

Western Immunoblotting Reagents: IgG Fraction of Rabbit Anti-D₅ Receptor

WR-3552

Lot # 9084

The antiserum was raised in a rabbit which was immunized with D₅ (Ac-23-35) 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 the D₅ 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 ₅ Dopamine Receptor (23-35)	100
D ₅ 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 ₃ Dopamine Receptor (2-10)	0
D ₃ Dopamine Receptor	0
D ₄ Dopamine Receptor (176-185)	0
D ₄ 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 using TBS/Tween-20 buffer as diluent.
2. Wash x 2 with TBS/Tween-20.
3. For blocked antibody controls dissolve 150 nmoles of peptide PS-3553B in 600 μ l of reconstituted antibody. Incubate one hour. 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 at least 1:8 (Note: higher dilutions may be needed). 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.

PS-3553B: D₅ Dopamine Receptor [Ac-23-35-Cys³⁶]

Amino Acid Sequence:

Ac-Gln-Gly-Asn-Ala-Val-Gly-Gly-Ser-Ala-Gly-Ala-Pro-Pro-Cys-COOH

Mol. Wt.: 1227.3

Peptide Purity: > 95%

Peptide Quantity: 150 nmoles

Lot # 9124

HPLC Analysis: See attached chart recording

Solvent System: A. 0.1% TFA in water
 B. 90% Acetonitrile + 10% water

Solvent Program:	<u>Time</u>	<u>Flow</u>	<u>% A</u>	<u>% B</u>
	0.0	1.2	100	0
	40.0		0	100
	41.0		0	100
	42.0		100	0
	45.0		100	0

Detection: Optical Density at 225nm

Results: Single peak at R_t = 11.839 min