Store at -20°C

Tight Junction Antibody Sampler Kit

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Support: 877-678-TECH (8324)
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#8683

1 Kit (6 x 20 µl)

Orders: 877-616-CELL (2355)
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rev.06/16

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
CD2AP Antibody	2135	20 µl	80 kDa	Rabbit IgG
Afadin (D1Y3Z) Rabbit mAb	13531	20 µl	205 kDa	Rabbit IgG
Claudin-1 (D5H1D) XP® Rabbit mAb	13255	20 µl	20 kDa	Rabbit IgG
ZO-1 (D7D12) Rabbit mAb	8193	20 µl	220 kDa	Rabbit IgG
ZO-2 Antibody	2847	20 µl	150 kDa	Rabbit IgG
ZO-3 (D57G7) XP® Rabbit mAb	3704	20 µl	140 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

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

Description: The Tight Junction Antibody Sampler Kit provides an economical means to evaluate the presence of a number of proteins involved in tight junctions. The kit includes antibody to perform two western blot experiments with each primary antibody.

Background: Tight junctions, or zona occludens, form a continuous barrier to fluids across the epithelium and endothelium. They function in regulation of paracellular permeability and in the maintenance of cell polarity, blocking the movement of transmembrane proteins between the apical and basolateral cell surfaces (reviewed in 1). Tight junctions are composed of claudin and occludin transmembrane proteins, which join the junctions to the cytoskeleton (1,2). The claudin family is composed of 23 integral membrane proteins, and their expression, which varies among tissue types, may determine both the strength and properties of the epithelial barrier (2,3). Zona occludens proteins ZO-1, -2, and -3 (also known as TJP1, 2, and 3) are peripheral membrane adaptor proteins that link junctional transmembrane proteins such as occludin and claudin to the actin cytoskeleton (reviewed in 4). ZO-1 and ZO-2 are required for tight junction formation and function (5,6). In subconfluent proliferating cells, ZO-1 and ZO-2 have been shown to colocalize to the nucleus and play a role in transcriptional regulation (7-9). Exogenous expression of the amino terminal portion of ZO-3 exerts a dominant negative effect that interferes with assembly of tight junctions and adherens junctions (10). ZO-1 has been shown to interact with afadin prior to the formation of tight junctions (11). Recent work 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 (12,13). CD2AP is a scaffolding protein that is thought to link membrane proteins to the cytoskeleton (14-16). It plays a role in the formation of tight junctions in specialized cell types such as the slit diaphragm of the kidney glomerulus (17). CD2AP is also involved in the immunological synapse between CD2-expressing T cells and antigen presenting cells (18). Research studies have shown that interaction between CD2AP and other cytoskeletal proteins may regulate the endocytosis of EGFR (16).

Specificity/Sensitivity: CD2AP Antibody recognizes endogenous levels of total CD2AP protein. Claudin-1 (D5H1D) XP® Rabbit mAb recognizes endogenous levels of total claudin-1 protein. Based on sequence similarity, Claudin-1 (D5H1D) XP® Rabbit mAb may cross-react with claudin-2 protein. ZO-1 (D7D12) Rabbit mAb recognizes endogenous levels of total ZO-1 protein. ZO-2 Antibody recognizes endogenous levels of total ZO-2 protein. ZO-3 (D57G7) XP® Rabbit mAb detects endogenous levels of total ZO-3 protein. Afadin (D1Y3Z) Rabbit mAb recognizes endogenous levels of total afadin protein and based on the protein sequence, this antibody is expected to recognize all afadin isoforms.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding 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, the carboxy terminus of human claudin-1 protein, Arg1117 of human afadin protein or to residues surrounding Pro714 of human β-catenin protein.

Background References:

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(3) Hewitt, K.J. et al. (2006) *BMC Cancer* 6, 186.

(4) Matter, K. and Balda, M.S. (2007) *J Cell Sci* 120, 1505-11.

(5) Hernandez, S. et al. (2007) *Exp Cell Res* 313, 1533-47.

(6) Umeda, K. et al. (2006) *Cell* 126, 741-54.

(7) Betanzos, A. et al. (2004) *Exp Cell Res* 292, 51-66.

(8) Traweger, A. et al. (2003) *J Biol Chem* 278, 2692-700.

(9) Huerta, M. et al. (2007) *Mol Biol Cell* 18, 4826-36.

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(11) Ooshio, T. et al. (2010) *J Biol Chem* 285, 5003-12.

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

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

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(15) Kirsch, K.H. et al. (2001) *J Biol Chem* 276, 4957-63.

(16) Lynch, D.K. et al. (2003) *J Biol Chem* 278, 21805-13.

(17) Kawachi, H. et al. (2006) *Nephrology (Carlton)* 11, 274-81.

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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 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.