



cell signaling solutions

Certificate of Analysis

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Anti-nNOS/NOS I

(rabbit antiserum)

Catalog # 07-571

Lot # 27897

Immunogen: Synthetic peptide corresponding to N-terminus of rat nNOS (neuronal nitric oxide synthase).

Specificity: Recognizes nNOS, Mr 165kDa.

Species Cross-Reactivity: Human, mouse and rat. Other species cross-reactivity is unknown.

Formulation: 200 μ l of rabbit antiserum with 0.05% sodium azide. Frozen solution.

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

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

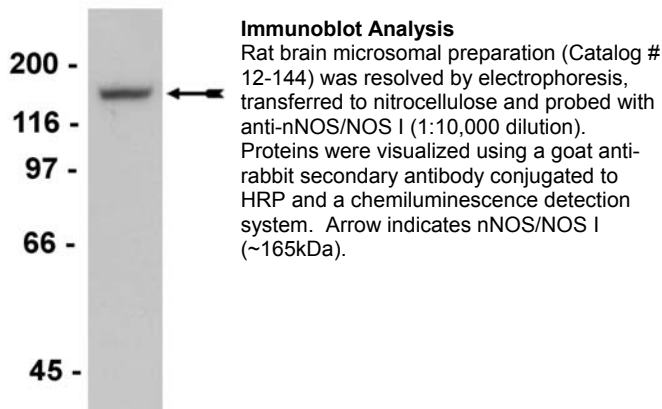
Quality Control Testing

Immunoblot Analysis: A 1:4,000-1:10,000 dilution of this lot detected nNOS (Mr ~165kDa) in a rat brain microsomal preparation (Catalog # 12-144).

The use of fresh extract preparations is recommended.

Additional Research Applications

Immunocytochemistry: This antibody has been reported by an independent laboratory to detect nNOS in C2C12 myotubes.¹



Application References:

1. Abdelmoity, A., *et al.*, *FEBS Lett.* **482**: 65-70, 2000.
2. Lau, K.S., *et al.*, *Physiol. Genomics* **2**: 21-27, 2000.
3. Sander, M., *et al.*, *Proc. Natl. Acad. Sci.* **97**: 13818-13823, 2000.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a freshly prepared neuronal extract and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared 3% nonfat dry milk (Catalog # 20-200) in PBS (PBS-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with a **1:4,000-1:10,000 dilution of anti-nNOS/NOS I**, 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 with agitation at room temperature.
6. Wash the nitrocellulose twice with water.
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).