

**Western Immunoblotting Reagents:
IgG Fraction of Rabbit Anti-D₁ Receptor**

WR-3512

Lot # 7967

The antiserum was raised in a rabbit which was immunized with D₁ (Ac-9-21) 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 the western blot detection of the D₁ 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 ₁ Dopamine Receptor (9-21)	100
D ₁ Dopamine Receptor	90
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
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 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-3511 in 600 µl 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 dilution to 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-3511: D₁ Dopamine Receptor (Ac-9-21)

Amino Acid Sequence:

Ac-Met-Asp-Gly-Thr-Gly-Leu-Val-Val-Glu-Arg-Asp-Phe-Ser-COOH

Mol. Wt.: 1467.6

Peptide Quantity: 150 nmoles

Peptide Purity: > 95% by HPLC

Lot Number: 9294

HPLC Analysis: See Attached Chart Recording

Solvent System: A. 0.1% TFA in H₂O
 B. 70% AcCN + 30% Water

Solvent Program:	<u>Time</u>	<u>Flow</u>	<u>%A</u>	<u>%B</u>
	0	1.0	100	0
	30	1.0	25	75
	31	1.0	25	75
	32	1.0	100	0
	35	1.0	100	0

Detection: optical density at 225 nm

Results: Major peak at R_t = 17.001 min