# abcam

## Product datasheet

# Anti-Synaptophysin antibody [YE269] ab32127





重组 RabMAb

★★★★★ 36 Abreviews 255 References 15 图像

概述

产品名称 Anti-Synaptophysin抗体[YE269]

描述 兔单克隆抗体[YE269] to Synaptophysin

宿主 Rabbit

经测试应用 适用于: WB, IHC-P, ICC/IF 种属反应性 与反应: Mouse, Rat, Human

预测可用于: Donkey, Cow \_\_\_\_\_

免疫原 Synthetic peptide within Human Synaptophysin aa 250 to the C-terminus (C terminal). The exact

sequence is proprietary. Residues in the cytoplasmic domain of human Synaptophysin.

Database link: P08247

(Peptide available as ab189853)

阳性对照 WB: PC-12 and HEK-293T cell lysates; Human fetal brain, mouse brain and rat brain lysates;

> 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.

常规说明 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® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

性能

形式 Liquid

存放说明 Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

存储溶液 pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

纯**度** Protein A purified

 克隆
 单克隆

 克隆编号
 YE269

 同种型
 IqG

#### 应用

#### The Abpromise guarantee Abpromise™承诺保证使用ab32127于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	<b>★★★★★</b> (7)	1/20000 - 1/100000. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa). Can be blocked with <b>Synaptophysin peptide (ab189853)</b> .
IHC-P	<b>★★★★★</b> (13)	1/400 - 1/800. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.  See IHC antigen retrieval protocols.
ICC/IF	<b>★★★★</b> (3)	Use a concentration of 0.1 µg/ml.

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和	仦

功能 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.

组织特异性 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.

疾病相关 Mental retardation, X-linked, SYP-related

**序列相似性** Belongs to the synaptophysin/synaptobrevin family.

Contains 1 MARVEL domain.

结**构域** The calcium-binding activity is thought to be localized in the cytoplasmic tail of the protein.

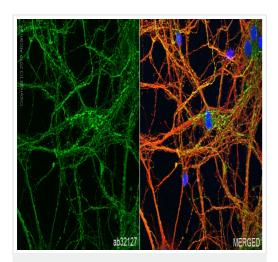
翻译后修饰 Ubiquitinated; mediated by SIAH1 or SIAH2 and leading to its subsequent proteasomal

degradation.

细胞定位 Cytoplasmic vesicle, secretory vesicle, synaptic vesicle membrane. Cell junction, synapse,

synaptosome.

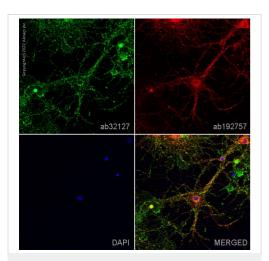
## 图片



Immunocytochemistry/ Immunofluorescence - Anti-Synaptophysin antibody [YE269] (ab32127)

Immunocytochemistry/ Immunofluorescence analysis of mouse primary neuron cells labeling Synaptophysin with purified ab32127 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.

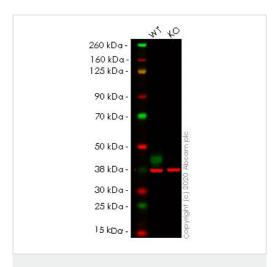


Immunocytochemistry/ Immunofluorescence - Anti-Synaptophysin antibody [YE269] (ab32127)

ab32127 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 ab32127 at 0.1µg/ml and ab192757, Mouse mono Anti-PSD95 antibody [K28/43] - Synaptic Marker. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse lgG - 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 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.



Western blot - Anti-Synaptophysin antibody [YE269] (ab32127)

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

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

Lane 2: SYP knockout HEK-293T cell lysate

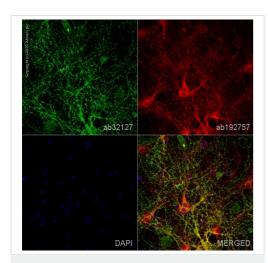
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

**Lanes 1-2:** Merged signal (red and green). Green - ab32127 observed at 38 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) 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 lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

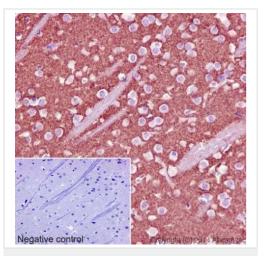


Immunocytochemistry/ Immunofluorescence - Anti-Synaptophysin antibody [YE269] (ab32127)

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 0.1µg/ml and ab192757, Mouse mono Anti-PSD95 antibody [K28/43] - Synaptic Marker. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse lgG - 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.

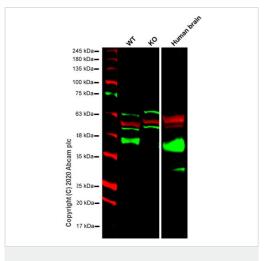


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Synaptophysin antibody [YE269] (ab32127)

Immunohistochemical staining of paraffin embedded mouse 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.



