

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

AS-1647

Lot # 7914

The antiserum was raised in a rabbit which was immunized with hiNOS(Cys<sup>1137</sup>-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. The IgG fraction of the rabbit antiserum 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 antiserum, or with additional buffer for more dilute antiserum.

**Antiserum Specificity**

<b>Polypeptide</b>	<b>% Cross Reactivity</b>
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 dilutions 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 1:3000.

**Western Blotting Protocol**

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such as 12% gels) and electrophoretic transfer to PVDF 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 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.