (2,3). α-catenin also plays a role in regulating β-catenin-dependent transcriptional activity, affecting differentiation and response to Wnt signaling. α-catenin binds to β-catenin in the nucleus, preventing it from regulating transcription, and levels of both proteins appear to be regulated via proteasome-dependent degradation (4).

Afadin has two splice variants: l-afadin, which is ubiquitously expressed, and s-afadin, which is expressed predominantly in neural tissue. s-afadin is a shorter form lacking one of the three proline-rich regions found in l-afadin, as well as the carboxyl-terminal F-actin binding region (5). Human s-afadin is identical to AF-6, the ALL-1 fusion partner involved in acute myeloid leukemias (6). Recent research has also shown that afadin is involved in controlling the directionality of cell movement when it is localized at the leading edge of moving cells (7,8).

Specificity/Sensitivity: Each antibody in this kit detects endogenous levels of total protein for the specified target and does not cross-react with other family members unless indicated. The α-E-Catenin (23B2) Rabbit mAb may cross-react with neuronal α-N-catenin. The γ-Catenin Antibody does not cross-react with β-catenin. Based on sequence homology, the Afadin (D1Y3Z) Rabbit mAb is expected to recognize all isoforms of afadin.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the carboxy terminus of human γ-catenin protein, or the sequence of human catenin δ-1 protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the amino-terminal sequence of human α-E-catenin, Arg1117 of human afadin protein, or to residues surrounding Pro714 of human β-catenin protein.

Background References:

(1) Kobielak, A. and Fuchs, E. (2004) *Nat Rev Mol Cell Biol* 5, 614-25.

(2) Yamada, S. et al. (2005) *Cell* 123, 889-901.

(3) Drees, F. et al. (2005) *Cell* 123, 903-15.

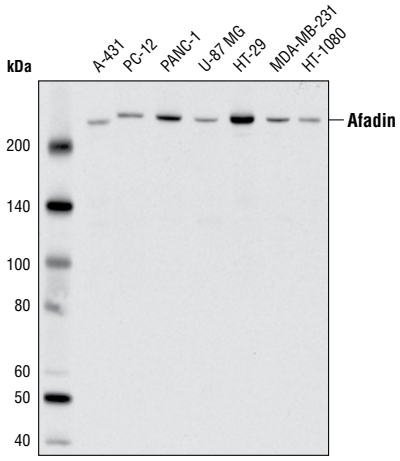
(4) Hwang, S.G. et al. (2005) *J Biol Chem* 280, 12758-65.

(5) Mandai, K. et al. (1997) *J Cell Biol* 139, 517-28.

(6) Prasad, R. et al. (1993) *Cancer Res* 53, 5624-8.

(7) Miyata, M. et al. (2009) *J Cell Sci* 122, 4319-29.

(8) Miyata, M. et al. (2009) *J Biol Chem* 284, 24595-609.



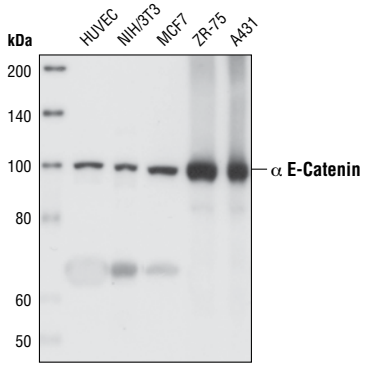
Western blot analysis of extracts from various cell lines using ***Afadin (D1Y3Z) Rabbit mAb #13531****.*

U. S. Patent No. 5,675,063

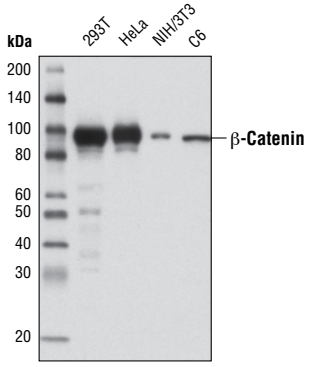
Thank you for your recent purchase. If you would like to provide a review visit www.cellsignal.com/comments.

Cell Signaling TECHNOLOGY®

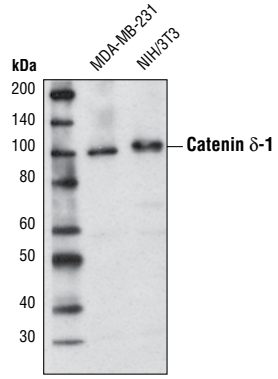
Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **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.



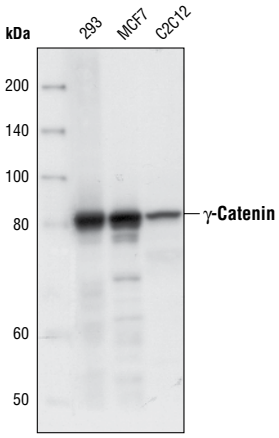
Western blot analysis of extracts from various cell lines using ***α-E-Catenin (23B2) Rabbit mAb #3240****.*



Western blot analysis of extracts from various cell lines using ***β-Catenin (D10A8) XP® Rabbit mAb #8480****.*



Western blot analysis of extracts from MDA-MB-231 and NIH/3T3 cells using ***Catenin δ-1 Antibody #4989****.*



Western blot analysis of extracts from 293, MCF-7 and C2C12 cells using ***γ-Catenin Antibody #2309****.*

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.