

## Anti-Mouse iNOS

(rabbit polyclonal IgG)

Catalog # 06-573

Lot # 15441

**Immunogen:** Partial fusion protein raised against the N-terminus of murine iNOS.

**Specificity:** Recognizes iNOS, does not cross-react with nNOS.

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

**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.

**Formulation:** 200mg of protein A purified IgG in 200ml of 0.1M Tris-glycine pH 7.0 containing 0.05% sodium azide. Frozen solution.

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

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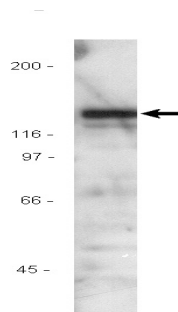
### Quality Control Testing

**Immunoblot Analysis:** 1µg/ml of this lot detected iNOS (~125kDa) in RIPA lysates from L8 and L929B cells which had been stimulated with 10ng/ml IFN $\gamma$  and 1µg/ml LPS.

**Immunoprecipitation:** 4µg of this lot immunoprecipitated iNOS from 500µg of L929B cells which had been stimulated with 10 ng/ml IFN $\gamma$  and 1µg/ml LPS RIPA lysate.

### Additional Research Applications

**Immunohistochemistry:** This antibody has been reported to detect iNOS in 2% paraformaldehyde-fixed sheep lung.<sup>1</sup>



#### Immunoblot Analysis

L8 cell lysate, pretreated with IFN $\gamma$  and LPS, was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-mouse iNOS (1µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates iNOS.

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### 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 a 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 $\mu$ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 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 (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **1mg/ml of anti-Mouse iNOS**, 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, 1:3000 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 $\mu$ g/ $\mu$ l total cell protein in a microcentrifuge tube with PBS.
2. Add **4mg of anti-Mouse iNOS** to 500 $\mu$ g-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 $\mu$ l of washed Protein A agarose bead slurry (50 $\mu$ l packed beads).
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 50 $\mu$ l 2X Laemmli sample buffer.
8. The agarose beads can either be frozen for later use or suspended in Laemmli sample buffer and boiled for 5 minutes. Collect the beads by a microcentrifuge pulse. SDS-PAGE and subsequent immunoblot analysis can be performed on a sample of the supernatant.