

**Western Immunoblotting Reagents**  
**IgG Fraction of Rabbit Anti-Nicotinic  $\beta$ 4 Receptor Sera**

WR-5656

Lot # 9923

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  $\beta$ 4 receptor (residues #490-498) attached onto a carrier protein. 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 the nicotinic  $\beta$ 4 receptor in western immunoblots.

**Antiserum Specificity**

<b>Polypeptide</b>	<b>% Cross Reactivity</b>
Nicotinic $\beta$ 4 receptor (490-498)	100
Nicotinic $\beta$ 4 receptor	~80
Nicotinic $\alpha$ 3 receptor	0
Nicotinic $\alpha$ 4 receptor	0
Nicotinic $\alpha$ 5 receptor	0
Nicotinic $\alpha$ 7 receptor	0
Nicotinic $\beta$ 2 receptor	0
Nicotinic $\beta$ 3 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-5656 in 600  $\mu$ l of reconstituted stock antibody. Incubate one hour. Then add 5.4 ml of 1% 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 1% normal goat serum in TBS/Tween-20 with 1% 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 1% 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-5656: human  $\beta$ 4 Nicotinic Receptor (Cys<sup>489</sup>-490-498)**

Amino Acid Sequence:

NH<sub>2</sub>-Cys-Glu-Gly-Pro-Tyr-Ala-Ala-Gln-Arg-Asp-COOH

Mol. Wt.: 1108.2

Peptide Quantity: 150 nmoles

Peptide Purity: > 98%

Date: September 19, 1998

Lot Number: 9297

HPLC Analysis: See Attached Chart Recording

Solvent System:      A. 0.05 M KH<sub>2</sub>PO<sub>4</sub>, 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<sub>t</sub> = 10.535 min