ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Life Technologies™ Your Molecular & Cell Technology Partner

Anti- S-Nitroso-Cys (SNO-Cys)

☐ **Cat #** NISC11-A	Rabbit anti-S-Nitrosylated-Cysteine IgG	**SIZE**: 100 ug
☐ **Cat #** SNOBSA-N-100	Nitrosylated-BSA protein conjugate for ELISA	**SIZE**: 100 ug

Nitric oxide (NO), generated by cell type-specific NO-synthase (NOS) isoforms, is a freely diffusible intercellular messenger that functions in target cells in NOS-dependent signaling. S-nitrosylation of cysteine thiols in proteins by the highly labile NO radical has been identified as a important effectors of NO-related bioactivity both in NOS-containing cells and intercellular signaling. Most cells contain low levels of nitrosylated proteins that are thought to be regulated by S-nitrosylation and denitrosylation. S-nitrosylation of proteins serves as a ubiquitous post-translational modification that dynamically regulates a broad functional spectrum of proteins. The majority of these proteins are regulated by S-nitrosylation on a single critical cysteine residue within an acidic/basic or hydrophobic structural motif that may also be subject to oxygen- or glutathione-dependent modification. NO-sensitive ion channels including the cardiac and skeletal muscle ryanodine receptor (RyR1), N-methyl-D-aspartate receptor (NMDAR) complex, cyclic-nucleotide gated ion channel, are modulated by S-nitrosylation. S-nitrosylation of capsase-3 inhibits apoptosis signaling. S-nitrosylation activates matrix metalloproteinase-9 (MMP-9) and induces neuronal apoptosis. The small G-protein p21Ras and Jun kinase are regulated by S-nitrosylation. The activity of transcription factors such as NF·B, c-jun, and c-fos is modulated by S-nitrosylation. In addition, the formation of S-nitrosylated glutathione (GSNO) has been proposed to be one of the major storage forms of NO *in vivo*.

Source of Antigen and Antibodies

Antigen	Nitrosylated-Cysteine-KLH (hemocyanin)
Ab Host/type	Rabbit, Polyclonal IgG, purified over SNO-BSA-agarose affinity column, supplied in PBS, ph 7.4 and 0.1% BSA in liquid or in powder form. Recostitute powder in 100 ul water and store at -20oC.
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
-ve control IgG	# 20009-1, Rabbit (non-immune) IgG, purified, suitable for ELISA, Western, IHC as –ve control

SNO-Cys-BSA protein conjugate (**cat # SNOBSA-N-100**) was prepare by coupling Cysteine to BSA followed by nitrosylation of cysteines. It is supplied in PBS at 1 mg/ml. This can be used to coat the ELISA plates to check antibody titer, or run on SDS-PAGE for Western (may see multiple bands due to modification of BSA) or used for antibody blocking (use 10 ul or 10 ug/1 ug of antibody). Store at –20oC in suitable size aliquots.

Form & Storage

Lyophilized products should be reconstituted in 100 ul PBS and gently mixed for 15 min at room temp. All peptide/antibody received in solution or reconstituted from lyophilized vials should be stored frozen at -20°C or below in suitable aliquots. It is not recommended to store diluted solutions. Avoid repeated freeze and thaw.

Recommended Usage

Western Blotting: An initial testing of antibodies is recommended at 1:500-1:2,000 using nitrosylated-Cysteine–BSA (**Cat # SNOBSA-N-100**) as a control. Users must optimize antibody dilution depending upon the nature of samples and other technical conditions.

ELISA (1:1K-5K; using 50-100 ng control proteins/well).

Histochemistry & Immunofluorescence: An initial testing of 2-10 ug/ml is recommended. Bovine endothelial cells treated with Ca+2 ionophore A23187 can be used as control. Users must optimize antibody dilution depending upon the nature of samples and other technical conditions.

Specificity & Cross-reactivity

Antibodies react with nitrosylated-cysteine modified proteins from all species. No significant reactivity is observed with unmodified BSA or Cysteine-BSA (not nitrosylated).

General References: Gow AJ et al (2002) JBC 158, 308-314; Sun J et al (2001) PNAS 98, 11158-11162; Gu Z et al (2002) Science 297, 1186-1190; Park HS et al (2000) PNAS 97, 14382-14387

*This product is for In vitro research use only.

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