Product Datasheet

SLC6A3/DAT1 Antibody
NBP2-22164SS

Unit Size: 0.025 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

Publications: 11

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NBP2-22164SS

SLC6A3/DAT1 Antibody (mAb16)

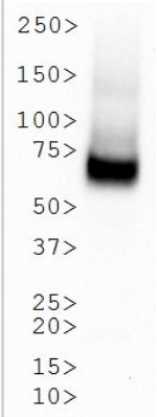
Product Information	
Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	mAb16
Preservative	0.05% Sodium Azide
Isotype	IgG1
Purity	Protein G purified
Buffer	Tris-glycine, 150 mM NaCl

Product Description	
Host	Mouse
Gene ID	6531
Gene Symbol	SLC6A3
Species	Mouse, Rat, Human (Negative)
Species Reactivity	Mouse and rat. Little to no human reactivity has been observed.
Immunogen	Synthetic peptide corresponding to the N-terminus of rat DAT1. [UniProt# P23977]

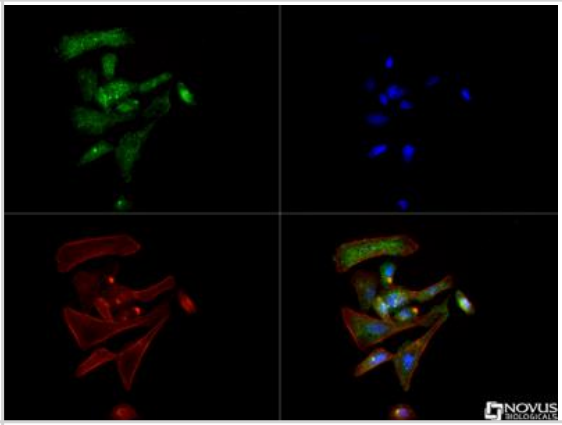
Product Application Details	
Applications	Western Blot, ELISA, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	ELISA, Immunocytochemistry/Immunofluorescence 1:250, Immunohistochemistry 1:100, Immunohistochemistry-Paraffin 1:100, Immunoprecipitation, Western Blot 1:1000
Application Notes	This DAT1 (mAb16) antibody is useful for Western blot, Immunocytochemistry/Immunofluorescence, Immunohistochemistry on paraffin-embedded sections and ELISA. In WB a band can been seen at ~70-85 kDa. Use in Immunoprecipitation reported in scientific literature (PMID: 20643191).

Images

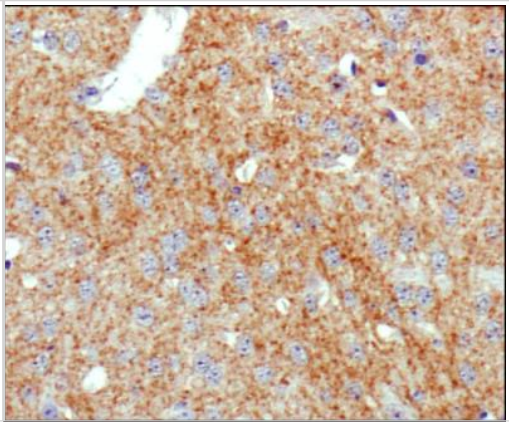
Western Blot: DAT1 Antibody (mAb16) [NBP2-22164] - Analysis of DAT1 expression in rat striatal membrane tissue lysate.



Immunocytochemistry/Immunofluorescence: DAT1 Antibody (mAb16) [NBP2-22164] - The DAT1 antibody was tested in PC12 cells at a 1:250 dilution against DyLight 488 (Green). Actin and nuclei were counterstained with Phalloidin-AlexaFluor 568 (Red) and DAPI (Blue), respectively.



Immunohistochemistry-Paraffin: DAT1 Antibody (mAb16) [NBP2-22164] - IHC analysis of DAT1 in mouse brain using DAB with hematoxylin counterstain.



Publications

Foster JD, Vaughan RA. Palmitoylation controls dopamine transporter kinetics, degradation, and protein kinase C-dependent regulation. J Biol Chem 2011 Feb 18 [PMID: 21118819] (WB, Rat)

Moritz AE, Foster JD, Gorentla BK et al. Phosphorylation of dopamine transporter serine 7 modulates cocaine analog binding. J Biol Chem 2013 Jan 4 [PMID: 23161550] (WB, Rat)

Foster JD, Yang JW, Moritz AE et al. Dopamine transporter phosphorylation site threonine 53 regulates substrate reuptake and amphetamine-stimulated efflux. J Biol Chem 2012 Aug 24 [PMID: 22722938] (WB, Rat, Mouse)

Chen R, Zhang M, Park S, Gnegy ME. C57BL/6J mice show greater amphetamine-induced locomotor activation and dopamine efflux in the striatum than 129S2/SvHsd mice. Pharmacol Biochem Behav 2007 May [PMID: 17524461] (WB, Mouse)

Gorentla BK, Moritz AE, Foster JD, Vaughan RA. Proline-directed phosphorylation of the dopamine transporter N-terminal domain. Biochemistry 2009 Feb 10 [PMID: 19146407] (WB, Rat)

Perry ML, Leinninger GM, Chen R et al. Leptin promotes dopamine transporter and tyrosine hydroxylase activity in the nucleus accumbens of Sprague-Dawley rats. J Neurochem 2010 Aug [PMID: 20412389] (WB, Rat)

Cervinski MA, Foster JD, Vaughan RA. Syntaxin 1A regulates dopamine transporter activity, phosphorylation and surface expression. Neuroscience 2010 Oct 13 [PMID: 20643191] (WB, IP, Rat)

Furman CA, Chen R, Guptaroy B et al. Dopamine and amphetamine rapidly increase dopamine transporter trafficking to the surface: live-cell imaging using total internal reflection fluorescence microscopy. J Neurosci 2009 Mar 11 [PMID: 19279270] (WB, Rat)

Johnson LA, Guptaroy B, Lund D et al. Regulation of amphetamine-stimulated dopamine efflux by protein kinase C beta. J Biol Chem 2005 Mar 25 [PMID: 15647254] (WB, Rat)

Johnson LA, Furman CA, Zhang M et al. Rapid delivery of the dopamine transporter to the plasmalemmal membrane upon amphetamine stimulation. Neuropharmacology 2005 Nov [PMID: 16212991] (WB, Rat)

Gaffaney JD, Vaughan RA. Uptake inhibitors but not substrates induce protease resistance in extracellular loop two of the dopamine transporter. Mol Pharmacol 2004 Mar [PMID: 14978248] (WB, Rat)

Procedures

IHC-P Protocol Specific for NBP2-22164: DAT1 Antibody (mAb16)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

WB Protocol Specific for NBP2-22164: DAT1 Antibody (mAb16)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.
Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

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ICC/IF Protocol Specific for NBP2-22164: DAT1 Antibody (mAb16)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

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Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

For more information on our guarantee, please visit www.novusbio.com/guarantee.