

# **Product datasheet**

# Anti-GABA Transporter 3 / GAT 3 antibody [EPR25153-38] (BSA and Azide free) ab300560

Recombinant RabMAb

12 Images

Overview	
Product name	Anti-GABA Transporter 3 / GAT 3 antibody [EPR25153-38] (BSA and Azide free)
Description	Rabbit monoclonal [EPR25153-38] to GABA Transporter 3 / GAT 3 - BSA and Azide free
Host species	Rabbit
Tested applications	Suitable for: IHC-Fr, WB, IHC-P, IP
Species reactivity	Reacts with: Mouse, Rat Does not react with: Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Tissue lysates: Mouse and rat brain, rat thalamus, rat spinal cord. IHC-P: Mouse and rat brain tissue lysates. IHC-Fr.: Mouse and rat thalamus. IP: Mouse and rat thalamus.
General notes	ab300560 is the carrier-free version of <b>ab300559</b> .
	ab300559 does not react in WB and IHC-P with human tissues.
	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>conjugation kits</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
	<ul> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> </ul>
	- Animal-free production
	For more information <u>see here</u> .

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>.

## **Properties**

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C.
Storage buffer	pH: 7.20 Constituent: 100% PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR25153-38
lsotype	lgG

### Applications

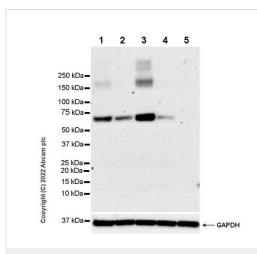
The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab300560 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-Fr		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration.
IHC-P		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.

Target	
Function	Terminates the action of GABA by its high affinity sodium-dependent reuptake into presynaptic terminals.
Tissue specificity	Widespread distribution in the brain.
Sequence similarities	Belongs to the sodium:neurotransmitter symporter (SNF) (TC 2.A.22) family. SLC6A11 subfamily.
Cellular localization	Membrane.

#### Images



Western blot - Anti-GABA Transporter 3 / GAT 3 antibody [EPR25153-38] (BSA and Azide free) (AB300560) All lanes : Anti-GABA Transporter 3 / GAT 3 antibody [EPR25153-38] (<u>ab300559</u>) at 1/1000 dilution

Lane 1 : Mouse brain tissue lysate

- Lane 2 : Rat brain tissue lysate
- Lane 3 : Rat thalamus tissue lysate
- Lane 4 : Rat spinal cord tissue lysate
- Lane 5 : Rat liver tissue lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

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

Performed under non-reducing conditions.

Observed band size: 280,70, 160 kDa

Exposure time: 3 minutes

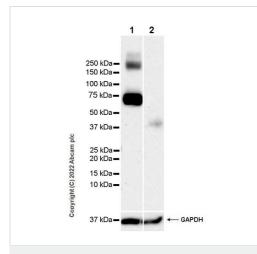
This data was developed using 300559, the same antibody clone in a different buffer formulation.

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

Samples are non-boiled as boiling may cause protein aggregates.

Negative control: Rat liver (PMID: 7854065).

Exposure time: 3 minutes.



Western blot - Anti-GABA Transporter 3 / GAT 3 antibody [EPR25153-38] (BSA and Azide free) (AB300560) All lanes : Anti-GABA Transporter 3 / GAT 3 antibody [EPR25153-38] (ab300559) at 1/1000 dilution

Lane 1 : Mouse thalamus tissue lysate at 20 µg Lane 2 : Mouse liver tissue lysate at 40 µg

#### Secondary

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

Performed under non-reducing conditions.

Observed band size: 70, 160 kDa

Exposure time: 3 minutes

This data was developed using 300559, the same antibody clone in a different buffer formulation.

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

This blot was developed using a high sensitivity ECL substrate.

Samples are non-boiled as boiling may cause protein aggregates.

Negative control: Mouse liver (PMID: 7854065).

70- kDa GAT-3 is observed. The molecular weight is consistent with what has been described in the literature (PMID: 29742425).

Bands around 160 kDa and 280 kDa may be GAT-3 dimer and tetramer.

