R&D ® Ab

Research & Diagnostic Antibodies

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Western Immunoblotting Reagents: IgG Fraction of Rabbit Anti-hiNOS (1137-1153 Serum

WR-1646 Lot # 8108

The antiserum was raised in a rabbit which was immunized with a peptide analogue of the carboxyl terminal of hiNOS covalently attached onto a carrier protein. The IgG fraction of the rabbit antiserum was prepared by precipitation, dialysis, and column chromatography. Rehydrate the lyophilized IgG fraction with 5.0 ml of TBS/Tween 20 that contains 1% normal goat serum(NGS). The stock solution should be further diluted 1:8 with additional buffer prior to use (see below). This should be sufficient for at least 20 lanes. This antiserum has been found to stain specifically hiNOS in western immunoblots of A-172 cells induced to produce hiNOS. The antiserum was tested for recognition of the other NOS subtypes by ELISA and western blotting techniques.

Antiserum Specificity

Polypeptide	% Cross Reactivity
hiNOS (1137-1153)	100
rhiNOS (Type 2)	~50
rhnNOS (Type 1)	0
rheNOS (Type 3)	0

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 4% normal goat serum using TBS/Tween-20 buffer as diluent.
2. Wash x 2 with TBS/Tween-20.
3. For blocked antibody controls dissolve 150 nmole of peptide PS-1644 in 600 μ l of reconstituted stock antibody. Incubate one hour. Then add 5.4 ml of 2% normal goat serum in TBS/Tween-20 and use 2.0 ml per lane this should be sufficient for 3 blocked control lanes. DO NOT ADD THE PEPTIDE TO THE STOCK POLYCLONAL ANTIBODY. THIS WILL BLOCK ALL BINDING.
4. Apply the rabbit polyclonal antibody after dilution to at least 1:8 (Note: higher dilutions may be needed). Use 2% normal goat serum in TBS/Tween-20 with 2% NGS as buffer for the primary antibody. Let the primary antibody bind for 2-4 hours.
5. Wash x 3 with TBS/Tween-20.
6. Apply affinity purified HRP-goat anti-rabbit 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 5 minutes per wash cycle of TBS/Tween-20.
8. Develop color using the enhanced DAB reaction.