upstate | CHEMICON | Linco
THE EXPERTISE OF UPSTATE®, CHEMICON® AND LINCO® IS NOW A PART OF MILLIPORE

RAT ANTI-DOPAMINE TRANSPORTER MONOCLONAL ANTIBODY

CATALOG NUMBER: MAB369

LOT NUMBER:

QUANTITY: 100 µL

ALTERNATE NAMES: DAT

EPITOPE: N-terminus

CLONE NAME: DAT-Nt

HOST/ISOTYPE: Rat IgG_{2a} kappa

BACKGROUND: The transmembrane dopamine transporter (DAT) is located on the presynaptic nerve terminal and is responsible for terminating dopaminergic transmission by transporting dopamine from the synaptic cleft into the dopaminergic neuron (reuptake). Dopaminergic pathways have been strongly implicated in reward and addiction, motivation, alcoholism, ADHD and degenerative motor diseases such as Parkinson's, Huntington's and Chorea.

SPECIFICITY: Dopamine transporter, N-terminus. Shows no cross reactivity to the closely related serotonin and norepinephrine transporters (Miller, 1997). Immunolocalization of DAT on paraformaldehyde fixed frozen sections of human brain using MAB369 shows dense punctate staining throughout the caudate, putamen and accumbens (Miller, 1997).

IMMUNOGEN: N-terminus of human dopamine transporter fused to Glutathione S-transferase.

APPLICATIONS: Western blot: (not recommended for use on rat) Recognizes a diffuse band at approximately 70-85 kDa on western blots of extracts (20 µg total protein) from human caudate, putamen, and nucleus accumbens.
Immunohistochemistry: 4% paraformaldehyde fixed tissue (care should be taken not to over-fix tissue); perfusion followed by less than 90 minutes post-fixation, then cryoprotect. Suggested working dilution 1:1,000-1:10,000. If using on rat tissue, absorbed anti-rat secondary antibodies are recommended, and the use of rat PAP systems, or ABC detection will enhance sensitivity.
Immunocytochemistry: 1:5,000 to 1:10,000.

IMMUNOHISTOCHEMISTRY PROTOCOL FOR MAB369
This antibody has been used successfully on 30 µm, free floating, 4% paraformaldehyde fixed mouse brain tissue. All steps are performed under constant agitation. Suggested protocol follows.
1) 3 x 10 minute washes in TBS (with or without 0.25% Triton).
2) Incubate for 30 minutes in TBS with 3% serum (same as host from secondary antibody).
3) Incubate primary antibody diluted appropriately in TBS with 1% serum (same as host from secondary antibody) (with or without 0.25% Triton) for 2 hours at room temperature followed by 16 hours at 4°C.
4) 3 x 10 minute washes in TBS.
5) Incubate with secondary antibody diluted appropriately in TBS with 1% serum (same as host from secondary antibody).

6) 3 x 10 minute washes in TBS.
7) ABC Elite (1:200 Vector Labs) in TBS.
8) 2 x 10 minute washes in TBS. (continued)
9) 1 x 10 minute wash in phosphate buffer (no saline).
10) DAB reaction with 0.06% NiCl added for intensification.
11) 2 x 10 minute washes in PBS.
12) 1 x 10 minute wash in phosphate buffer (no saline).

Optimal working dilutions and protocols must be determined by end user.

SPECIES REACTIVITY: Reacts with Human, Monkey and Rodent. Reactivity with other species has not been determined.

CONTROL: POSITIVE CONTROL: Brain (caudate, putamen, and nucleus accumbens)

PRESENTATION: Tissue culture supernatant. Liquid. Contains no preservative.

STORAGE/HANDLING: Maintain at -20°C in undiluted aliquots for up to 6 months after date of receipt. Avoid repeated freeze/thaw cycles.

REFERENCES:

Roy, N., et al., *Nature Medicine* (2006) **12**:1259-1268.

Garzon, M. and V. Pickel, *J Comparative Neurology* (2005) **498**:821-839.

Jakowec, M.W., et al., *J of Neuroscience Research* (2004) **76**:539-550.

Fisher, B.E., et al., *J of Neuroscience Research* (2004) **77**:378-390.

Sonntag, K., et al., *European Journal of Neuroscience* (2004) **19**:1141-1152.

Battaglia, G. et al., *J. Neuroscience* (2004) **24**:828-835.

Decker, M. J. et al. (2003). Episodic neonatal hypoxia evokes executive dysfunction and regionally specific alterations in markers of dopamine signaling. J. Neurosci. 117:417-425.

Morón J. A., et al., (2003). Mitogen-ActivatedProtein Kinase Regulates Dopamine Transport Capacity. J. Neurosci. 23(24):8480-8488.

Loder, MK et al (2003). The Dopamine Transporter Constitutively Internalizes and Recycles in a Protein Kinase C-regulated Manner in Stably Transfected PC12 Cell Lines. J Biol Chem 278(24):22168-22174.

Storch, A et al. (2003). Functional characterization of dopaminergic neurons derived from rodent mesencephalic progenitor cells. J Chem Neuroanatomy 26:133-142.

Reveron ME et al (2002). L-Dopa does not cause neurotoxicity in VMAT2 heterozygote knockout mice. Neurotoxicology 23:611-619.

Tillerson, JL. et al. (2002). Forced Nonuse in Unilateral Parkinsonian Rats Exacerbates Injury. J Neurosci 22(15):6790-6799.

Lee, FJS et al. (2001). Direct binding and functional coupling of -synuclein to the dopamine transporters accelerate dopamine-induced apoptosis . FASEB J 15:916-926.

Mundorf, ML et al. (2001). Catecholamine release and uptake in the mouse prefrontal cortex. J Neurochem 79(1):130-142.

Fallon, J. et al. (2000). In vivo induction of massive proliferation, directed migration, and differentiation of neural cells in the adult mammalian brain. P.N.A.S. USA 97(26):14686-14691.

Padungchaichot, P et al. (2000). Early direct and transneuronal effects in mice with targeted expression of a toxin gene to D1 dopamine receptor neurons. Neurosci 95(4):1025-1033.

Khan, Z. U. et al. (1998). Prominence of the dopamine d2 short isoform in dopaminergic pathways. P.N.A.S. USA 95:7731-7736.

Betarbet, R. et al. (1997). Dopaminergic neurons intrinsic to the primate striatum. J. Neuroscience 17:6761-6768.

Miller et al. (1997). Immunochemical analysis of dopamine transporter protein in Parkinson's disease. Annals of Neurology 41:530-539.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

28820 Single Oak Drive • Temecula, CA 92590
Technical Support: T: 1-800-MILLIPORE (1-800-645-5476) • F: 1-800-437-7502
www.millipore.com