

Product Information



iNOS Electrophoresis Standard

Catalog No. 360862

Synonyms:	Inducible Nitric Oxide Synthase; NOS II
Source:	Isolated from activated murine macrophage cells ¹
M_r:	130 kDa/subunit
Purity:	≥95%
Stability:	≥1 year at -80°C
Supplied as:	5 µg purified iNOS supplied in 50 µl of 100 mM HEPES buffer, pH 7.5, containing 20% glycerol
Applications:	Western blot and gel staining (This enzyme may not be catalytically active).

Nitric oxide synthase (NOS) catalyzes the biosynthesis of nitric oxide from L-arginine. Constitutively expressed NOS is found in brain (nNOS) and endothelial cells (eNOS). iNOS (NOS II) is a soluble enzyme found in a variety of tissues including macrophages, hepatocytes, vascular smooth muscle cells, and chondrocytes.^{2,3} iNOS expression is increased by a variety of factors including LPS, IFN- γ , IL-1 β , and TNF- α , whereas expression is decreased by dexamethasone.⁴⁻⁸ The enzyme has been cloned from several species including mouse, rat, and human with homology of at least 80% between these species.³ The calculated molecular weight of the protein from the deduced amino acid sequence is 130,000-135,000.²

Laboratory Procedures

iNOS electrophoresis standard isolated from activated mouse macrophages is stable for at least six months when stored at -80°C. Avoid repeated freezing and thawing of the enzyme. To use the enzyme in different experiments, it is recommended that the standard be thawed once and aliquoted into the required number of vials and refrozen. A vial(s) can be thawed as needed, mixed with the appropriate volume of reducing sample buffer, and prepared for electrophoresis as described below.

To use the electrophoresis standard, follow the instructions described below for the appropriate application. Store unused standard in the SDS reducing buffer at -20°C.

1. Gel Staining:

Dilute the standard 1:2 with 2X SDS reducing sample buffer. Heat for 5 minutes at 95°C and then cool on ice. The concentration of the standard is 50 ng/µl. Load 10 µl per lane (0.5 µg) for mini gels or 20 µl (1.0 µg) for full length gels. After electrophoresis, stain the gel with Coomassie Blue to visualize the proteins. If the gel is to be silver stained, less protein can be loaded; approximately 0.05 µg per lane for minigels or 0.1 µg per lane for full length gels.

2. Western blotting:

Dilute the standard 1:2 with 2X SDS reducing sample buffer. Heat for 5 minutes at 95°C and then cool on ice. The concentration of the standard is 50 ng/µl. Further dilute the 50 ng/µl standard 1:5 with 1X SDS sample buffer. The concentration of this standard is 10 ng/µl. Load 2-10 µl (20-100 ng) of standard per lane. The amount of standard loaded per lane for western blotting purposes may need to be optimized depending on the sensitivity of the antibody and the detection method used. (When using the Cayman Chemical iNOS polyclonal antibody (Catalog No. 160862), 20-50 ng of standard will give a dark band using ECL for detection.) After electrophoresis, transfer to nitrocellulose or PVDF membranes for western blot analysis.

References

1. Hevel, J.M., White, K.A., and Marletta, M.A. *J. Biol. Chem.* **266**, 22789-22791 (1991).
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3. Michel, T., Xie, Q.-W., and Nathan, C. *in* Methods in nitric oxide research. Feelisch, M. and Stamler, J.S., editors. John Wiley & Sons, Chichester, 161-175 (1996).
4. Lorsbach, R.B., Murphy, W.J., Lowenstein, C.J., *et al.* *J. Biol. Chem.* **268**, 1908-1913 (1993).
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6. Schmidt, H.H.H.W., Warner, T.D., Nakane, M., *et al.* *Mol. Pharmacol.* **41**, 615-624 (1992).
7. Ogura, T. and Esumi, H. *Mol. Neurosci.* **7**, 853-856 (1996).
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Related Products

iNOS (murine macrophage) - Cat. No. 60862 • iNOS (murine recombinant) - Cat. No. 60864 • iNOS Polyclonal Antiserum - Cat. No. 160862 • iNOS (human) Polyclonal Antiserum - Cat. No. 160863

WARNING: THIS PRODUCT IS FOR LABORATORY RESEARCH ONLY: NOT FOR ADMINISTRATION TO HUMANS. NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

MATERIAL SAFETY DATA

This material should be considered hazardous until information to the contrary becomes available. Do not ingest, swallow, or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. This information contains some, but not all, of the information required for the safe and proper use of this material. Before use, the user must review the complete Material Safety Data Sheet, which has been sent under separate cover to the MSDS supervisor at your institution.

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