

#9324 Store at -20°C

Phospho-RSK2 (Tyr529) Antibody

- Small 100 µl (10 Western mini-blot)
- Petite 40 µl (4 Western mini-blot)



Orders ■ 877-616-CELL (2355)
orders@cellsignal.com

Support ■ 877-678-TECH (8324)
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New 12/07

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, Mk	90 kDa	Rabbit

Background: The 90 kDa ribosomal S6 kinases (RSK1–4) are a family of widely expressed serine/threonine kinases characterized by two nonidentical, functional kinase domains (1) and a C-terminal docking site for extracellular signal-regulated kinases (ERKs) (2). Several sites both within and outside of the RSK kinase domain, including Ser380, Thr359, Ser363 and Thr573, are important for kinase activation (3). RSK1–3 are activated via coordinated phosphorylation by MAPKs, by autophosphorylation, and by phosphoinositide-3-OH kinase (PI3K) in response to many growth factors, polypeptide hormones and neurotransmitters (3).

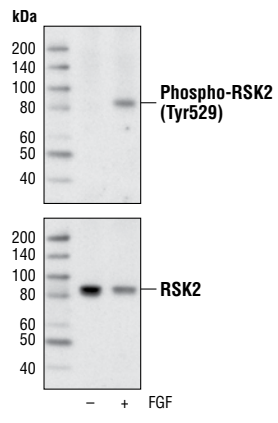
Phosphorylation of RSK2 on Tyr529 was identified at Cell Signaling Technology (CST) using PhosphoScan®, our LC-MS/MS platform for phosphorylation site discovery. This study identifies RSK2 as a key substrate of FGFR3 in human t(4;14)-positive multiple myeloma (MM) cells. Constitutively active FGFR3 directly phosphorylates RSK2 on Tyr529, which primes RSK2 for activation by ERK1/2 (4). RSK2 can also be phosphorylated at Tyr529 by Src family tyrosine kinases Src and Fyn in response to EGF stimulation (5). Mutations in the RSK2 gene are associated with Coffin-Lowry syndrome (CLS), an X-linked disorder characterized by mental retardation and the presence of characteristic facial anomalies (6).

Specificity/Sensitivity: Phospho-RSK2 (Tyr529) Antibody detects endogenous RSK2 protein only when phosphorylated at Tyr529. This antibody cross-reacts with some tyrosine phosphorylated proteins including EGFR.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phosphopeptide (KLH-coupled) corresponding to residues surrounding Tyr529 of human RSK2. Antibodies are purified by protein A and peptide affinity chromatography.

Selected Application References:
Kang, S. et al. (2007) FGFR3 activates RSK2 to mediate hematopoietic transformation through tyrosine phosphorylation of RSK2 and activation of the MEK/ERK pathway. *Cancer Cell* 12, 201–14. Application: W.

Kang, S. et al. (2007) *J Biol Chem* Submitted for publication. Application: W.



Western blot analysis of extracts from untreated or FGF-treated SK-N-MC cells, using Phospho-RSK2 (Tyr529) Antibody (upper) or RSK2 Antibody #9340 (lower).

Background References:

- (1) Fisher, T.L. and Blenis, J. (1996) *Mol Cell Biol* 16, 1212–9.
- (2) Smith, J.A. et al. (1999) *J Biol Chem* 274, 2893–8.
- (3) Dalby, K.N. et al. (1998) *J Biol Chem* 273, 1496–505.
- (4) Kang, S. et al. (2007) *Cancer Cell* 12, 201–14.
- (5) Kang, S. et al. (2007) *J Biol Chem* Submitted for Publication
- (6) Delaunoy, J.P. et al. (2006) *Clin Genet* 70, 161–6.

Entrez-Gene ID # 6197
Swiss-Prot Acc. # P51812

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:**
- Phospho-p90RSK (Ser380) (9D9) Rabbit mAb #9335
 - Phospho-p90RSK (Ser380) Antibody #9341
 - Phospho-p90RSK (Thr359/Ser363) Antibody #9344
 - Phospho-p90RSK (Thr573) Antibody #9346
 - Phospho-RSK3 (Thr356/Ser360) Antibody #9348
 - RSK1/RSK2/RSK3 (32D7) Rabbit mAb #9355
 - RSK1/RSK2/RSK3 Antibody #9347
 - RSK1 Antibody #9333
 - RSK2 Antibody #9340
 - RSK3 Antibody #9343
 - 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 #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
 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.