# abcam

## Product datasheet

## Anti-NPTX2 antibody [EPR24020-38] - BSA and Azide free ab277533



#### 15 Images

#### Overview

**Product name** Anti-NPTX2 antibody [EPR24020-38] - BSA and Azide free

**Description** Rabbit monoclonal [EPR24020-38] to NPTX2 - BSA and Azide free

**Host species** Rabbit

**Tested applications** Suitable for: ICC/IF, IHC-P, WB, IHC-Fr, IP, Flow Cyt (Intra)

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human brain, pancreas and testis tissue lysates; SH-SY5Y whole cell lysate; Mouse brain,

> kidney and testis tissue lysates; Rat brain and testis tissue lysates. IHC-P: Human pancreas and cerebrum tissue; Mouse cerebrum tissue; Rat cerebrum tissue. ICC/IF: Beta-TC-6 cells. Flow Cyt (intra): Beta-TC-6 cells. IP: Human brain tissue lysate; Mouse brain tissue lysate. IHC-Fr: Frozen

human cerebral cortex, pancreas, and heart tissue sections.

**General notes** ab277533 is the carrier-free version of ab277523.

> Our carrier-free 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 cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits 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® 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 **RabMAb**<sup>®</sup> **patents**.

## **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C.

Storage buffer Constituent: 100% PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR24020-38

**Isotype** IgG

## **Applications**

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab277533 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
ICC/IF		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.
WB		Use at an assay dependent concentration. Detects a band of approximately 50-60 kDa (predicted molecular weight: 47 kDa).
IHC-Fr		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.

## **Target**

**Function** Likely to play role in the modification of cellular properties that underlie long-term plasticity. Binds

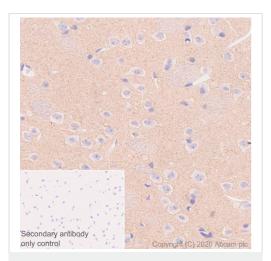
to agar matrix in a calcium-dependent manner.

**Tissue specificity** Brain, pancreas, liver, heart and skeletal muscle. Highest levels are seen in the testis.

Sequence similarities Contains 1 pentaxin domain.

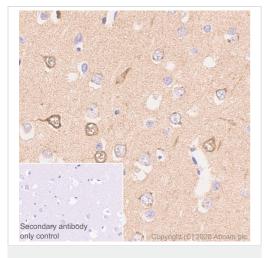
Cellular localization Secreted.

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Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NPTX2 antibody

[EPR24020-38] - BSA and Azide free (ab277533)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NPTX2 antibody
[EPR24020-38] - BSA and Azide free (ab277533)

This data was developed using <u>ab277523</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Mouse cerebrum tissue labeling NPTX2 with <a href="mailto:ab277523">ab277523</a> at 1/2000 (0.229 ug/ml) followed by a ready to use LeicaDS9800 (Bond® Polymer Refine Detection). Positive staining on mouse cerebrum (PMID:31772567). The section was incubated with <a href="mailto:ab277523">ab277523</a> for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond<sup>®</sup> Polymer Refine Detection) was used.

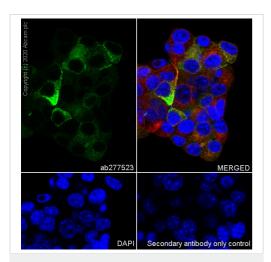
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

This data was developed using <u>ab277523</u>, the same antibody clone in a different buffer formulation.

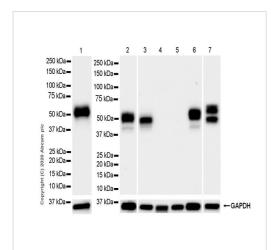
Immunohistochemical analysis of paraffin-embedded Human cerebrum tissue labeling NPTX2 with <a href="mailto:ab277523">ab277523</a> at 1/2000 (0.229 ug/ml) followed by a ready to use LeicaDS9800 (Bond® Polymer Refine Detection) was used. Positive staining on human cerebrum. The section was incubated with <a href="mailto:ab277523">ab277523</a> for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond<sup>®</sup> Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-NPTX2 antibody [EPR24020-38] - BSA and Azide free (ab277533)



Western blot - Anti-NPTX2 antibody [EPR24020-38]

- BSA and Azide free (ab277533)

This data was developed using <u>ab277523</u>, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 100% Methanol permeabilized Beta-TC-6 cells labelling NPTX2 with <a href="mailto:ab277523">ab277523</a> at 1/50 (9.16 ug/ml) dilution, followed by <a href="mailto:ab150077">ab150077</a> Goat Anti-Rabbit lgG H&L (Alexa Fluor®488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in Beta-TC-6 cells. <a href="mailto:ab195889">ab195889</a> Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) at 1/1000 dilution.