Exposure time: 3 minutes.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GABA Transporter 3 / GAT 3 antibody [EPR25153-38] (BSA and Azide free) (AB300560)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GABA Transporter 3 / GAT 3 antibody [EPR25153-38] (BSA and Azide free) (AB300560) This data was developed using <u>ab300559</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded mouse brain tissue labeling GABA Transporter 3 / GAT 3 with **ab300559** at 1/2000 dilution (0.254 µg/mL) followed by a ready to use LeicaDS9800 (Bond<sup>™</sup> Polymer Refine Detection kit). Positive staining on mouse brain is observed. The section was incubated with **ab300559** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: PBS was used instead of primary antibody, followed by a ready to use secondary antibody LeicaDS9800 (Bond™ Polymer Refine Detection kit).

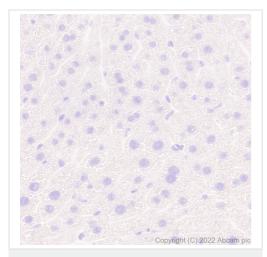
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins was used.

This data was developed using <u>ab300559</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded rat brain tissue labeling GABA Transporter 3 / GAT 3 with **ab300559** at 1/2000 dilution (0.254 µg/mL) followed by a ready to use LeicaDS9800 (Bond<sup>™</sup> Polymer Refine Detection kit). Positive staining on rat brain is observed (PMID: 26390912, 7854065). The section was incubated with **ab300559** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: PBS was used instead of primary antibody, followed by a ready to use secondary antibody LeicaDS9800 (Bond™ Polymer Refine Detection kit).

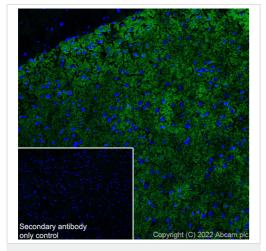
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins was used.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GABA Transporter 3 / GAT 3 antibody [EPR25153-38] (BSA and Azide free) (AB300560) This data was developed using <u>ab300559</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded mouse liver tissue labeling GABA Transporter 3 / GAT 3 with <u>ab300559</u> at 1/2000 dilution (0.254 µg/mL) followed by a ready to use LeicaDS9800 (Bond<sup>™</sup> Polymer Refine Detection kit). Negative control: no staining on mouse liver is observed. The section was incubated with <u>ab300559</u> for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>™</sup> RX instrument. Counterstained with Hematoxylin.

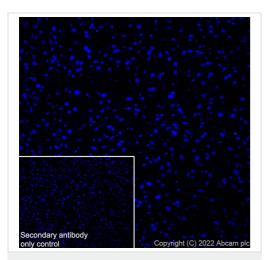
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins was used.



Immunohistochemistry (Frozen sections) - Anti-GABA Transporter 3 / GAT 3 antibody [EPR25153-38] (BSA and Azide free) (AB300560) This data was developed using <u>ab300559</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen mouse thalamus (fresh) tissue labeling GABA Transporter 3 / GAT 3 with <u>ab300559</u> at 1/50 dilution (10.14 µg/ml) followed by <u>ab150081</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed at 1/1000 dilution (2 µg/ml) (Green). Positive staining on mouse thalamus is observed. The nuclear counterstain was DAPI (Blue).

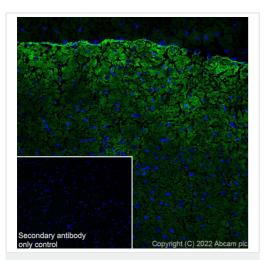
Secondary antibody control: PBS was used instead of primary antibody, followed by preadsorbed secondary antibody <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) 1/1000 dilution (2  $\mu$ g/ml).



Immunohistochemistry (Frozen sections) - Anti-GABA Transporter 3 / GAT 3 antibody [EPR25153-38] (BSA and Azide free) (AB300560) This data was developed using <u>ab300559</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen mouse liver (fresh) tissue labeling GABA Transporter 3 / GAT 3 with **ab300559** at 1/50 dilutio (10.14  $\mu$ g/ml) followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed at 1/1000 dilution (2  $\mu$ g/ml) (Green). Negative control: no staining on mouse liver is observed (PMID: 22896705). The nuclear counterstain was DAPI (Blue).

Secondary antibody control: Secondary antibody is <u>**ab150081**</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup>488) preadsorbed at 1/1000 dilution (2  $\mu$ g/ml).

