

## Certificate of Analysis

### Anti-phospho-nNOS/NOS I (Ser1417)

(rabbit immunoaffinity purified IgG)

Catalog # 07-544

Lot # DAM1437152

**Immunogen:** KLH-conjugated, synthetic peptide corresponding to amino acids surrounding Ser1417 of human neuronal Nitric Oxide Synthase (nNOS), with an N-terminal cysteine added for conjugation purposes, where pS is phosphorylated serine corresponding to the AMPK phosphorylation site. This site also corresponds to Ser1451 of the nNOS $\mu$  isoform. The immunizing sequence is identical in mouse, rat, zebrafish and frog.

**Specificity:** Recognizes phosphorylated nNOS/NOS I, Mr 170kDa.

**Species Cross-reactivity:** Human and rat. Predicted to cross-react with mouse, zebrafish and frog based on sequence homology.

**Formulation:** 100 $\mu$ g of immunoaffinity purified rabbit IgG in 100 $\mu$ l of 0.014M phosphate buffer, pH 7.6, 0.175M NaCl, 0.07% sodium azide, and 30% glycerol. Liquid at -20°C.

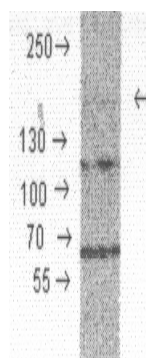
**Storage and Stability:** Stable for 1 year at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

**NOTE:** This product has been incorrectly labeled as Ser1416.

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

### Quality Control Testing

**Immunoblot Analysis:** 1:500 dilution of this lot detected affinity purified bacterial expressed rat nNOS/NOS I that had been phosphorylated by purified AMPK *in vitro*.



#### Immunoblot Analysis

Lambda phosphatase treated (Lane 1) and untreated (Lane 2) affinity purified bacterial expressed rat nNOS/NOS I that had been phosphorylated by purified AMPK was resolved by electrophoresis, transferred to pvd and probed with anti-phospho-nNOS/NOS I (Ser1417) (1:500). Proteins were visualized using a donkey anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phosphorylated nNOS/NOS I (~170kDa).

### General References:

Chen, Z.-P., *et al.*, *Am. J. Physiol. Endocrinol. Metab.* **279**: E1202-E1206, 2000.

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>, 1mM NaF) and transfer the proteins to pvdf. Wash the blotted pvdf twice with water.
2. Block the blotted pvdf in freshly prepared 5% nonfat dry milk (Catalog # 20-200) in TBS with 0.05% Tween<sup>®</sup>-20 (TBS-MLK) for 1 hour at room temperature with constant agitation.
3. Incubate the pvdf with **1:250-1:500 of anti-phospho-nNOS/NOS I (Ser1417)**, diluted in freshly prepared TBS-MLK overnight with agitation at 4°C.
4. Wash the pvdf twice with water.
5. Incubate the pvdf in the secondary reagent of choice (donkey anti-rabbit IgG HRP, Catalog # AP-182P, 1:50000 dilution was used) in TBS-MLK for 1.0 hour with agitation at room temperature.
6. Wash the pvdf twice with water.
7. Wash the pvdf in TBS-0.05% Tween<sup>®</sup>-20 for 3-5 minutes.
8. Rinse the pvdf in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals."