

#3276 Store at -20°C

APPL1 Antibody

✓ 100 µl
(10 Western mini-blot)



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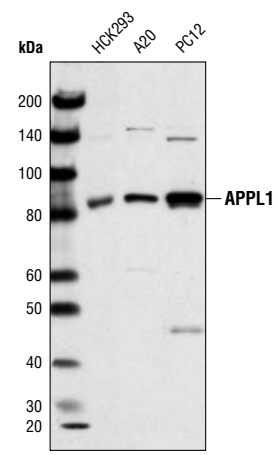
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W	H, M, R	82 kDa	Rabbit

Background: The APPL1 multidomain adaptor protein is a BAR-domain protein family member that is involved in membrane trafficking within a number of signal transduction pathways (1). The N-terminal BAR domain mediates the formation of crescent-shaped APPL1 homodimers (or APPL1 and APPL2 heterodimers) that are important in lipid binding and membrane curvature sensing (1). The PH domain of APPL1 is required for binding of the adaptor protein to Rab5 GTPase (2). In response to extracellular stimuli, Rab5 GTP hydrolysis releases APPL1 from the endosome and allows translocation of APPL1 to the nucleus where it joins a protein complex that controls chromatin remodeling and gene expression (3). The C-terminal PTB domain of APPL1 enables an interaction between APPL1 and the TrkA neurotrophin receptor. An association between these two proteins and the TrkA-interacting protein GIPC1 within endosomes is required for nerve growth factor (NGF) mediated signaling (4). APPL1 also binds follicle-stimulating hormone (FSH) receptors, which may provide a relay of FSH signaling to the PI3K/Akt pathway (5). The APPL1 adaptor protein is implicated in insulin signaling, as interaction between APPL1 and Akt2 is required for insulin-stimulated translocation of GLUT4 receptor proteins. Both induced overexpression and knockdown of APPL1 inhibit insulin-stimulated GLUT4 translocation (6). APPL1 binds the adiponectin receptor and acts as a downstream effector in the adiponectin pathway that mediates NO production (7,8). APPL1 interacts with DCC (deleted in colorectal cancer) protein and may play a role in DCC-induced apoptosis (9).

Specificity/Sensitivity: APPL1 Antibody detects endogenous levels of total APPL1 protein.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding residue surrounding Ala672 of human APPL1. Antibodies are purified by peptide affinity chromatography.



Western blot analysis of extracts from HEK293, A20 and PC12 cells using APPL1 Antibody.

Background References:

- (1) Habermann, B. (2004) *EMBO Rep* 5, 250-5.
- (2) Zhu, G. et al. (2007) *EMBO J* 26, 3484-93.
- (3) Miaczynska, M. et al. (2004) *Cell* 116, 445-56.
- (4) Lin, D.C. et al. (2006) *Mol Cell Biol* 26, 8928-41.
- (5) Nechamen, C.A. et al. (2004) *Biol Reprod* 71, 629-36.
- (6) Saito, T. et al. (2007) *J Biol Chem* 282, 32280-7.
- (7) Mao, X. et al. (2006) *Nat Cell Biol* 8, 516-23.
- (8) Cheng, K.K. et al. (2007) *Diabetes* 56, 1387-94.
- (9) Liu, J. et al. (2002) *J Biol Chem* 277, 26281-5.

Entrez-Gene ID # 26060
Swiss-Prot Acc. # Q9UKG1

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

*Species cross-reactivity is determined by Western blot.

Recommended Antibody Dilutions:
Western blotting 1:1000

Companion Products:
 Phototope[®]-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
 Anti-rabbit IgG, HRP-linked Antibody #7074
 Biotinylated Protein Ladder #7727
 Prestained Protein Marker, Broad Range (Premixed Format) #7720
 20X LumiGLO[®] Reagent and 20X Peroxide #7003
 Anti-biotin, HRP-linked Antibody #7075

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

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus
 F—Flow cytometry E—ELISA D—DELFA[®]
 Z—zebra fish B—bovine All—all species expected
 Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Western Immunoblotting Protocol (Primary Antibody Incubation in Nonfat Dry Milk)

For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 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% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk 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.