

**IgG Fraction Rabbit Anti-human endothelial
Nitric Oxide Synthase (Myristylated form) Serum**

AS-1689

Lot # 8016

This antiserum was raised in a rabbit which was immunized with heNOS (caproyl-2-11Cys¹²) covalently attached onto a carrier protein. This antiserum is specific for the myristylated form of hecNOS. It is suitable for immunocytochemical and western immunoblotting detection of myristylated heNOS. The antiserum has been characterized by western blotting, ELISA, and cell staining techniques using synthetic peptides and recombinant whole proteins. Dilute the lyophilized antiserum with 0.1 ml of 10 mg/ml BSA in PBS for the equivalent of whole antiserum, or with additional buffer for more dilute antiserum.

Antiserum Specificity

Polypeptide	% Cross Reactivity
heNOS (cap-2-11Cys ¹²)	100
recombinant myristylated heNOS (myristylated eNOS type III)	100
heNOS (2-11Cys ¹²)	0
recombinant heNOS (NOS type III)	0
recombinant hnNOS (NOS type I)	0
recombinant hiNOS (NOS type II)	0

Immunocytochemical Staining

This antiserum has been found to stain fixed cultured cells known to express myristylated eNOS by indirect immunofluorescence at a dilutions of 1:400.

Western Immunoblot

Western immunoblots using homogenate from cells expressing myristylated hecNOS resulted in a single band being detected at ~ 135 kDa at a dilution of 1:500.

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such as 7.5% gels) and electrophoretic transfer to PVDF membrane, block the membrane overnight with 4% normal goat serum in TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the rabbit polyclonal antibody after dilution to at least 1:500 (Note: higher dilutions may be needed). Use 2% normal goat serum in TBS/Tween-20 as buffer for the primary antibody. Let the primary antibody bind for 2 - 4 hours.
4. Wash x 3 with TBS/Tween-20.
5. Apply affinity purified HRP-goat anti-rabbit IgG antiserum diluted 1:2500 (dilution may vary depending upon supplier) in 2% normal goat serum in TBS/Tween-20. Incubate 1 - 2 hours. Note: greater sensitivity may be achieved using ABC techniques.
6. Wash x 4 for 5 min per wash cycle in a fairly large volume of TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.