

Western Immunoblotting Reagents: IgG Fraction of Rabbit Anti-D₂ Receptor Antiserum

WR-3526

Lot # 8046

The antiserum was raised in a rabbit which was immunized with D₂(Cys²⁷¹-272-282) 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 western blot detection of both the D_{2S} and D_{2L} dopamine receptors. 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 ₂ Dopamine Receptor (272-282)	100
D ₂ Dopamine Receptor	60
D ₁ Dopamine Receptor (9-21)	0
D ₁ Dopamine Receptor	0
D ₃ Dopamine Receptor (2-10)	0
D ₃ 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 as diluent.
2. Wash x 2 with TBS/Tween-20.
3. For blocked antibody controls dissolve 150 nmole of peptide PS-3526B with 600 µl of reconstituted antibody. Incubate for 60 min, then add 5.4 ml of 1% normal goat serum in TBS/Tween-20 buffer. 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, 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 TBS/Tween-20 buffer. Incubate 1-2 hours.
7. Wash x 4 for at least 5 min per wash cycle in TBS/Tween-20.
8. Develop color using enhanced DAB reaction.

PS-3526B: D₂ Dopamine Receptor [Cys²⁷¹-272-282]

Amino Acid Sequence:

NH₂-Cys-Ala-Ala-Arg-Arg-Ala-Gln-Glu-Leu-Glu-Met-Glu-COOH

Mol. Wt.: 1406.6

Peptide Purity: >95%

Peptide Quantity: 150 nmoles

Lot # 7062

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
	40		0	100
	44		0	100
	46		100	0
	50		100	0

Detection: optical density at 225 nm

Results: Major peak at R_t = 23.897 min