

#2951 Store at -20°C

β-Catenin Antibody Sampler Kit



✓ 1 Kit
(5 x 20 µl)

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
β-Catenin (D10A8) XP® Rabbit mAb	8480	20 µl	92 kDa	Rabbit IgG
Phospho-β-Catenin (Ser33/37/Thr41) Antibody	9561	20 µl	92 kDa	Rabbit IgG
Phospho-β-Catenin (Thr41/Ser45) Antibody	9565	20 µl	92 kDa	Rabbit IgG
Phospho-β-Catenin (Ser552) (D8E11) Rabbit mAb	5651	20 µl	92 kDa	Rabbit IgG
Phospho-β-Catenin (Ser675) (D2F1) XP® Rabbit mAb	4176	20 µl	92 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.

Description: The β-Catenin Antibody Sampler Kit provides an economical means of detecting total β-catenin as well as β-catenin phosphorylated at various residues. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: β-Catenin is a key downstream effector in the Wnt signaling pathway (1). It is implicated in two major biological processes in vertebrates: early embryonic development (2) and tumorigenesis (3). CK1 phosphorylates β-catenin at Ser45. This phosphorylation event primes β-catenin for subsequent phosphorylation by GSK-3β (4-6). GSK-3β destabilizes β-catenin by phosphorylating it at Ser33, Ser37, and Thr41 (7). Mutations at these sites result in the stabilization of β-catenin protein levels and have been found in many tumor cell lines (8).

Both Akt and PKA were shown to phosphorylate β-catenin at Ser552 and Ser675. Phosphorylation at Ser552 and Ser675 induces β-catenin accumulation in the nucleus and increases its transcriptional activity (9-12).

Specificity/Sensitivity: Each antibody in this kit recognizes only its specific target and does not cross-react with other family members.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser33, Ser37 and Thr41 of human β-catenin or residues surrounding Thr41 and Ser45 of human β-catenin. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Pro714 of human β-catenin protein, a synthetic phosphopeptide corresponding to residues surrounding Ser552 of human β-catenin protein, or a synthetic phosphopeptide corresponding to residues surrounding Ser675 of human β-catenin.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. 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.

Selected rabbit monoclonal antibodies are produced under license (granting certain rights including those under U. S. Patent No. 5,675,063 and/or U.S.S.N. 11/476,277) from Epitomics, Inc. U.S.S.N. 11/476,277) from Epitomics, Inc.

Background References:

- (1) Cadigan, K.M. and Nusse, R. (1997) *Genes Dev.* 11, 3286-3305.
- (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.
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- (5) Lin, C. et al. (2002) *Cell* 108, 837-847.
- (6) Yanagawa, S. et al. (2002) *EMBO J.* 21, 1733-1742.
- (7) Yost, C. et al. (1996) *Genes Dev.* 10, 1443-1454.
- (8) Morin, P.J. (1997) *Science* 275, 1787-1790.
- (9) Taurin, S. et al. (2006) *J. Biol. Chem.* 281, 9971-9976.
- (10) Fang, D. et al. (2007) *J. Biol. Chem.* 282, 11221-11229.
- (11) He, X.C. et al. (2007) *Nat. Genet.* 39, 189-198.
- (12) Hino, S. et al. (2005) *Mol. Cell Biol.* 25, 9063-9072.

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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—zebra fish 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.

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.