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Certificate of Analysis

Anti-nNOS/NOS I
(rabbit antiserum)
Catalog # 07-571
Lot #DAM1394814

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.

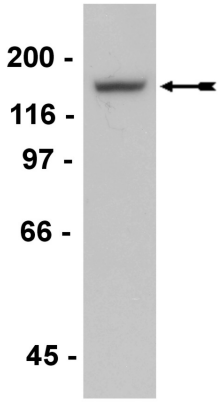
Formulation: **200µl** of rabbit antiserum with 0.05% sodium azide. Frozen solution.

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.



Immunoblot Analysis
Rat brain microsomal preparation (Catalog # 12-144) was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-nNOS/NOS I (1:10,000 dilution). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates nNOS/NOS I (~165kDa).

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℃.
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).