

3050 Spruce Street Saint Louis, Missouri 63103 USA Telephone (800) 325-5832 (314) 771-5765 Fax (314) 286-7828 email: techserv@sial.com sigma-aldrich.com

ProductInformation

ANTI-GUANYLYL CYCLASE §1, SOLUBLE (ER-19)

Developed in Rabbit, Affinity Isolated Antibody

Product Number G 4405

Product Description

Anti-Soluble Guanylyl Cyclase $\beta 1$ is developed in rabbit using a synthetic peptide corresponding to amino acid residues 189-207 of rat soluble Guanylyl cyclase $\beta 1$ with N-terminal added lysine conjugated to KLH with glutaraldehyde as immunogen. The sequence differs in human, bovine and mouse by only 1 amino acid. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-Soluble Guanylyl Cyclase $\beta 1$ (sGC $\beta 1$) specifically recognizes sGC $\beta 1$ by immunoblotting and immunoprecipitation (~70 kDa). Additional weak bands may be detected in some preparations of brain extracts by immunoblotting. Staining of the sGC $\beta 1$ band is specifically inhibited with the immunizing peptide. The product is also useful for the detection of sGC $\beta 1$ by immunohistochemistry. The epitope(s) recognized by the antibody is compatible with routine formalin-fixation and paraffin-embedding. The antibody reacts with sGC $\beta 1$ of human, bovine, rat and mouse origin.

Soluble guanylyl cyclase [sGC; GTP pyrophosphate-lyase (cyclizing); (EC 4.6.1.2)] catalyses in a Mg^{2+} or Mn^{2+} dependent manner, the conversion of guanosine-5'-triphosphate (GTP) to cyclic guanosine-3',5-monophosphate (cGMP) and pyrophosphate. 1,2 sGC is an obligate hemoprotein enzyme consisting of a and β subunits of ~80 kDa and ~70 kDa respectively, both required for catalyic activity. 3 At least two main different α and two β subunits have been identified in human tissues. 4

The enzyme is a major physiological receptor for nitric oxide (NO), an important intra- and intercellular membrane-permeant signaling molecule. Gaseous NO binds to Fe²⁺ in the prosthetic heme group of sGC. NO binding is followed by disruption of the $\beta 1$ subunit histidine 105 bond to iron and activation of the enzyme. 1,2,5 The N-terminal domains of the subunits are

essential for the stimulation of the enzyme by NO. A central portion of the sGC molecule mediates dimerization. The C-terminus domain of both subunits, also conserved in plasma membrane-bound guanylyl cyclase and in adenylyl cyclases, forms the catalytic domain. cGMP regulates various effector proteins, such as protein kinases (e.g. PKG), phosphodiesterases and ion-channels. ⁶ The NO-sGC-cGMP signaling pathway is important in regulating a great variety of physiological processes including smooth muscle relaxation, inflammation, platelet adhesion and aggregation, pulmonary physiology and neuronal function. 1,2,6,7 sGC is constitutively expressed in a wide variety of animals including vertebrates, insects and molluscs. It is found in the cell cytoplasm of virtually all mammalian cells although the β2 subunit might also be associated with cell membranes. Non-uniform distribution of sGC isoforms $\alpha 1$ and $\beta 1$ has been found in rat brain. ⁸ Due to its ubiquitous nature, sGC may have considerable pathophysiological significance. It is an important target for NO-releasing and non-NO-releasing activator drugs in human cardiovascular therapy. 4,9

Reagent

Anti-Soluble Guanylyl Cyclase $\beta 1$ is supplied as an affinity isolated antibody in 10 mM phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide.

Protein concentration is approximately 1 mg/ml.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution.

Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

For continuous use, store at 2 °C to 8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

A minimum working dilution of 1:2,000 is determined by immunoblotting using a cytosolic fraction of rat brain.

sGC β 1 is immunoprecipitated from 60 to 120 μ g of a cytosolic fraction of rat brain using 2 to 3 μ g of the antibody.

A minimum working dilution of 1:400 is determined by indirect immunoperoxidase staining of trypsin-digested, formalin-fixed, paraffin-embedded human, bovine and mouse heart sections.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilution by titration test.

References

- Hobbs, A.J., TiPS, 18, 484-491 (1997).
- 2. Denninger, J.W., and Marletta, M.A., Biochim. Biophys. Acta, **1411**, 334-350 (1999).
- Kamisaki, Y., et al., J. Biol. Chem., 261, 7236-724 (1986).
- 4. Zabel, U., et al., Biochem. J., **335**, 51-57 (1998).
- Murad, F., J. Am. Med. Assoc., 276, 1189-1192 (1996).
- 6. Zhan, X., et al., J. Histochem. Cytochem., **47**, 1369-1374 (1999).
- 7. Schmidt, H.H.H.W., and Walter, U., Cell, **78**, 919-925 (1994).
- 8. Furuyama, T., et al., Brain Res. Mol. Brain Res., **20**, 335-344 (1993).
- Vallance, P., and Moncada, S., J. R. Coll. Phys., 28, 209-219 (1994).

lpg 2/01