## abcam

### **Product datasheet**

# Anti-Dopamine Transporter antibody [EPR19695] - BSA and Azide free ab221845

אילשעבע RabMAb

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<u>1 References</u> 画像数 13

製品の概要	
製品名	Anti-Dopamine Transporter antibody [EPR19695] - BSA and Azide free
製品の詳細	Rabbit monoclonal [EPR19695] to Dopamine Transporter - BSA and Azide free
由来種	Rabbit
特異性	Human species is recommended based on IHC-P results, we do not guarantee IHC-Fr, WB and IP for human.
アプリケーション	適用あり: IHC-P, IP, WB, IHC-Fr
種交差性	交差種: Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: Unboiled Mouse and rat striatum lysates. IHC-P: Mouse and rat striatum tissues, normal human striatum, normal human substantia nigra. IHC-Fr: Mouse brain (Coronal section), rat brain (sagittal section). IP: Mouse striatum whole cell lysate.
特記事項	ab221845 is the carrier-free version of <b>ab184451</b> .
	Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.
	This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.
	Use our <b><u>conjugation kits</u></b> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.
	This product is compatible with the Maxpar <sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar <sup>®</sup> is a trademark of Fluidigm Canada Inc.
	This product is a recombinant monoclonal antibody, which offers several advantages including:
	- High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production

#### For more information see here.

Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.

#### 製品の特性

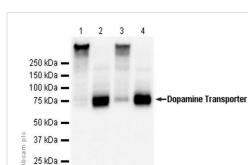
製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C. Do Not Freeze.
パッファー	pH: 7.2 Constituent: PBS
キャリア・フリー	はい
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR19695
アイソタイプ	lgG

アプリケーション

The Abpromise guaranteeAbpromise保証は、次のテスト済みアプリケーションにおけるab221845の使用に適用されますアプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
ІНС-Р		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 70-85 kDa (predicted molecular weight: 69 kDa).
IHC-Fr		Use at an assay dependent concentration.

ターゲット情報	
機能	Amine transporter. Terminates the action of dopamine by its high affinity sodium-dependent reuptake into presynaptic terminals.
関連疾患	Defects in SLC6A3 are the cause of dystonia-parkinsonism infantile (DYTPRI) [MIM:613135]. It is a neurodegenerative disorder characterized by infantile onset of parkinsonism and dystonia. Other neurologic features include global developmental delay, bradikinesia and pyramidal tract signs.
配列類似性	Belongs to the sodium:neurotransmitter symporter (SNF) (TC 2.A.22) family. SLC6A3 subfamily.
細胞内局在	Membrane.



Western blot - Anti-Dopamine Transporter antibody

[EPR19695] - BSA and Azide free (ab221845)

All lanes : Anti-Dopamine Transporter antibody [EPR19695] (ab184451) at 1/1000 dilution

Lane 1 : Mouse striatum lysate boiledLane 2 : Mouse striatum lysate unboiledLane 3 : Rat striatum lysate boiled

Lane 4 : Rat striatum lysate unboiled

Lysates/proteins at 20 µg per lane.

#### Secondary

-GAPDH (ab181602)

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

#### Predicted band size: 69 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab184451**).

We recommend not to boil the samples after lysis to get desired WB results.

ab181602 was used as a GAPDH loading control.

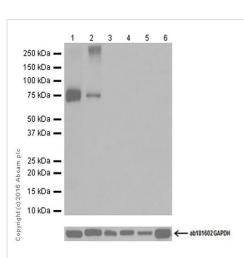
All lanes : Anti-Dopamine Transporter antibody [EPR19695] (ab184451) at 1/1000 dilution

Lane 1 : Mouse striatum lysate
Lane 2 : Rat striatum lysate
Lane 3 : Mouse kidney lysate
Lane 4 : Rat liver lysate
Lane 5 : Neuro-2a (Mouse neuroblastoma cell line) whole cell lysate

Lane 6: C6 (Rat glial tumor cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary



Western blot - Anti-Dopamine Transporter antibody [EPR19695] - BSA and Azide free (ab221845)

20 kDa — 15 kDa — 10 kDa — All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 69 kDa Observed band size: 70-85 kDa

Exposure time: 3 minutes

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab184451**).

Blocking/Dilution buffer: 5% NFDM/TBST.

ab181602 was used as GAPDH loading control.

Dopamine transporters are distributed widely across the brain in regions with high dopaminergic activity, such as the striatum and substantia nigra (PMID:1765147), so non-related tissues and cells were chosen as negative controls; and DAT is a glycoprotein with molecular weight of 70-85KD (PMID:25356398, PMID: 20643191).

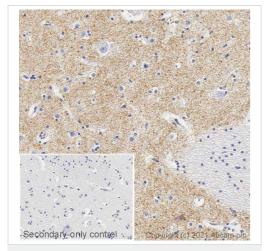
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab184451**).

IHC image of Dopamine Transporter staining in a section of formalin fixed paraffin embedded normal human striatum\* performed on a Leica BOND<sup>™</sup>. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with **ab184451**, 5ugml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen.

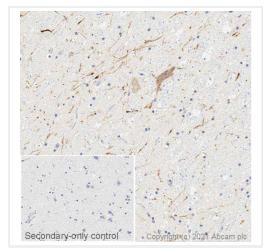
The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

\*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Dopamine Transporter antibody [EPR19695] - BSA and Azide free (ab221845)



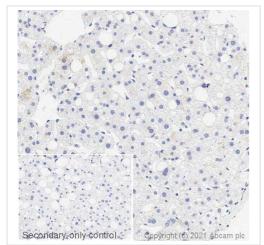
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Dopamine Transporter antibody [EPR19695] - BSA and Azide free (ab221845)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab184451</u>).

