

#4978 Store at -20°C

Raptor Antibody

100 µl
 (10 Western mini-blot)

Orders ■ 877-616-CELL (2355)
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Support ■ 877-678-TECH (8324)
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rev. 02/06/08

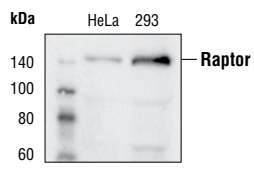
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W	H, M, R, Mk	150 kDa	Rabbit

Background: The regulatory associated protein of mTOR (Raptor) was identified as an mTOR binding partner that mediates mTOR signaling to downstream targets (1,2). Raptor binds to mTOR substrates, including 4E-BP1 and p70 S6 kinase, through their TOR signaling (TOS) motifs and is required for mTOR-mediated phosphorylation of these substrates (3,4). Binding of the FKBP12-rapamycin complex to mTOR inhibits the mTOR-raptor interaction, suggesting a mechanism for rapamycin's specific inhibition of mTOR signaling (5). This mTOR-raptor interaction and its regulation by nutrients and/or rapamycin is dependent on a protein called GβL (6). GβL is also part of the rapamycin-insensitive complex between mTOR and rictor (rapamycin-insensitive companion of mTOR), and may mediate rictor-mTOR signaling to downstream targets including PKCα (7). Furthermore, the rictor-mTOR complex has been identified as the previously elusive PDK2 responsible for the phosphorylation of Akt/PKB on Ser473, facilitating phosphorylation of Akt/PKB on Thr308 by PDK1 and required for the full activation of Akt/PKB (8).

Specificity/Sensitivity: Raptor Antibody detects endogenous levels of total raptor protein. This antibody also cross-reacts with an unidentified protein of 90 kD.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues near the amino-terminus of human Raptor. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from HeLa and 293 cells using Raptor Antibody.

Background References:

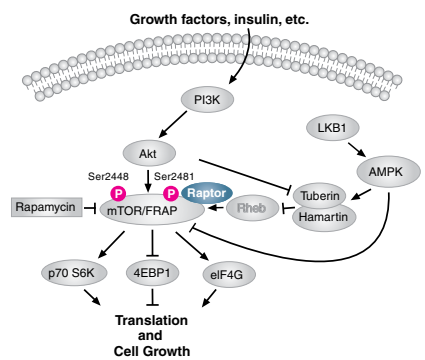
- (1) Hara, K. et al. (2002) *Cell* 110, 177–189.
- (2) Kim, D. et al. (2002) *Cell* 110, 163–175.
- (3) Beugnet, A. et al. (2003) *J. Biol. Chem.* 278, 40717–40722.
- (4) Nojima, H. et al. (2003) *J. Biol. Chem.* 278, 15461–15464.
- (5) Oshiro, N. et al. (2004) *Genes Cells* 9, 359–366.
- (6) Kim, D. H. et al. (2003) *Mol. Cell* 11, 895–904.
- (7) Sarbassov, D. et al. (2004) *Curr. Biol.* 14, 1296–1302.
- (8) Sarbassov, D.D. et al. (2005) *Science* 307, 1098–1101.

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 antibody.*

***Species cross-reactivity is determined by Western blot.**

Recommended Antibody Dilutions:
 Western blotting 1:1000

- Companion Products:**
 Raptor (24C12) Rabbit mAb #2280
 Raptor (W990) Antibody #2214
 Rictor (53A2) Rabbit mAb #2114
 Rictor Antibody #2140
 GβL Antibody #3227
 GβL (86B8) Rabbit mAb #3274
 Phospho-mTOR (Ser2448) Antibody #2971
 mTOR Antibody #2972
 Phospho-mTOR (Ser2481) Antibody #2974
 4E-BP1 (53H11) Rabbit mAb #9644
 Rapamycin (FRAP/mTOR Inhibitor) #9904
 p70 S6 Kinase Antibody #9202
 Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
 Anti-rabbit IgG, HRP-linked Antibody #7074
 Prestained Protein Marker, Broad Range (Premixed Format) #7720
 Biotinylated Protein Ladder Detection Pack #7727
 20X LumiGLO® Reagent and 20X Peroxide #7003



IMPORTANT: 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.

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
 Species enclosed in parentheses are predicted to react based on 100% sequence homology.

F—Flow cytometry E—ELISA D—DELFIATM
 Z—zebra fish B—bovine All—all species expected

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.