# **Research & Diagnostic Antibodies**

2645 W. Cheyenne Ave, N. Las Vegas, NV 89032

Phone: 702-638-7800

# Rabbit Anti-D<sub>4</sub> Receptor Sera

AS-3545S

Lot # 10742

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. This antiserum has been found to be highly specific for this peptide sequence and is suitable for immunocytochemical detection of the  $D_4$  dopamine receptor. Rehydrate the lyophilized antibody 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.

#### **Antiserum Specificity**

Polypeptide	% Cross Reactivity
D <sub>4</sub> Dopamine Receptor(Ac-176-185)	100
D <sub>4</sub> Dopamine Receptor	60
$D_1$ Dopamine Receptor(9-21)	0
D <sub>1</sub> Dopamine Receptor	0
$D_2$ Dopamine Receptor(272-282)	0
D <sub>2</sub> Dopamine Receptor	0
D <sub>3</sub> Dopamine Receptor(2-10)	0
D <sub>3</sub> Dopamine Receptor	0
D <sub>5</sub> Dopamine Receptor(23-35)	0
D <sub>5</sub> Dopamine Receptor	0

#### **Immunocytochemical Staining**

This antiserum has been found to stain specific cells in various regions of PLP fixed rat brain sections at a dilution of 1:10,000. 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 1:500 dilution.

## Western Blotting Protocol

- 1. After SDS-PAGE on 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 rabbit polyclonal antibody after dilution to at least 1:500 (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.
- 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.