# abcam

### Product datasheet

## Anti-Synaptophysin antibody [YE269] - BSA and Azide free ab187259



יובענין RabMAb

11 References 画像数 15

#### 製品の概要

製品名 Anti-Synaptophysin antibody [YE269] - BSA and Azide free

製品の詳細 Rabbit monoclonal [YE269] to Synaptophysin - BSA and Azide free

由来種 Rabbit

アプリケーション 適用あり: ICC/IF, WB, IHC-P 種交差性 交差種: Mouse. Rat. Human

交差が予測される動物種: Donkey, Cow 🔷

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

WB: PC-12 and HEK-293T cell lysates; Human fetal brain, mouse brain and rat brain lysates; ポジティブ・コントロール Neurons from iPS cells lysate. ICC/IF: PC-12 cells; Human iPS cell derived neurons, primary mouse neurons/glia, DN14 cells. IHC-P: Human pancreas, mouse cerebral cortex, rat cerebral

cortex, medullablastoma, lung neuroendocrine tumor tissues; Sheep gut tissue.

ab187259 is the carrier-free version of ab32127. 特記事項

> 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<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 **RabMAb**<sup>®</sup> **patents**.

#### 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.

**バッファー** pH: 7.20

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** YE269

アイソタイプ lgG

#### アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab187259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
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa). Can be blocked with Synaptophysin peptide (ab189853).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.

#### ターゲット情報

機能 Possibly involved in structural functions as organizing other membrane components or in targeting

the vesicles to the plasma membrane. Involved in the regulation of short-term and long-term

synaptic plasticity.

組織特異性 Characteristic of a type of small (30-80 nm) neurosecretory vesicles, including presynaptic

vesicles, but also vesicles of various neuroendocrine cells of both neuronal and epithelial

phenotype.

**関連疾患** Mental retardation, X-linked, SYP-related

**配列類似性** Belongs to the synaptophysin/synaptobrevin family.

Contains 1 MARVEL domain.

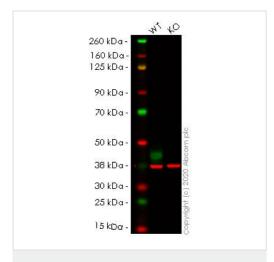
ドメイン The calcium-binding activity is thought to be localized in the cytoplasmic tail of the protein. 翻訳後修飾 Ubiquitinated: mediated by SIAH1 or SIAH2 and leading to its subsequent proteasomal

Ubiquitinated; mediated by SIAH1 or SIAH2 and leading to its subsequent proteasomal degradation.

Cytoplasmic vesicle, secretory vesicle, synaptic vesicle membrane. Cell junction, synapse, synaptosome.

#### 画像

細胞内局在



Western blot - Anti-Synaptophysin antibody [YE269]

- BSA and Azide free (ab187259)

**All lanes :** Anti-Synaptophysin antibody [YE269] (<u>ab32127</u>) at 1/1000 dilution

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: SYP knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

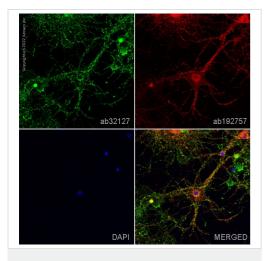
Performed under reducing conditions.

**Predicted band size:** 34 kDa **Observed band size:** 38 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab32127</u>).

Lanes 1-2: Merged signal (red and green). Green - <u>ab32127</u> observed at 38 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) observed at 37 kDa.

ab32127 was shown to react with Syp in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line ab255356 (knockout cell lysate ab263862) was used. Wild-type HEK-293T and SYP knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab32127 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



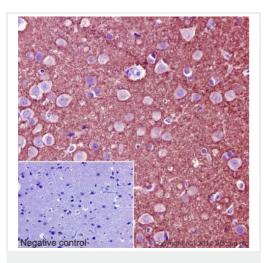
Immunocytochemistry/ Immunofluorescence - Anti-Synaptophysin antibody [YE269] - BSA and Azide free (ab187259)

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab32127).

<u>ab32127</u> staining Synaptophysin in primary mouse neurons/glia, DIV14 (prepared from E18 mouse hippocampal brain area, obtained from Transnetyx Tissue by BrainBits, LLC, cat.no. C57EHP) cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with <u>ab32127</u> at 0.1μg/ml and <u>ab192757</u>, Mouse mono Anti-PSD95 antibody [K28/43] - Synaptic Marker. Cells were then incubated with <u>ab150081</u>, Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor<sup>®</sup> 488), pre-adsorbed at 1/1000 dilution (shown in green) and <u>ab150120</u>, Goat polyclonal Secondary Antibody to Mouse lgG - H&L (Alexa Fluor<sup>®</sup> 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 100% methanol (5 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



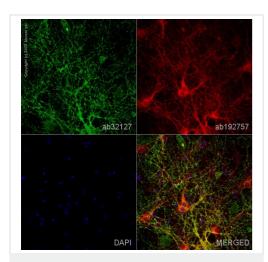
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Synaptophysin antibody [YE269] - BSA and Azide free (ab187259)

Immunohistochemical staining of paraffin embedded rat cerebral cortex with purified **ab32127** at a dilution of 1/400.

