R&D® Ab
Research & Diagnostic Antibodies
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IgG Fraction of Rabbit Anti-human endothelial Nitric Oxide Synthase Serum

AS-1687 Lot # 7948

The pooled antisera were raised in rabbits which were immunized with heNOS(2-11) covalently attached onto a carrier protein. The pooled antisera have been characterized by western blotting, ELISA, and cell staining techniques using synthetic peptides and recombinant whole proteins. The antibodies are specific for the amino terminal of heNOS, and have been found to be suitable for the immunocytochemical and western blot detection of heNOS. The antibodies recognize the non-myristylated form of heNOS best, but they do cross-react ~50% with the myristylated form of heNOS. The IgG fraction of the pooled rabbit antisera was prepared by precipitation, dialysis, and column chromatography. Dilute the lyophilized antibody with 0.1 ml of 10 mg/ml BSA in PBS for the equivalent of whole antisera, or with additional buffer for more dilute antibody.

Each vial contains 2.6 mg of purified IgG.

Antisera Specificity

Polypeptide	% Cross Reactivity
heNOS (2-11)	100
recombinant heNOS (NOS type III)	100
heNOS (cap-2-11Cys^{12})	~ 50
recombinant myristylated heNOS (myristylated NOS type III)	~ 50
recombinant hnNOS (NOS type I)	0
recombinant hiNOS (NOS type II)	0

Immunocytochemical Staining

This IgG fraction has been found to stain formalin fixed cultured cells known to express heNOS by indirect immunofluorescence at a concentration of 50 μg/ml.

Western Immunoblot

Western immunoblots using homogenate from cells expressing heNOS resulted in a single band being detected at ~ 135 kDa at a concentration of 25 μg/ml.

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such at 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 IgG fraction after diluting to at least 25 μg/ml (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 at least 5 min per wash cycle in TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.