R&D® Ab
Research & Diagnostic Antibodies
2645 W. Cheyenne Ave, N. Las Vegas, NV 89032 Phone: 702-638-7800

Western Immunoblotting Reagents
IgG Fraction of Rabbit Anti-Nicotinic β2 Receptor Sera

WR-5646 Lot # 9429

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 β2 receptor (residues #493-502) 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 β2 receptor in western immunoblots.

Antiserum Specificity

Polypeptide	% Cross Reactivity
Nicotinic β2 receptor (493-502)	100
Nicotinic β2 receptor	~90
Nicotinic α3 receptor	0
Nicotinic α4 receptor	0
Nicotinic α5 receptor	0
Nicotinic α7 receptor	0
Nicotinic β3 receptor	0
Nicotinic β4 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-5646 in 600 µl 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.

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PS-5646: human β2 Nicotinic Receptor (Cys^{492}-493-502)

Amino Acid Sequence:

NH_2-Cys-His-Ser-Asp-His-Ser-Ala-Pro-Ser-Ser-Lys-COOH

Mol. Wt.: 1154.2
Peptide Quantity: 150 nmoles
Peptide Purity: > 98%
Date: September 20, 1998
Lot Number: 9298

HPLC Analysis: See Attached Chart Recording

Solvent System: A. 0.05 M KH_2PO_4, pH 3.0
B. 70% AcCN + 30% A

Solvent Program:

Time	Flow	%A	%B
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 = 6.400 min