

p-RGS9-1 (Ser 475): sc-19829

BACKGROUND

In vertebrate photoreceptors, photoexcited rhodopsin interacts with the G protein transducin causing it to bind GTP and stimulate the enzyme cGMP phosphodiesterase. Rapid termination of the active state of this pathway is dependent upon RGS9-1 (regulator of G protein signaling), a photoreceptor-specific regulator of G protein signaling that serves as a GTPase activating protein (GAP) for transducin. RGS9-1 is the retina-specific isoform derived from an alternative splicing event on the RGS9 gene. Dark-adapted retinas contain significant levels of RGS9-1 phosphorylation of Ser 475, whereas light-adapted retinas show much lower levels. The yet unidentified kinase responsible for phosphorylating RGS9-1 at Ser 475 is a peripheral membrane protein that is not inhibited or activated by PKA, PKG, rhodopsin kinase, CaM kinase II, CKII or Cdk5, or by the addition of phorbol ester. However, the kinase can be inhibited by bisindolylmaleimide I and by lowering Ca²⁺ to nanomolar levels with EGTA, suggesting that RGS9-1 phosphorylation of Ser 475 is regulated by light and Ca²⁺ concentration.

REFERENCES

1. Guan, K.L., et al. 1999. A G protein signaling network mediated by an RGS protein. *Genes Dev.* 13: 1763-1767.
2. Zhang, K., et al. 1999. Structure, alternative splicing and expression of the human RGS9 gene. *Gene* 240: 23-34.
3. Hepler, J.R. 1999. Emerging roles for RGS proteins in cell signalling. *Trends Pharmacol. Sci.* 20: 376-382.
4. Balasubramanian, N., et al. 2001. Phosphorylation of the regulator of G protein signaling RGS9-1 by protein kinase A is a potential mechanism of light- and Ca²⁺-mediated regulation of G protein function in photoreceptors. *Biochemistry* 40: 12619-12627.
5. Hu, G., et al. 2001. Phosphorylation of RGS9-1 by an endogenous protein kinase in rod outer segments. *J. Biol. Chem.* 276: 22287-22295.

CHROMOSOMAL LOCATION

Genetic locus: RGS9 (human) mapping to 17q24.1; Rgs9 (mouse) mapping to 11 E1.

SOURCE

p-RGS9-1 (Ser 475) is available as either goat (sc-19829) or rabbit (sc-19829-R) polyclonal affinity purified antibody raised against a short amino acid sequence containing Ser 475 phosphorylated RGS9-1 of human origin.

PRODUCT

Each vial contains 200 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-19829 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

RESEARCH USE

For research use only, not for use in diagnostic procedures.

APPLICATIONS

p-RGS9-1 (Ser 475) is recommended for detection of Ser 475 phosphorylated RGS9-1 of mouse and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000); non cross-reactive with RGS9-2.

p-RGS9-1 (Ser 475) is also recommended for detection of correspondingly phosphorylated RGS9-1 in additional species, including canine and bovine.

Suitable for use as control antibody for RGS9 siRNA (h): sc-36412, RGS9 siRNA (m): sc-36413, RGS9 shRNA Plasmid (h): sc-36412-SH, RGS9 shRNA Plasmid (m): sc-36413-SH, RGS9 shRNA (h) Lentiviral Particles: sc-36412-V and RGS9 shRNA (m) Lentiviral Particles: sc-36413-V.

Molecular Weight of p-RGS9-1: 55 kDa.

RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: for goat primary antibody (sc-19829): use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), for rabbit primary antibody (sc-19829-R): use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent) and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: for goat primary antibody (sc-19829): use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941, for rabbit primary antibody (sc-19829-R): use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

STORAGE

Store at 4° C, ****DO NOT FREEZE****. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.