

IgG Fraction of Rabbit Anti-D₅ Receptor Sera

AS-3552G

Lot # 9078

The antiserum was raised in a rabbit which was immunized with D₅(Ac-23-35-Cys³⁶) 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 and western blot detection of the D₅ 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 2.19 mg of purified rabbit IgG.

Antiserum Specificity

Polypeptide	% Cross Reactivity
D ₅ Dopamine Receptor(Ac-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

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 10 µg/ml. This includes the Medial septum, Nucleus accumbens, Dentate gyrus, Globus pallidus, Medial forebrain bundle, Cortex regions 1-3, Substantia Nigra reticulata, and the Ventral tegmental area. 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 band being detected at ~49 KDa at a concentration of 100 µg/ml.

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such as 12% gels) and electrophoretic transfer to PVDF membrane, block the membrane overnight with 4% normal goat serum in TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the IgG fraction after dilution to at least 100 µg/ml (Note: higher dilutions may be needed). 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.