Western blot - Anti-Synaptophysin antibody [YE269] (ab32127)

**All lanes :** Anti-Synaptophysin antibody [YE269] (ab32127) 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

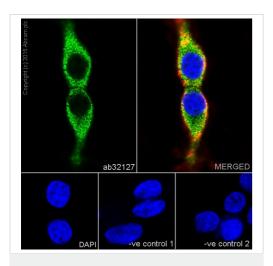
Lane 3: Human brain tissue lysate

Performed under reducing conditions.

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

**Lanes 1-3:** Merged signal (red and green). Green - ab32127 observed at 38 kDa. Red - loading control, **ab7291** observed at 50 kDa.

ab32127 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 <a href="mailto:ab267272">ab267272</a>
(knockout cell lysate <a href="mailto:ab257060">ab257060</a>) was used. Wild-type and Synaptophysin knockout samples were subjected to SDS-PAGE. ab32127 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (<a href="mailto:ab7291">ab7291</a>) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti- Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<a href="mailto:ab216773">ab216773</a>) and Goat anti- Mouse lgG H&L (IRDye® 680RD) preadsorbed (<a href="mailto:ab216776">ab216776</a>) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.

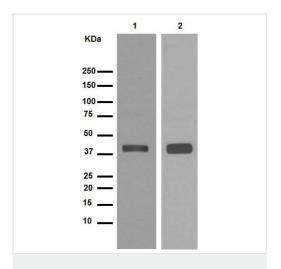


Immunocytochemistry/ Immunofluorescence - Anti-Synaptophysin antibody [YE269] (ab32127)

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.



Western blot - Anti-Synaptophysin antibody [YE269] (ab32127)

**All lanes :** Anti-Synaptophysin antibody [YE269] (ab32127) at 1/100000 dilution (purified)

Lane 1 : Mouse brain
Lane 2 : Rat brain

**Secondary** 

All lanes: HRP goat anti-rabbit at 1/1000 dilution

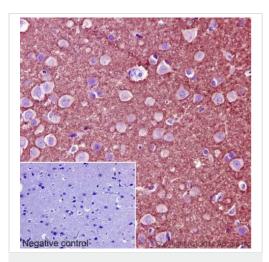
Predicted band size: 34 kDa Observed band size: 38 kDa

Blocking/Dilution buffer: 5% NFDM/TBST

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Synaptophysin antibody [YE269] (ab32127)

Unpurified ab32127 showing positive staining in medulloblastoma tissue.

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

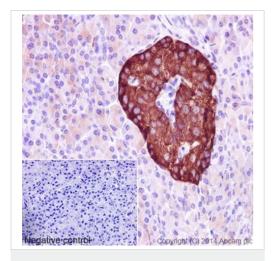


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Synaptophysin antibody [YE269] (ab32127)

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.

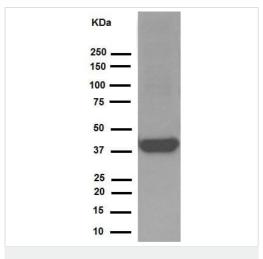


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Synaptophysin antibody [YE269] (ab32127)

Immunohistochemical staining of paraffin embedded human pancreas 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.



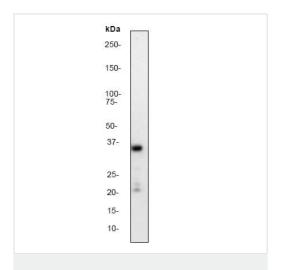
Western blot - Anti-Synaptophysin antibody [YE269] (ab32127)

Anti-Synaptophysin antibody [YE269] (ab32127) at 1/100000 dilution (purified) + Human fetal brain at 20 µg

#### Secondary

HRP goat anti-rabbit (H+L) at 1/1000 dilution

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



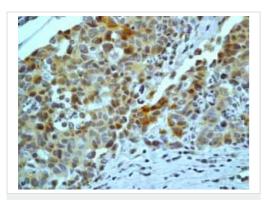
Western blot - Anti-Synaptophysin antibody [YE269] (ab32127)

Anti-Synaptophysin antibody [YE269] (ab32127) at 1/10000 dilution (unpurified) + PC-12 (rat adrenal gland pheochromocytoma cell line) cell lysate

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

Additional bands at: 21 kDa, 22 kDa. We are unsure as to the

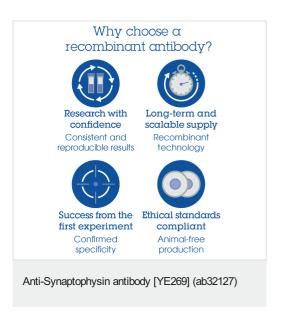
identity of these extra bands.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Synaptophysin antibody [YE269] (ab32127)

Unpurified ab32127 showing positive staining in lung neuroendocrine tumor tissue.

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



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