

β2-Chimerin (2E3) Rat mAb

✓ 100 µl
(10 Western mini-blot)

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

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

Web ■ www.cellsignal.com

rev. 05/23/07

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	Isotype
W Endogenous	H, M, R	47 kDa	Rat**	IgG2a

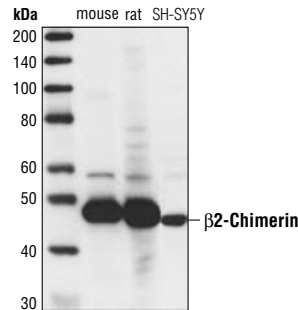
Background: Chimerins are a family of GTPase-activating proteins (GAPs) that facilitate GTP hydrolysis by the small GTPase Rac, rendering it inactive and regulating cell shape, spreading and motility. Regulation of chimerin proteins occurs in response to growth factor receptor or G-protein coupled receptor activation followed by phospholipase C activation. Chimerins are among the growing number of phorbol ester and diacylglycerol (DAG) effector molecules that do not belong to the PKC family of isoenzymes (reviewed in 1,2). β2-chimerin is highly expressed in brain and pancreas, and its expression is down-regulated in malignant gliomas (3). β2-chimerin is also down-regulated in breast cancer, and its expression causes GAP activity-dependent cell cycle arrest in MCF-7 breast cancer cells (4). Signaling from the epidermal growth factor receptor (EGFR) activates β2-chimerin and allows its association with Rac1 at the plasma membrane (5). Also in response to EGF, diacylglycerol kinase (DGK) γ interacts with β2-chimerin, promotes its translocation to the plasma membrane, and regulate its GAP activity (6).

Specificity/Sensitivity: β2-Chimerin (2E3) Rat mAb recognizes endogenous levels of total β2-chimerin protein.

Source/Purification: Monoclonal antibody is produced by immunizing rats with full-length recombinant human β2-Chimerin.

Background References:

- (1) Yang, C. and Kazanietz, M.G. (2007) *Biochem J.* 403, 1–12.
- (2) Brose, N. and Rosenmund, C. (2002) *J. Cell Sci.* 115, 4399–4411.
- (3) Yuan, S. et al. (1995) *Cancer Res.* 55, 3456–3461.
- (4) Yang, C. et al. (2005) *J. Biol. Chem.* 280, 24363–24370.
- (5) Wang, H. et al. (2006) *EMBO J.* 25, 2062–2074.
- (6) Yasuda, S. et al. (2007) *FEBS Lett.* 581, 551–557.



Western blot analysis of extracts from mouse brain, rat brain and SH-SY5Y cells using β2-Chimerin (2E3) Rat 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-rat secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting 1:1000

Companion Products:

Anti-rat IgG, HRP-linked Antibody #7077

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 Z—zebra fish B—bovine All—all species expected

Western Immunoblotting Protocol (Primary Ab 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%).
- Western Blot Detection Reagents:** Anti-rat IgG, HRP-linked Antibody #7077, Biotinylated Protein Ladder Detection Pack #7727, Prestained Protein Marker, Broad Range (Premixed Format) #7720, 20X LumiGLO® Reagent and 20X Peroxide #7003.
- 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.