IHC image of Dopamine Transporter staining in a section of formalin fixed paraffin embedded normal human substantia nigra\* performed on a Leica BOND<sup>™</sup>. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was incubated with **ab184451**, 5ugml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

\*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

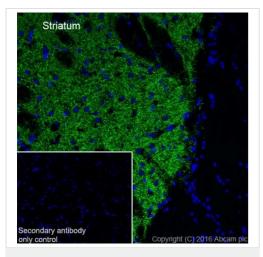


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Dopamine Transporter antibody [EPR19695] - BSA and Azide free (ab221845) This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab184451</u>).

Negative control image: IHC image of Dopamine Transporter staining in a section of formalin fixed paraffin embedded normal human liver\* performed on a Leica BOND<sup>™</sup>. The section was pretreated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins..The section was incubated with <u>ab184451</u>, 5ugml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

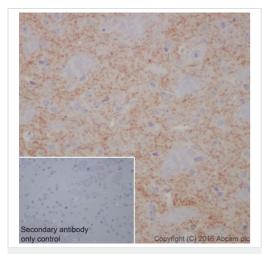
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

\*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Immunohistochemistry (Frozen sections) - Anti-Dopamine Transporter antibody [EPR19695] - BSA and Azide free (ab221845)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen Rat brain (sagittal section) tissue labeling Dopamine Transporter with <u>ab184451</u> at 1/500 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor<sup>®</sup> 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution (green). The result showed cytoplasmic staining on rat striatum. The nuclear counterstain is DAPI (blue). Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <u>ab150077</u> at 1/1000 dilution. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and



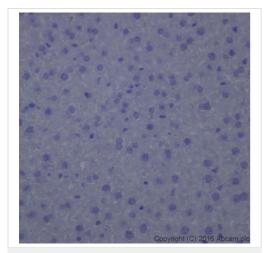
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Dopamine Transporter antibody [EPR19695] - BSA and Azide free (ab221845) Immunohistochemical analysis of paraffin-embedded Mouse striatum tissue labeling Dopamine Transporter with <u>ab184451</u> at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

sodium azide (ab184451).

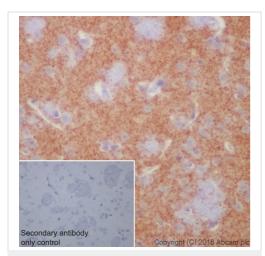
Membrane and cytoplasm staining on mouse striatum is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <u>ab97051</u> at 1/500 dilution. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab184451</u>).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Dopamine Transporter antibody [EPR19695] - BSA and Azide free (ab221845)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Dopamine Transporter antibody [EPR19695] - BSA and Azide free (ab221845) Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling Dopamine Transporter with <u>ab184451</u> at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

Counter stained with Hematoxylin.

Negative control: Negative staining on mouse liver.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <u>ab97051</u> at 1/500 dilution. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab184451</u>).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Immunohistochemical analysis of paraffin-embedded Rat striatum tissue labeling Dopamine Transporter with <u>ab184451</u> at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

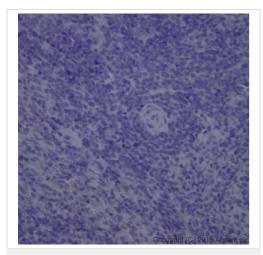
Cytoplasm staining on rat striatum is observed.

Counter stained with Hematoxylin.

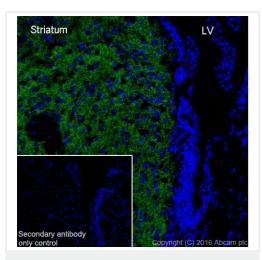
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab184451**).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Dopamine Transporter antibody [EPR19695] - BSA and Azide free (ab221845)



Immunohistochemistry (Frozen sections) - Anti-Dopamine Transporter antibody [EPR19695] - BSA and Azide free (ab221845) Immunohistochemical analysis of paraffin-embedded Rat spleen tissue labeling Dopamine Transporter with <u>ab184451</u> at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

Counter stained with Hematoxylin.

Negative control: Negative staining on rat spleen.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <u>ab97051</u> at 1/500 dilution. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab184451</u>).

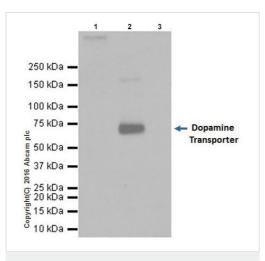
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen Mouse brain (Coronal section) tissue labeling Dopamine Transporter with <u>ab184451</u> at 1/500 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor<sup>®</sup> 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution (green). The result showed cytoplasmic staining on mouse striatum but negative on lateral ventricle (LA).

The nuclear counterstain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab150077** at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab184451</u>).



Immunoprecipitation - Anti-Dopamine Transporter antibody [EPR19695] - BSA and Azide free (ab221845) Dopamine Transporter was immunoprecipitated from 0.35mg of Mouse striatum whole cell lysate with **ab184451** at 1/30 dilution.

Western blot was performed from the immunoprecipitate using <u>ab184451</u> at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

Lane 1: Mouse striatum whole cell lysate, 10µg (Input).

Lane 2: ab184451 IP in Mouse striatum whole cell lysate.

Lane 3: Rabbit IgG, monoclonal [EPR25A] - Isotype Control (<u>ab172730</u>) instead of <u>ab184451</u> in Mouse striatum whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 30 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab184451**).



Anti-Dopamine Transporter antibody [EPR19695] -BSA and Azide free (ab221845)

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