

**Rabbit Anti-human neuronal Nitric Oxide Synthase Serum**

AS-1656

Lot # 9257

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. 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**

<b>Polypeptide</b>	<b>% Cross Reactivity</b>
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 as 7.5% 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 1:2000 (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 use TBS/Tween-20. Incubate 1-2 hours. Note: greater sensitivity may be achieved using ABC techniques.
6. Wash x 4 for 5 minutes wash cycle with TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.