A pre-diluted HRP polymer for rabbit/mouse IgG was used as the secondary antibody and the sample was counterstained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0.

PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab32127</u>).



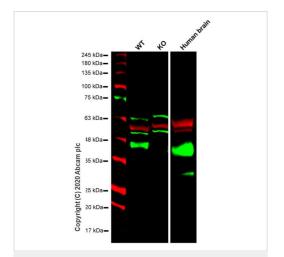
Immunocytochemistry/ Immunofluorescence - Anti-Synaptophysin antibody [YE269] - BSA and Azide free (ab187259)

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab32127)

ab32127 staining Synaptophysin in primary hippocampal rat neurons/glia, (obtained from Neuromics, cat. no. PC35101), DIV14. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab32127 at 0.1?g/ml and ab192757, Mouse mono Anti-PSD95 antibody [K28/43] - Synaptic Marker. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a high-content analyser (Operetta CLS,
Perkin Elmer) and a maximum intensity projection of confocal
sections is shown.



Western blot - Anti-Synaptophysin antibody [YE269] - BSA and Azide free (ab187259)

**All lanes :** Anti-Synaptophysin antibody [YE269] (<u>ab32127</u>) at 1/1000 dilution

Lane 1: Wild-type HEK-293T cell lysate at 20 µg

Lane 2: SYP knockout HEK-293T cell lysate at 20 µg

Lane 3: Human brain tissue lysate

Performed under reducing conditions.

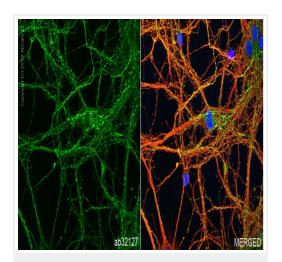
Predicted band size: 34 kDa Observed band size: 38 kDa

This data was developed using the same antibody clone in a different buffer formulation (ab32127).

Lanes 1-3: Merged signal (red and green). Green - ab32127

observed at 38 kDa. Red - loading control, **ab7291** observed at 50 kDa.

<u>ab32127</u> Anti-Synaptophysin antibody [YE269] was shown to specifically react with Synaptophysin in wild-type HEK293T cells. Loss of signal was observed when knockout cell line <u>ab267272</u> (knockout cell lysate <u>ab257060</u>) was used. Wild-type and Synaptophysin knockout samples were subjected to SDS-PAGE. <u>ab32127</u> and Anti-alpha Tubulin antibody [DM1A] - Loading Control (<u>ab7291</u>) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti- Rabbit IgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) and Goat anti- Mouse IgG H&L (IRDye® 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.

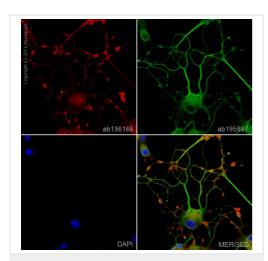


Immunocytochemistry/ Immunofluorescence - Anti-Synaptophysin antibody [YE269] - BSA and Azide free (ab187259)

Immunocytochemistry/ Immunofluorescence analysis of mouse primary neuron cells labeling Synaptophysin with purified <u>ab32127</u> at 1/100 (2.7 $\mu$ g/mL). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) 1/200 (2.5  $\mu$ g/mL). Goat anti rabbit lgG (Alexa Fluor<sup>®</sup> 488, <u>ab150077</u>) was used as the secondary antibody at 1/1000 (2  $\mu$ g/mL) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

Confocal scanning Z step was set as 0.3 µm followed by image processing with maximum Z projection.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32127).



Immunocytochemistry/ Immunofluorescence - Anti-Synaptophysin antibody [YE269] - BSA and Azide free (ab187259)

ab196379 ab195889

Immunocytochemistry/ Immunofluorescence - Anti-Synaptophysin antibody [YE269] - BSA and Azide free (ab187259)

Clone YE269 (ab187259) has been successfully conjugated by Abcam. This image was generated using Anti-Synaptophysin antibody [YE269] (Alexa Fluor® 647). Please refer to <a href="mailto:ab196166">ab196166</a> for protocol details.

