3050 Spruce Street, St. Louis, MO 63103 USA Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757 email: techservice@sial.com sigma-aldrich.com

# **Product Information**

## Anti-Nitric Oxide Synthase, Inducible antibody, Mouse monoclonal clone NOS-IN, purified from hybridoma cell culture

cione NOS-IN, purified from hybridoma cello

Product Number SAB4200766

### **Product Description**

Anti-Nitric Oxide Synthase, Inducible antibody, Mouse monoclonal (mouse IgG1 isotype) is derived from the NOS-IN hybridoma, produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mouse immunized with a synthetic peptide corresponding to the C terminal region of nitric oxide synthase (NOS) of mouse macrophage origin (iNOS, also termed macNOS), conjugated to KLH. The isotype is determined by ELISA using Mouse Monoclonal Antibody Isotyping Reagents, Product Number ISO2. The antibody is purified from culture supernatant of hybridoma cells.

Anti-Nitric Oxide Synthase, Inducible antibody, Mouse monoclonal reacts specifically with inducible-type nitric oxide synthase (iNOS, also termed macNOS and mNOS). It does not react with NOS derived from brain (bNOS) or with endothelial-type NOS (eNOS). The antibody recognizes iNOS from human<sup>1</sup>, mouse<sup>2</sup>, rat<sup>3</sup> and plant<sup>4</sup> origin. The product may be used in several immunochemical techniques including Immunoblotting<sup>1,3</sup> (~130kDa), Immunofluorescence<sup>5</sup>, FACS<sup>3</sup> and Immunohistochemistry<sup>6</sup>.

Nitric oxide synthase (NOS) is an enzyme involved in the synthesis of nitric oxide (NO), a free radical generated under physiological conditions by virtually all mammalian cells.<sup>7-8</sup> NO is formed from arginine by NOS which oxidizes a guanidino nitrogen of arginine, releasing NO and citrulline. NO is a messenger molecule mediating diverse functions including vasodilatation, neurotransmission, antimicrobial and anti-tumor activities.9 In addition, NO has been implicated as a pathogenic mediator in a variety of conditions, including central nervous system (CNS) disease states, such as the animal model of multiple sclerosis (MS) and experimental allergic encephalomyelitis.<sup>10</sup> NOS protein isoforms contain consensus sequences for the binding of NADPH, flavins and calmodulin.

At least three types of NOS have been classified on the basis of molecular mass, subcellular location and Ca2+ dependency. Type I NOS (NOS-1) found in neurons, is a 150-160 kD protein, also called neuronal NOS (nNOS). Type II, best characterized in macrophages, is

a 130 kDa protein, also known as inducible NOS (iNOS) or macrophage NOS (mNOS). Type III found in endothelial cells, is a 135 kDa protein, also called endothelial NOS (eNOS, or ecNOS). nNOS and eNOS are constitutively expressed and are dependent on Ca2+/calmodulin for NO production, whereas iNOS is Ca2+-independent and is expressed in activated macrophages and some glial cells after stimulation.<sup>11</sup> Nevertheless, evidence indicates that the various types of NOS may serve a variety of diverse biological pathways.<sup>7,12-13</sup> For instance, iNOS is not found only in macrophages but also in several other cell types including hepatocytes, chondrocytes, endothelial cells and fibroblasts.<sup>13</sup>

Isoform-specific antibodies to iNOS may serve as a tool for iNOS isoform identification in a specific cell or tissue.<sup>14</sup>

### Reagent

Supplied as a solution in 0.01 M phosphate buffered saline pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody Concentration: ~ 1.0 mg/mL

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage/Stability

For continuous use, store at 2–8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing 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

Immunoblotting: a working concentration of  $2-4 \mu g/mL$  is recommended using mouse macrophage RAW 264.7 cell line activated with lipopolysaccharide (LPS) and interferon- $\gamma$ .

<u>Immunohistochemistry:</u> a working concentration of 10–20 µg/mL is recommended using heat-retrieved formalin-fixed, paraffin-embedded human pancreas sections.

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

#### References

- 1. Anand T., et al., *Cytotechnology*, **66**, 823-38 (2014).
- de Luca D., et al., Sci World J., 2013:208705 (2013).
- Jain K., et al., Cell Stress Chaperones, 19, 801-12 (2014).
- 4. Liu X., et al., *Chinese Sci Bull.*, **52**, 84-90 (2007).
- 5. Chen M., et al., *J Mol Neurosci.*, **34**, 89-100 (2008).

- Boczoń K., et al., *Folia Histochem Cytobiol.*, **42**, 209-13 (2004).
- 7. Dinerman JL., et al., *Proc Natl Acad Sci U S A*, **91**, 4214-8 (1994).
- Bredt DS. and Snyder SH., *Proc Natl Acad Sci* U S A, 87, 682-5 (1990).
- Förstermann U. and Sessa WC., *Eur Heart J.*, **33**, 829-37 (2012).
- Bagasra O., et al., *Proc Natl Acad Sci U S A.*, 92, 12041-5 (1995).
- 11. Ricciardolo FL., *Physiol Rev.*, **84**, 731-65 (2004).
- 12. Snyder SH., Nature, 372, 504-5 (1994).
- 13. Snyder SH., Nature, 377, 196-7 (1995)
- 14. Pollock JS., et al., *Histochemical J.*, **27**, 738-44 (1995).

SG,DR\_OKF/LV,PHC 09/17-1