

Western Immunoblotting Reagents

IgG Fraction of Rabbit Anti-Nicotinic $\alpha 3$ Receptor Serum

WR-5611

Lot # 9400

The antiserum was raised in a rabbit which was immunized with a peptide analogue of the carboxyl terminal of the nicotinic $\alpha 3$ receptor 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 the nicotinic $\alpha 3$ receptor in western immunoblots.

Antiserum Specificity

| Polypeptide | % Cross Reactivity |
|---|--------------------|
| Nicotinic $\alpha 3$ receptor (466-474) | 100 |
| Nicotinic $\alpha 3$ receptor | ~ 90 |
| 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 |
| 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-5611 in 600 μ 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-5612: human $\alpha 3$ Nicotinic Receptor (Tyr⁴⁶⁵-466-474)

Amino Acid Sequence:

NH₂-Tyr-Gln-Pro-Leu-Met-Ala-Arg-Glu-Asp-Ala-COOH

Mol. Wt.: 1193.4

Peptide Quantity: 150 nmole

Peptide Purity: > 98%

Date: March 1, 2001

Lot Number: 10157

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 = 14.433 min