**ab196166** staining Synaptophysin in PC12 cells. The cells were fixed with 100% methanol (5 min), permeabilised in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab196166** at 1/100 dilution (shown in red) and **ab195887**, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor<sup>®</sup> 488, shown in green) at 1/167 dilution overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

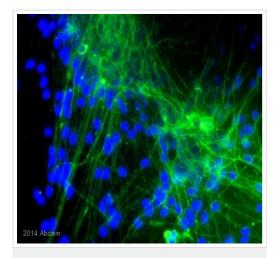
This product gave a positive signal in 5% formaldehyde (10 min) fixed PC12 cells under the same testing conditions.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Clone YE269 (ab187259) has been successfully conjugated by Abcam. This image was generated using Anti-Synaptophysin antibody [YE269] (Alexa Fluor® 488). Please refer to <a href="mailto:ab196379">ab196379</a> for protocol details.

**ab196379** staining Synaptophysin in PC12 cells. The cells were fixed with 100% methanol (5 min), permeabilised in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with **ab196379** at 1/100 dilution (shown in green) and **ab195889**, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor<sup>®</sup> 594, shown in red) at 1/167 dilution overnight at +4°C.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



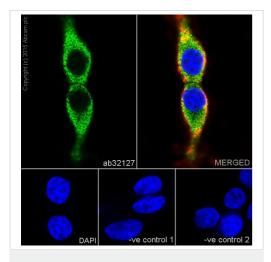
Immunocytochemistry/ Immunofluorescence - Anti-Synaptophysin antibody [YE269] - BSA and Azide free (ab187259)

This image is courtesy of an anonymous Abreview.

<u>ab32127</u> staining synaptophysin in human iPS cell derived neurons by immunocytochemistry/immmunofluorescence.

Samples were fixed with paraformaldehyde and blocked with 1% serum for 30 minutes at room temperature. Samples were incubated with primary antibody at 1/250 dilution for 1 hour. <a href="mailto:ab150061">ab150061</a> was used as the secondary antibody at 1/250 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab32127</u>).



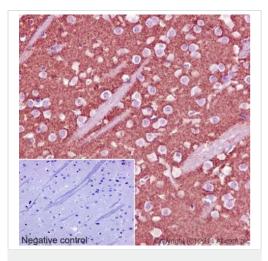
Immunocytochemistry/ Immunofluorescence - Anti-Synaptophysin antibody [YE269] - BSA and Azide free (ab187259)

Immunofluorescent staining of PC-12 (rat adrenal gland pheochromocytoma cell line) cells (fixed in 4% PFA, permeabilized with 0.1% Triton X 100) using purified **ab32127** at a dilution of 1/50.

An Alexa Fluor<sup>®</sup> 488 goat anti-rabbit antibody was used as the secondary at a dilution of 1/500 and the cells were counterstained with DAPI.

The negative control is shown in the bottom right hand panel - for the negative control, Alex Fluor<sup>®</sup> 594 goat anti-mouse was used at a dilution of 1/500.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab32127</u>).



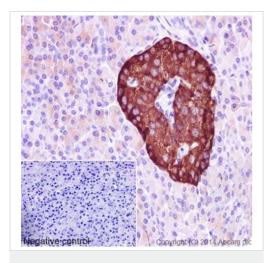
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Synaptophysin antibody [YE269] - BSA and Azide free (ab187259)

Immunohistochemical staining of paraffin embedded mouse cerebral cortex with purified <u>ab32127</u> at a dilution of 1/400.

A pre-diluted HRP polymer for rabbit/mouse IgG was used as the secondary antibody and the sample was counterstained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0.

PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32127).



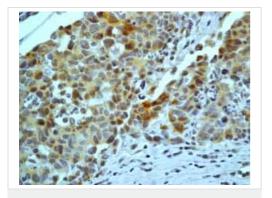
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Synaptophysin antibody [YE269] - BSA and Azide free (ab187259)

Immunohistochemical staining of paraffin embedded human pancreas with purified <u>ab32127</u> at a dilution of 1/400.

A pre-diluted HRP polymer for rabbit/mouse IgG was used as the secondary antibody and the sample was counterstained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0.

PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32127).

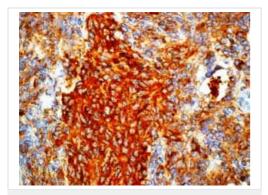


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Synaptophysin antibody [YE269] - BSA and Azide free (ab187259)

Unpurified <u>ab32127</u> showing positive staining in lung neuroendocrine tumor tissue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32127).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Synaptophysin antibody [YE269] - BSA and Azide free (ab187259)

This IHC data was generated using the same anti-Synaptophysin antibody clone, YE269, in a different buffer formulation (cat# **ab32127**).

Unpurified <u>ab32127</u> showing positive staining in Medulloblastoma tissue.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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Azide free (ab187259)

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