**All lanes :** Anti-NPTX2 antibody [EPR24020-38] (<u>ab277523</u>) at 1/1000 dilution

**Lane 1 :** Beta-TC-6 (mouse pancreas insulinoma beta cell) whole cell lysate

Lane 2 : Mouse brain tissue lysate

Lane 3: Mouse testis tissue lysate

Lane 4: Mouse kidney tissue lysate

Lane 5: Mouse lung tissue lysate

Lane 6: Rat brain tissue lysate

Lane 7: Rat testis tissue lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (ab97051) at 1/50000 dilution

Predicted band size: 47 kDa

Observed band size: 50-60 kDa

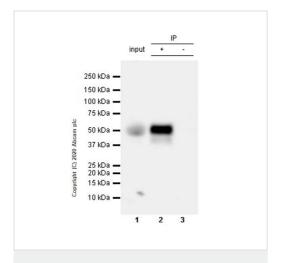
This data was developed using <u>ab277523</u>, the same antibody clone in a different buffer formulation.

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

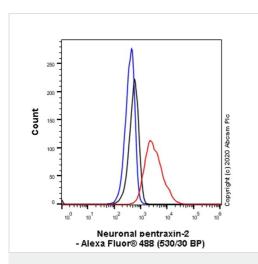
NPTX2 is a glycoprotein. The molecular weight observed is consistent with what has been described in the literature (PMID:

8786423, 28440221).

Exposure time: 15 seconds.



Immunoprecipitation - Anti-NPTX2 antibody
[EPR24020-38] - BSA and Azide free (ab277533)



Flow Cytometry (Intracellular) - Anti-NPTX2 antibody [EPR24020-38] - BSA and Azide free (ab277533)

This data was developed using <u>ab277523</u>, the same antibody clone in a different buffer formulation.

NPTX2 was immunoprecipitated from 0.35 mg Mouse brain tissue lysate with <u>ab277523</u> at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using <u>ab277523</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(<u>ab131366</u>) was used at 1/5000 dilution.

Lane 1: Mouse brain tissue lysate 10 ug

Lane 2: ab277523 IP in Mouse brain tissue lysate

**Lane 3:** Rabbit monoclonal  $\lg G$  (<u>ab172730</u>) instead of <u>ab277523</u> in mouse brain tissue lysate

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

Exposure time: 10 seconds.

This data was developed using <u>ab277523</u>, the same antibody clone in a different buffer formulation.

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized Beta-TC-6 (Mouse pancreas insulinoma beta cell) cells labelling NPTX2 with <a href="mailto:ab277523">ab277523</a> at 1/50 dilution (1ug) (Red) compared with a Rabbit monoclonal lgG (<a href="mailto:ab172730">ab172730</a>) isotype control (Black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

A Goat anti rabbit lgG (Alexa Fluor  $^{\otimes}$  488, <u>ab150077</u>) at 1/2000 dilution was used as the secondary antibody.

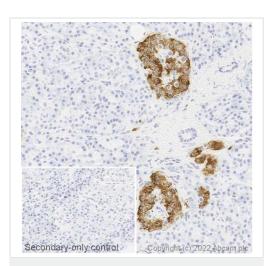


Immunohistochemistry (Frozen sections) - Anti-NPTX2 antibody [EPR24020-38] - BSA and Azide free (ab277533)

IHC image of NPTX2 staining in a section of frozen normal human cerebral cortex performed on a Leica Biosystems BOND® RX instrument. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with <a href="mailto:ab277523">ab277523</a>, 0.5 ug/ml, 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.

This data was developed using <u>ab277523</u>, the same antibody clone in a different buffer formulation.

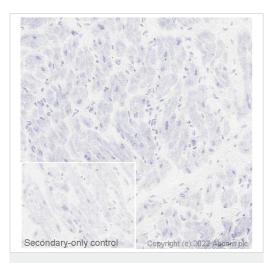


Immunohistochemistry (Frozen sections) - Anti-NPTX2 antibody [EPR24020-38] - BSA and Azide free (ab277533)

IHC image of NPTX2 staining in a section of frozen normal human pancreas performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with <u>ab277523</u>, 0.5 ug/ml, 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.

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This data was developed using <u>ab277523</u>, the same antibody clone in a different buffer formulation.



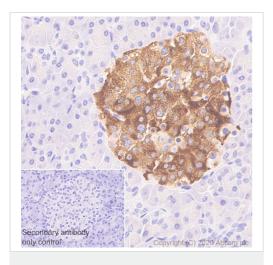
Immunohistochemistry (Frozen sections) - Anti-NPTX2 antibody [EPR24020-38] - BSA and Azide free (ab277533)

Negative tissue image: IHC image of NPTX2 staining in a section of frozen normal human heart performed on a Leica Biosystems BOND® RX instrument. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with <a href="mailto:ab277523">ab277523</a>, 0.5 ug/ml, 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.