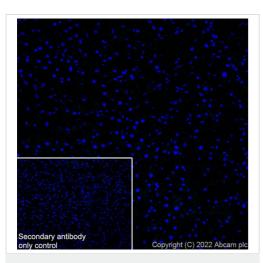


Immunohistochemistry (Frozen sections) - Anti-GABA Transporter 3 / GAT 3 antibody [EPR25153-38] (BSA and Azide free) (AB300560)

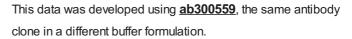
This data was developed using <u>ab300559</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen rat thalamus (fresh) tissue labeling GABA Transporter 3 / GAT 3 with **ab300559** at 1/50 dilution (10.14 µg/ml) followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed at 1/1000 dilution (2 µg/ml) (Green). Positive staining on rat thalamus is observed. The nuclear counterstain was DAPI (Blue).

Secondary antibody control: PBS was used instead of primary antibody, followed by a preadsorbed secondary antibody <u>ab150081</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) at 1/1000 dilution (2 µg/ml).

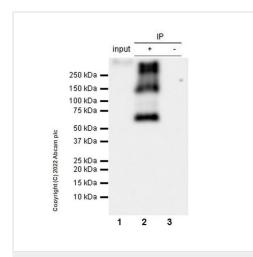


Immunohistochemistry (Frozen sections) - Anti-GABA Transporter 3 / GAT 3 antibody [EPR25153-38] (BSA and Azide free) (AB300560)



Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen rat liver (fresh) tissue labeling GABA Transporter 3 / GAT 3 with **ab300559** at 1/50 dilution (10.14 µg/ml), followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed at 1/1000 dilution (2 µg/ml) (Green). Negative control: no staining on rat liver is observed (PMID: 22896705). The nuclear counterstain was DAPI (Blue).

Secondary antibody control: PBS was used instead of primary antibody, followed by a preadsorbed secondary antibody <u>ab150081</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) at 1/1000 dilution (2 µg/ml).



Immunoprecipitation - Anti-GABA Transporter 3 / GAT 3 antibody [EPR25153-38] (BSA and Azide free) (AB300560) This data was developed using <u>ab300559</u>, the same antibody clone in a different buffer formulation.

GABA Transporter 3 / GAT 3 was immunoprecipitated from 0.35 mg mouse thalamus tissue lysate with **ab300559** at 1/30 dilution (2 µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using **ab300559** at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: Mouse thalamus tissue lysate 10  $\mu g$ 

Lane 2: ab300559 IP in mouse thalamus tissue lysate

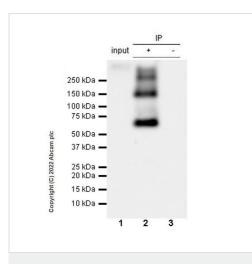
Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab300559</u> in mouse thalamus tissue lysate

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

Exposure time: 3.25 seconds

70- kDa GAT-3 band is observed. The molecular weight is consistent with what has been described in the literature (PMID: 29742425). Bands around 160 kDa and 280 kDa may be GAT-3 dimer and tetramer.

Observed MW (kDa): 70, 160, 280.



Immunoprecipitation - Anti-GABA Transporter 3 / GAT 3 antibody [EPR25153-38] (BSA and Azide free) (AB300560) This data was developed using <u>ab300559</u>, the same antibody clone in a different buffer formulation.

GABA Transporter 3 / GAT 3 was immunoprecipitated from 0.35 mg rat thalamus tissue lysate with <u>ab300559</u> at 1/30 dilution (2 μg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using <u>ab300559</u> at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(<u>ab131366</u>) was used at 1/5000 dilution.

Lane 1: Rat thalamus tissue lysate 10 µg

Lane 2: ab300559 IP in Rat thalamus tissue lysate

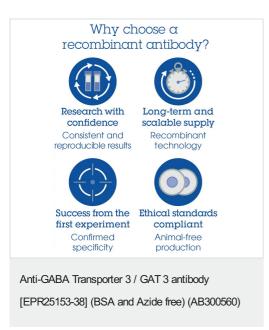
Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab300559</u> in rat thalamus tissue lysate

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

Exposure time: 3.25 seconds

70- kDa GAT-3 band is observed. The molecular weight is consistent with what has been described in the literature (PMID: 29742425). Bands around 160 kDa and 280 kDa may be GAT-3 dimer and tetramer.

Observed MW (kDa): 70, 160, 280.



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <u>https://www.abcam.com/abpromise</u> or contact our technical team.

## **Terms and conditions**

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors