

# Phospho-Gab2 (Ser159) Antibody

✓ 100 µl  
(10 Western mini-blots)



Cell Signaling  
TECHNOLOGY®

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

Support ■ 877-678-TECH (8324)  
info@cellsignal.com

Web ■ www.cellsignal.com

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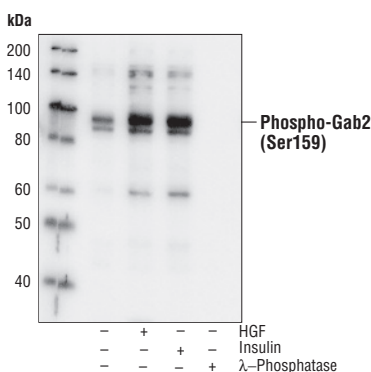
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications W	Species Cross-Reactivity H, M, R	Molecular Wt. 98 kDa	Source Rabbit
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**Background:** The GRB-associated binder (Gab) family is a family of adaptor proteins recruited by a wide variety of receptor tyrosine kinases (RTKs) such as EGFR, HGFR, insulin receptor, cytokine receptor and B cell antigen receptors. Upon stimulation of RTKs by their cognate ligand, Gab is recruited to the plasma membrane, undergoes phosphorylation and functions as a multiprotein assembly center (1–4). Multiple tyrosine phosphorylation sites of Gab1 protein have been identified (5). Phosphorylation of Tyr472 regulates its binding to p85 PI3 kinase (6,7). Phosphorylation of Gab1 at Tyr307, Tyr373 and Tyr407 modulates its association to PLCγ (8). Phosphorylation of Tyr627 and Tyr659 is required for Gab1 binding to and activation of the protein tyrosine phosphatase SHP2 (6,9). Akt phosphorylates Gab2 at Ser159. This phosphorylation inhibits Gab2 tyrosine phosphorylation and downstream signal amplification (10).

**Specificity/Sensitivity:** Phospho-Gab2 (Ser159) Antibody detects endogenous levels of Gab2 only when phosphorylated at serine 159. This antibody does not cross-react with Gab1 or Gab3.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic phosphopeptide (KLH-coupled) corresponding to residues surrounding Ser159 of human Gab2. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from H-4-II-E cells, untreated, HGF-treated, insulin-treated, or treated with λ phosphatase after lysis, using Phospho-GAB2 (Ser159) Antibody.

### Background References:

- Holgado-Madruga, M. et al. (1996) *Nature* 379, 560–564.
- Weidner, K.M. et al. (1996) *Nature* 384, 173–176.
- Takahashi-Tezuka, M. et al. (1998) *Mol. Cell. Biol.* 18, 4109–4117.
- Ingham, R.J. et al. (2001) *J. Biol. Chem.* 276, 12257–12265.
- Lehr, S. et al. (1999) *Biochemistry* 38, 151–159.
- Rocchi, S. et al. (1998) *Mol. Endocrinol.* 12, 914–923.
- Yu, C.F. et al. (2001) *J. Biol. Chem.* 276, 32552–32558.
- Gual, P. et al. (2000) *Oncogene* 19, 1509–1518.
- Cunnick, J.M. et al. (2001) *J. Biol. Chem.* 276, 24380–24387.
- Lynch, D.K. and Daly, R. (2002) *EMBO J.* 21, 72–82.

**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.

### Recommended Antibody Dilutions:

Western blotting 1:1000

### Companion Products:

Phospho-Gab2 (Tyr452) Antibody #3881

Phospho-Gab1 (Tyr307) Antibody #3234

Phospho-Gab1 (Tyr627) Antibody #3231

Gab1 Antibody #3232

Phospho-Akt (Ser473) Antibody #9271

Phospho-Akt (Ser473) (587F11) Mouse mAb #4051

Phospho-Akt (Ser473) (193H12) Rabbit mAb #4058

Akt Antibody #9272

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

LumiGLO® Reagent and Peroxide #7003

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

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry F—Flow cytometry E—ELISA D—DELFIATM

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish All—all species expected  
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

## Western Immunoblotting Protocol

**For Western blots, incubate membrane with diluted anti-body in 5% 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.

- A1. ■ 1X Phosphate Buffered Saline (PBS)
- A2. ■ 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
- A3. ■ Transfer Buffer:  
25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- A4. ■ 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).
- A5. ■ Nonfat Dry Milk (weight to volume [w/v])
- A6. ■ 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%).
- A7. ■ Wash Buffer:  
1X TBS, 0.1% Tween-20 (TBS/T)
- A8. ■ Bovine Serum Albumin (BSA)
- A9. ■ 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%).
- A10. ■ Phototope<sup>®</sup>-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<sup>®</sup> chemiluminescent reagent and peroxide.
- A11. ■ Prestained Protein Marker, Broad Range (Premixed Format) #7720
- A12. ■ Biotinylated Protein Ladder Detection Pack #7727
- A13. ■ 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.

- B1. Treat cells by adding fresh media containing regulator for desired time.
- B2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- B3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- B4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- B5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- B6. Microcentrifuge for 5 minutes.
- B7. 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.*

- B8. Electrotransfer to nitrocellulose or PVDF membrane.

### C. Membrane Blocking and Antibody Incubations

*Note: Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.*

- C1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- C2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- C3. Wash three times for 5 minutes each with 15 ml of TBS/T.
- C4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- C5. Wash three times for 5 minutes each with 15 ml of TBS/T.
- C6. 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.
- C7. Wash three times for 5 minutes each with 15 ml of TBS/T.

### D. Detection of Proteins

- D1. Incubate membrane with 10 ml LumiGLO<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

*Note: LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.*

- D2. 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<sup>®</sup> incubation and declines over the following 2 hours.*