

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

AS-1686

Lot # 10542

The antiserum was raised in a rabbit which was immunized with human eNOS (2-11Cys<sup>12</sup>) covalently attached onto a carrier protein. This antiserum is specific for the amino terminal of heNOS. It recognizes the non-myristylated form best, but does crossreact ~50% with the myristylated form of heNOS. It is suitable for immunocytochemical and western immunoblotting detection of heNOS. 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>
heNOS (2-11Cys <sup>12</sup> )	100
recombinant heNOS (NOS type III)	100
heNOS (cap-2-11Cys <sup>12</sup> )	50
recombinant myristylated heNOS (myristylated NOS type III)	50
Recombinant hnNOS (NOS type I)	0
recombinant hiNOS (type II)	0

**Immunocytochemical Staining**

This antiserum has been found to stain fixed cultured cells known to express heNOS by indirect immunofluorescence at a dilutions of 1:400.

**Western Immunoblot**

Western immunoblots using homogenate from cells expressing hiNOS resulted in a single band being detected at ~ 135 kDa at a dilution of 1:800.

**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 1:800 (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 4 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 6 for 5 min per wash cycle with TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.