

Western Immunoblotting Reagents
IgG Fraction of Rabbit Anti-Nicotinic β 3 Receptor Sera

WR-5651

Lot # 9917

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 β 3 receptor (residues #450-458) 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 β 2 receptor in western immunoblots.

Antiserum Specificity

| Polypeptide | % Cross Reactivity |
|--|---------------------------|
| Nicotinic β 3 receptor (450-458) | 100 |
| Nicotinic β 3 receptor | ~85 |
| Nicotinic α 3 receptor | 0 |
| Nicotinic α 4 receptor | 0 |
| Nicotinic α 5 receptor | 0 |
| Nicotinic α 7 receptor | 0 |
| Nicotinic β 2 receptor | 0 |
| Nicotinic β 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-5651 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-5651: human β 3 Nicotinic Receptor (Cys⁴⁴⁹-450-458)

Amino Acid Sequence:

NH₂-Cys-Leu-Lys-Met-Trp-Leu-His-Ser-Tyr-His-COOH

Mol. Wt.: 1316.6

Peptide Quantity: 150 nmoles

Peptide Purity: > 98%

Date: September 13, 1998

Lot Number: 9283

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: Major peak at R_t = 17.477 min