R&D ® Ab

Research & Diagnostic Antibodies

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Rabbit Anti-human neuronal Nitric Oxide Synthase Receptor Serum

AS-1657 Lot # 9266

The antiserum was raised in a rabbit which was immunized with hnNOS(1411-1433) covalently attached onto a carrier protein. This antiserum is specific for the carboxyl end of hnNOS and is suitable for immunocytochemical and western immunoblotting detection of hnNOS. The antiserum has been characterized by western blotting, ELISA, and cell staining techniques using synthetic peptides and recombinant whole proteins. The IgG fraction of the rabbit antiserum was prepared by precipitation, dialysis, and column chromatography. Dilute the lyophilized antiserum with 0.1 ml of 10 mg/ml BSA is PBS for the equivalent of whole antiserum, or with additional buffer for more dilute antiserum.

Antiserum Specificity

Polypeptide	% Cross Reactivity
hnNOS(1411-1433)	100
recombinant hnNOS (NOS type I)	100
hiNOS(1137-1153)	0
recombinant hiNOS(NOS type II)	0
heNOS(1182-1203)	0
recombinant heNOS(type III)	0

Immunocytochemical Staining

This antiserum has been found to stain fixed cultured cells known to express hnNOS by indirect immunofluorescence at a dilutions of 1:1000 to 1:5000.

Western Immunoblot

Western immunoblots using homogenate from cells expressing hnNOS resulted in a single band being detected at ~ 160 kDa at a dilution of 1:2000.

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such at 12% gels) and elecrophoretic transfer to PVDF membrane overnight with 4% normal goat serum in 1:5 diluted evaporated goat milk, using TBS/Tween-20 buffer as diluent.
2. Wash x 2 with TBS/Tween-20.
3. Apply the rabbit polyclonal antibody after dilution to at least 1:800(Note:higher dilutions may be needed). Use 1% normal goat serum in 1:5 diluted evaporated goat milk mild as buffer for the primary antibody. Dilute the condensed goat milk with TBS/Tween-20. 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 1% normal goat serum in 1:5 diluted evaporated goat milk(useTBS/Tween-20 to dilute the goat milk). Incubate 1-2 hours. Note: greater sensitivity may be achieved using ABC techniques.
6. Wash x 3 and then soak the membrane overnight in a fairly large volume to TBS/Tween-20.
7. Develop color using the DAB reaction or the enhanced DAB reaction.