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-D_3 Receptor Antiserum

WR-3532 Lot # 9094

The antiserum was raised in a rabbit which was immunized with D_3(2-10-Cys^{11}) covalently attached onto a carrier protein, and it has been characterized by immunocytochemical, western immunoblot and ELISA techniques. The antiserum and the IgG fraction of the antiserum have been found to be highly specific for this peptide sequence and are suitable for the western blot detection of the D_3 dopamine receptor. Rehydrate the lyophilized IgG fraction with 5.0 ml of 10 mg/ml BSA in PBS. 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.

Antiserum Specificity

Polypeptide	% Cross Reactivity
D_3 Dopamine Receptor (2-10)	100
D_3 Dopamine Receptor	80
D_1 Dopamine Receptor (9-21)	0
D_1 Dopamine Receptor	0
D_2 Dopamine Receptor (272-282)	0
D_2 Dopamine Receptor	0
D_4 Dopamine Receptor (176-185)	0
D_4 Dopamine Receptor	0
D_5 Dopamine Receptor (23-35)	0
D_5 Dopamine 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 in TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. For blocked antibody controls dissolve 150 nmole of peptide PS-3537 in 600 µl of reconstituted antibody and incubate 60 min. Then add 5.4 ml of 1% normal goat serum in TBS/Tween-20. Using 2 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 polyclonal antibody after diluting the stock solution 1:8 (Note: higher dilutions may be necessary). Use 1% normal goat serum in TBS/Tween-20 as buffer for the primary antibody. Let the primary antibody bind for 2-4 hours.
5. Wash x 3 with TBS/Tween-20.
6. Apply affinity purified HRP-goat anti-rabbit IgG antiserum diluted 1:2500 (dilution may vary depending upon supplier) in 1% normal goat serum in TBS/Tween-20. Incubate 1-2 hours.
7. Wash x 4 for at least 5 min per wash cycle in TBS/Tween-20.
8. Develop color using the enhanced DAB reaction.

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PS-3537: Dopamine D_3 Receptor (2-10)

Amino Acid Sequence:

NH_2-Ala-Ser-Leu-Ser-Gln-Leu-Ser-Ser-His-COOH

Mol. Wt.: 928.99
Peptide Purity: 95%
Peptide Quantity: 150 nmoles
Lot Number: 7829

HPLC Analysis: See 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
20	1.2	50	50
21	1.2	0	100
22	1.2	100	0
25	1.2	100	0

Detection: optical density at 225 nm
Results: single peak at R_t = 14.357