R&D® Ab

Research & Diagnostic Antibodies

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IgG Fraction of Rabbit Anti-D_4 Receptor Sera

AS-3545G Lot # 10750

The antiserum was raised in a rabbit which was immunized with D_4(Ac-176-185) 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 immunocytochemical detection of the D_4 dopamine receptor. Rehydrate the lyophilized IgG fraction with 0.1 ml of PBS which contains 10 mg/ml BSA or with additional buffer (such as 10 mg/ml BSA in PBS) for more dilute antibody. Each vial contains 2.78 mg of purified rabbit IgG.

Antiserum Specificity

Polypeptide	% Cross Reactivity
D_4 Dopamine Receptor(Ac-176-185)	100
D_4 Dopamine Receptor	60
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_5 Dopamine Receptor(23-35)	0
D_5 Dopamine Receptor	0

Immunocytochemical Staining

This IgG fraction has been found to stain specific cells in various regions of PLP fixed rat brain sections at a concentration of 5 μg/ml. This includes the glomeruli of the olfactory bulb, staining of cortex layer 4 and dendritic staining in cortex layers 2 and 3. In layer 5 of the cortex apical dendrites of many neurons were stained. In the hippocampus diffuse immunoreactivity was observed in the molecular layer of the dentate gyrus, with fine dots associated with many neurons in the granule cell layer. For additional information on PLP fixative see the FAQ page on our web site at www.RDAbs.com

Western Immunoblot

Western immunoblots using whole rat brain homogenate resulted in a single wide band being detected at ~48-53 kD at a concentration of 40μg/ml.

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such at 12% gels) and electrophoretic transfer to PVDF membrane, block the membrane overnight with 2% normal goat serum in TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the rabbit polyclonal antibody after diluting to 40 μg/ml. Use 2% normal goat serum in TBS/Tween-20 as buffer for the primary antibody. Let the primary antibody bind for 2-4 hours.
4. Wash x 3 with TBS/Tween-20.
5. Apply affinity purified HRP-goat anti-rabbit IgG antiserum diluted 1:2500 (dilution may vary depending upon supplier) in 2% normal goat serum in TBS/Tween-20. Incubate 1-2 hours. Note: greater sensitivity may be achieved using ABC techniques.
6. Wash x 4 for 5 minute/wash with TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.