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_{2L} Receptor

WR-3524 Lot # 8050

The antiserum was raised in a rabbit which was immunized with D_{2L}(Ac-243-249) 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_{2L} 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_{2L} Dopamine receptor (243-249)	100
D_{2L} Dopamine receptor (243-254) cyclized	100
D_2 Dopamine receptor (Cys^{271}-272-282)	0
D_{2S} Dopamine receptor (Ac-240-247)	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 1:5 diluted evaporated goat milk, using TBS/Tween-20 buffer as diluent.
2. Wash x 2 with TBS/Tween-20.
3. For blocked antibody controls dissolve 150 nmole of the free peptide with 600 µ l of reconstituted antibody. Incubate 60-90 min. Then add 5.4 ml of 1% normal goat serum in 1:5 diluted evaporated goat milk. 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 1:5 diluted evaporated goat milk as buffer for the primary antibody. Dilute the condensed goat milk with TBS/Tween-20 and apply 2.0 ml per lane. 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 1:5 diluted evaporated goat milk (useTBS/Tween-20 to dilute the goat milk). Incubate 1-2 hours.
7. Wash x 3 and then soak the membrane overnight in a fairly large volume of TBS/Tween-20.
8. Develop color using the DAB reaction or the enhanced DAB reaction.

Note: if goat milk is not available, use 4% normal goat serum as substitute

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Quality Control Data Sheet

PS-3524-3: D_{2L} Dopamine Receptor Loop [Ac-243-249]

Amino Acid Sequence:
Ac-Asn-Cys-Thr-His-Pro-Glu-Asp-COOH

Mol. Wt.: 814.8
Peptide Purity: >98%
Peptide Quantity:150 nmoles
Date: March 27, 1995
Lot # 6516

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
20		50	50
21		0	100
22		100	0
25		100	0

Detection: optical density at 225 nm
Results: Major peak at R_t = 9.395 min

FAX: 702-638-7801 www.RDAbs.com e-mail: Info@RDAbs.com