This data was developed using <u>ab277523</u>, the same antibody clone in a different buffer formulation.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NPTX2 antibody

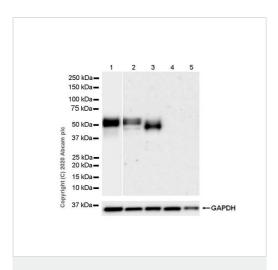
[EPR24020-38] - BSA and Azide free (ab277533)

This data was developed using <u>ab277523</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human pancreas tissue labeling NPTX2 with <u>ab277523</u> at 1/2000 (0.229 ug/ml) followed by a ready to use LeicaDS9800 (Bond<sup>®</sup> Polymer Refine Detection). Positive staining on islet of human pancreas. The section was incubated with <u>ab277523</u> 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: Secondary antibody is a ready to use LeicaDS9800 (Bond<sup>®</sup> Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Western blot - Anti-NPTX2 antibody [EPR24020-38] - BSA and Azide free (ab277533)

**All lanes :** Anti-NPTX2 antibody [EPR24020-38] (**ab277523**) at 1/1000 dilution

Lane 1: Human brain tissue lysate

Lane 2: Human pancreas tissue lysate

Lane 3: Human testis tissue lysate

Lane 4: Human kidney tissue lysate

Lane 5: Human lung tissue lysate

Lysates/proteins at 20 µg per lane.

#### **Secondary**

**All lanes :** VeriBlot for IP secondary antibody(HRP)(<u>ab131366</u>) at 1/1000 dilution

Predicted band size: 47 kDa

Observed band size: 50-60 kDa

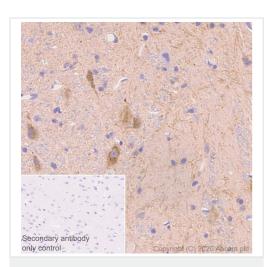
This data was developed using <u>ab277523</u>, the same antibody clone in a different buffer formulation.

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

NPTX2 is a glycoprotein. The molecular weight observed is consistent with what has been described in the literature (PMID: 8786423, 28440221).

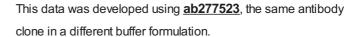
Negative: human kidney, human lung (PMID: 8530029, 8786423).

Exposure times: Lane 1: 15 secondsLane 2-5: 3 minutes.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NPTX2 antibody

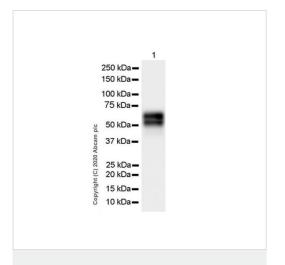
[EPR24020-38] - BSA and Azide free (ab277533)



Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labeling NPTX2 with <u>ab277523</u> at 1/2000 (0.229 ug/ml) followed by a ready to use LeicaDS9800 (Bond<sup>®</sup> Polymer Refine Detection). Positive staining on rat cerebrum. The section was incubated with <u>ab277523</u> 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: Secondary antibody is a ready to use LeicaDS9800 (Bond<sup>®</sup> Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Western blot - Anti-NPTX2 antibody [EPR24020-38]
- BSA and Azide free (ab277533)

Anti-NPTX2 antibody [EPR24020-38] (ab277523) at 1/1000 dilution + SH-SY5Y (human neuroblastoma epithelial cell) whole cell lysate at 20 µg

#### **Secondary**

Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (<u>ab97051</u>) at 1/50000 dilution

Predicted band size: 47 kDa

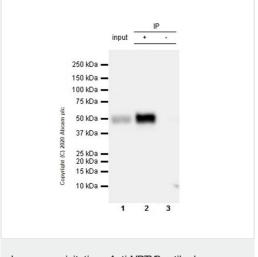
Observed band size: 50-60 kDa

This data was developed using <u>ab277523</u>, the same antibody clone in a different buffer formulation.

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

NPTX2 is a glycoprotein. The molecular weight observed is consistent with what has been described in the literature (PMID: 8786423, 28440221).

Exposure time: 15 seconds.



Immunoprecipitation - Anti-NPTX2 antibody
[EPR24020-38] - BSA and Azide free (ab277533)

This data was developed using <u>ab277523</u>, the same antibody clone in a different buffer formulation.

NPTX2 was immunoprecipitated from 0.35 mg Human brain tissue lysate with <u>ab277523</u> at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using <u>ab277523</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(<u>ab131366</u>) was used at 1/5000 dilution.

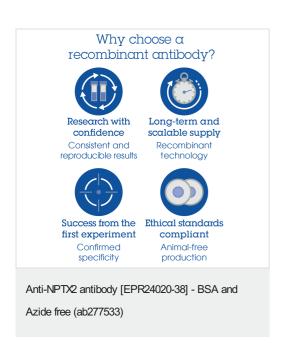
Lane 1: Human brain tissue lysate 10 ug

Lane 2: ab277523 IP in Human brain tissue lysate

**Lane 3:** Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab277523</u> in human brain tissue lysate

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

Exposure time: 10 seconds.



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

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