

#2114 Store at -20°C

Rictor (53A2) Rabbit mAb

✓ 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 W	Species Cross-Reactivity* H, M, R, Mk	Molecular Wt. 200 kDa	Source Rabbit**	Isotype IgG
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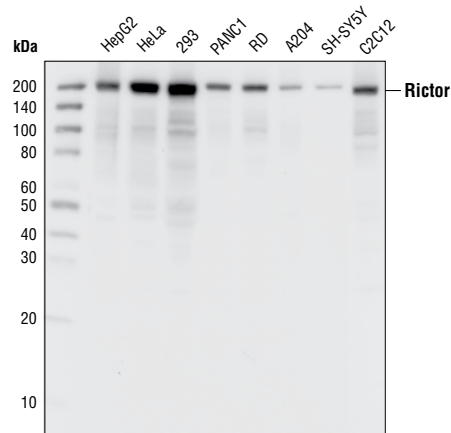
Background: Cell growth is a fundamental biological process whereby cells accumulate mass and increase in size. The mammalian TOR (mTOR) pathway regulates growth by coordinating energy and nutrient signals with growth factor-derived signals (1). mTOR is a large protein kinase with two different complexes. One complex contains mTOR, GβL and raptor, which is a target of rapamycin. The other complex, insensitive to rapamycin, includes mTOR, GβL and rictor (1). The mTOR-rictor complex phosphorylates Ser473 of Akt/PKB *in vitro* (2). This phosphorylation is essential for full Akt/PKB activation. Furthermore, an siRNA knockdown of rictor inhibits Ser473 phosphorylation in 3T3-L1 adipocytes (3). This complex has also been shown to phosphorylate the rapamycin-resistant mutants of S6K1, another effector of mTOR (4).

Specificity/Sensitivity: Rictor (53A2) Rabbit mAb detects endogenous levels of total Rictor protein.

Source/Purification: Rictor (53A2) Rabbit mAb is produced by immunizing rabbits with a synthetic peptide (KLH-coupled) derived from the sequence Gln1681 of human Rictor.

Background References:

- (1) Sarbassov, D.D. et al. (2004) *Curr. Biol.* 14, 1296–1302.
- (2) Sarbassov, D.D. et al. (2005) *Science* 307, 1098–1101.
- (3) Hresko, R.C. and Mueckler, M. (2005) *J. Biol. Chem.* 280, 40406–40416.
- (4) Ali, S.M. and Sabatini, D.M. (2005) *J. Biol. Chem.* 280, 19445–19448.



Western blot analysis of extracts from various cell lines, using Rictor (53A2) Rabbit mAb.

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 antibody.

*Species cross-reactivity is determined by Western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody

Recommended Antibody Dilutions:
Western blotting 1:1000

Companion Products:

- Rictor Antibody #2140
- Raptor (24C12) Rabbit mAb #2280
- Raptor Antibody #4978
- Phospho-mTOR (Ser2448) Antibody #2971
- Phospho-mTOR (Ser2448) (49F9) Rabbit mAb (IHC Specific) #2976
- Phospho-mTOR (Ser2481) Antibody #2974
- Phospho-mTOR (Ser2448) Blocking Peptide #1230
- mTOR Antibody #2972
- mTOR (7C10) Rabbit mAb #2983
- mTOR Blocking Peptide #1072
- SignalSilence® mTOR siRNA #6381
- SignalSilence® mTOR siRNA Kit #6380
- Rapamycin (FRAP/mTOR Inhibitor) #9904
- 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 F—Flow cytometry E—ELISA D—DELFIA®
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

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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.