



cell signaling solutions

Certificate of Analysis

10 Old Barn Road • Lake Placid, NY 12946
Technical Support: T: 800 548-7853 • F: 518 523-4513
email: techserv@upstate.com
Sales Department: T: 800 233-3991 • F: 781 890-7738
Licensing Dept.: 800 310-4659
www.upstate.com

Anti-iNOS/NOS II

(rabbit polyclonal IgG)

Catalog # 06-573

Lot # 24123

Immunogen: Partial fusion protein raised against the N-terminus of murine iNOS/NOS II.

Specificity: Recognizes iNOS, Mr 125kDa. Does not cross-react with nNOS/NOS I.

Species Cross-reactivity: Mouse, bovine, sheep and guinea pig. Other species cross-reactivity is unknown.

Formulation: 200µg of protein A purified IgG in 200µl of 0.1M Tris-glycine pH 7.4, 0.15 NaCl containing 0.05% sodium azide. Frozen solution.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap.

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

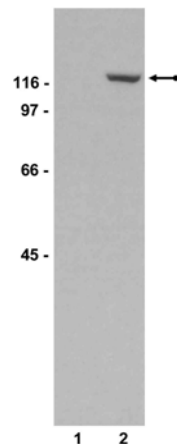
Quality Control Testing

Immunoblot Analysis: 0.5-2µg/ml of this lot detected iNOS/NOS II in RIPA lysates from L929B cells which had been stimulated with 10ng/ml IFN γ and 1µg/ml LPS for 24 hours. 1µg/ml of a previous lot detected iNOS/NOS II from similarly treated L8 cells.

Immunoprecipitation: 4µg of a previous lot immunoprecipitated iNOS/NOS II from 500µg of L929B cells which had been stimulated with 10ng/ml IFN γ and 1µg/ml LPS.

Additional Research Applications

Immunohistochemistry: This antibody has been reported to detect iNOS/NOS II in 2% paraformaldehyde-fixed sheep lung.¹



Immunoblot Analysis

L929B cell lysate from cells treated with (lane 2) or without (lane 1) IFN γ and LPB, were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-iNOS/NOS II (2µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates iNOS (~125kDa).

Application Reference:

1. Sherman, T.S., *et al.*, Am. J. Physiol. **276**: L383-L390, 1999.

General References:

Stuehr, D.J., *et al.*, Proc. Natl. Acad. Sci. **88** 7773-7777, 1991.

Xie, Q., *et al.*, Science **256**: 225-228, 1992.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on an IFN γ and LPS treated cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1 μ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na $_3$ VO $_4$; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Cat # 20-200), (PBS-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 μ g/ml of anti-iNOS/NOS II**, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a **goat anti-rabbit** HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Immunoprecipitation Protocol

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1 μ g/ μ l total cell protein in a microcentrifuge tube with PBS.
2. Add **4 μ g of anti-iNOS/NOS II** to 500 μ g-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 μ l (50 μ l packed beads) of washed Protein A agarose bead slurry (Cat # 16-125).
5. Gently rock the reaction mixture at 4°C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 60 μ l 2X Laemmli sample buffer.
8. Store the beads frozen for future analysis or boil the beads for 5 minutes.
9. Collect the beads after boiling using a microcentrifuge pulse.
10. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.