

Western Immunoblotting Reagents

IgG Fraction of Rabbit Anti-Nicotinic $\alpha 5$ Receptor Sera

WR-5621

Lot # 9904

This IgG fraction was prepared by precipitation, dialysis and column chromatography from a pool of antisera that were raised in rabbits which were immunized with a peptide analogue of the carboxyl terminal of the nicotinic $\alpha 5$ receptor (residues #460-468) attached onto a carrier protein. Rehydrate the lyophilized IgG fraction with 5.0 ml of TBS/Tween-20 that contains 2% 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 the nicotinic $\alpha 5$ receptor in western immunoblots.

Antiserum Specificity

Polypeptide	% Cross Reactivity
Nicotinic $\alpha 5$ receptor (460-468)	100
Nicotinic $\alpha 5$ receptor	~95
Nicotinic $\alpha 3$ receptor	0
Nicotinic $\alpha 4$ receptor	0
Nicotinic $\alpha 7$ receptor	0
Nicotinic $\beta 2$ receptor	0
Nicotinic $\beta 3$ receptor	0
Nicotinic $\beta 4$ receptor	0

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such as 10% gels) and electrophoretic transfer to PVDF membrane, block the membrane overnight with 4% normal goat serum using TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. For blocked antibody controls dissolve 150 nmole of peptide PS-5621 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 IgG fraction 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 1-2 hours.
5. Wash x 3 with TBS/Tween-20.
6. Apply affinity purified HRP-goat anti-rabbit IgG antiserum diluted 1:2500 (Note: dilution may vary depending upon supplier) in 2% normal goat serum in TBS/Tween-20. Incubate 1 hour.
7. Wash x 4 for 5 minutes per wash cycle with TBS/Tween-20.
8. Develop color using the enhanced DAB reaction.

PS-5621: human $\alpha 5$ Nicotinic Receptor (Cys⁴⁵⁹-460-468)

Amino Acid Sequence:

NH₂-Cys-Pro-Val-His-Ile-Gly-Asn-Ala-Asn-Lys-COOH

Mol. Wt.: 1051.2

Peptide Quantity: 150 nmoles

Peptide Purity: > 98%

Date: September 6, 1998

Lot Number: 9278

HPLC Analysis: See Attached Chart Recording

Solvent System: A. 0.05 M KH₂PO₄, pH 3.0
 B. 70% AcCN + 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 = 11.653 min