R&D® Ab

Research & Diagnostic Antibodies

2645 W. Cheyenne Ave, N. Las Vegas, NV 89032 702-638-7800

Western Immunoblotting Reagents: IgG Fraction of Rabbit Anti-D_{2S} Receptor

WR-3520 Lot # 8047

The antiserum was raised in a rabbit which was immunized with D_{2s}(Ac-240-247-Cys^{247}) covalently attached onto a carrier protein, and it has been characterized by immunocytochemical, western immunoblot and ELISA techniques. This antiserum and the IgG fraction of the antiserum have been found to be highly specific for this peptide sequence and are suitable for immunocytochemical detection of D_{2S} dopamine receptor. Rehydrate the lyophilized IgG fraction with 5.0 ml of 10 mgm/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_{2S} Dopamine receptor (Ac-240-247)	100
D_{2L}Dopamine receptor (243-254)cyclized	0
D_2 Dopamine receptor (Cys^{271}-272-282)	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 as diluent.
2. Wash x 2 with TBS/Tween-20.
3. For blocked antibody controls dissolve 150 nmole of peptide PS-3520-1with 600 µ l of reconstituted antibody. Incubate one hour. Then add 5.4 ml of 1% normal goat serum in TBS/Tween-20 and 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 1:8. 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 5 min. per wash cycle in TBS/Tween-20.
8. Develop color using the enhanced DAB reaction.

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Quality Control Data Sheet

PS-3520-1: D_{2S} Dopamine Receptor [Ac-239-244- Cys^{245}]

Amino Acid Sequence:
Ac-Pro-Leu-Lys-Glu-Ala-Ala-Cys-COOH

Mol. Wt.: 773.0
Peptide Purity: >95%
Peptide Quantity:150 nmoles
Date: Aug. 31, 1995
Lot# 7064

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: Major peak at R_t = 17.273 min