

**Western Immunoblotting Reagents:
Anti-human Inducible Nitric Oxide Synthase Monoclonal Antibody**
Supplied as culture supernatant

WR-5210

Lot # 8118

This culture supernatant contains mouse monoclonal anti-hiNOS clone 2D2-B2 antibody raised against recombinant hiNOS. The MAb has been epitope mapped using 96 overlapping 18 amino acid long synthetic peptides which cover the entire 1153 amino acid length of hiNOS and was found to bind to a defined region of the hiNOS sequence, residues 781-798. The 0.5 ml aliquot should be diluted to 40 ml (a 1:80 dilution) and should be sufficient for at least 20 lanes. This culture supernatant has been found to bind to iNOS by ELISA and immunocytochemistry and in western immunoblots. This monoclonal antibody has been tested for recognition of other iNOS isozymes by ELISA and western immunoblotting techniques.

Antiserum Specificity

Polypeptide/Protein	% Cross Reactivity
hiNOS (781-798)	100
rhNOS (Type 2)	100
hnNOS (1045-1062)	0
rhnNOS (Type 1)	0
heNOS (806-823)	0
rheNOS (Type 3)	0

Western Blotting Protocol

Western immunoblots resulted in a single band being detected at ~130 kDa at a dilution of 1:80.

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 4% normal goat serum in TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the mouse monoclonal antibody after preparing a 1:80 dilution. Use 2% normal goat serum in TBS/Tween-20 as buffer for the primary antibody. Let the primary bind for 1 - 2 hours.
4. For blocked antibody controls, add 6.0 ml of the 1:80 diluted monoclonal antibody to the contents of the vial containing the free peptide. Pre-incubate for 60 minutes before applying to the membrane. This should be sufficient for 3 blocked control lanes. **DO NOT ADD THE PEPTIDE TO THE STOCK MONOCLONAL ANTIBODY. THIS WILL BLOCK ALL BINDING.**
5. Wash x 3 with TBS/Tween-20.
6. Apply affinity purified HRP-goat anti-mouse IgG antiserum diluted 1:2500 (dilution may vary depending upon supplier) in 2% normal goat serum in TBS/Tween-20. Incubate 1 - 2 hours.
7. Wash x 4 for at least 5 min per wash cycle in TBS/Tween-20.
8. Develop color using the enhanced DAB reaction.

PS-5166B: hiNOS (781-798-amide)

Amino Acid Sequence:

NH₂-Pro-Ala-Leu-Val-Gln-Gly-Ile-Leu-Glu-Arg-Val-Val-Asp-Gly-Pro-Thr-Pro-His-amide

Mol. Wt.: 1897.2

Peptide Purity: 95%

Peptide Quantity: 150 nmoles

Peptide Lot # 7792

HPLC Analysis: See Attached Chart Recording

Solvent System: A. 0.05 M KH₂PO₄, pH 3.0

B. 70% Acetonitrile + 30% A

Solvent Program:

<u>Time</u>	<u>Flow</u>	<u>%A</u>	<u>%B</u>
0	1.2	100	0
30	1.2	25	75
31	1.2	0	100
32	1.2	100	0
35	1.2	100	0

Detection: optical density at 225 nm

Results: single peak at R_t = 24.652 min