R&D ® Ab
Research & Diagnostic Antibodies
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Rabbit Anti-human inducible Nitric Oxide Synthase Serum

AS-1646 Lot # 7913

The antiserum was raised in a rabbit which was immunized with hiNOS(Cys^{1137}-1138-1153) covalently attached onto a carrier protein. This antiserum is specific for the carboxyl end of hiNOS and is suitable for immunocytochemical and western immunoblotting detection of hiNOS. 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 is PBS for the equivalent of whole antiserum, or with additional buffer for more dilute antiserum.

Antiserum Specificity

Polypeptide	% Cross Reactivity
hiNOS(1137-1153)	100
recombinant hiNOS (NOS type II)	100
hnNOS(1411-1433)	0
recombinant hnNOS(NOS type I)	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 hiNOS by indirect immunofluorescence at a dilution of 1:1000 to 1:5000.

Western Immunoblot

Western immunoblots using homogenate from cells expressing hiNOS resulted in a single band being detected at ~ 130 kDa at a dilution of 1:1000.

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such at 12% gels) and electrophoretic transfer to PVDF membrane, block the membrane overnight with 2% 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 1:1000 (Note: higher dilutions may be needed). Use 1% 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 1% 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 with TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.