abcam

Product datasheet

Anti-Synaptophysin antibody [YE269] ab32127

KO VALIDATED Recombinant RabMAb

36 Abreviews 255 References 15 Images

Overview

Product name	Anti-Synaptophysin antibody [YE269]
Description	Rabbit monoclonal [YE269] to Synaptophysin
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Donkey, Cow
Immunogen	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**)
Positive control	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, DIV14 cells. IHC-P: Human pancreas, mouse cerebral cortex, rat cerebral cortex, medullablastoma, lung neuroendocrine tumor tissues; Sheep gut tissue.
General notes	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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	YE269
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32127 in the following tested applications.

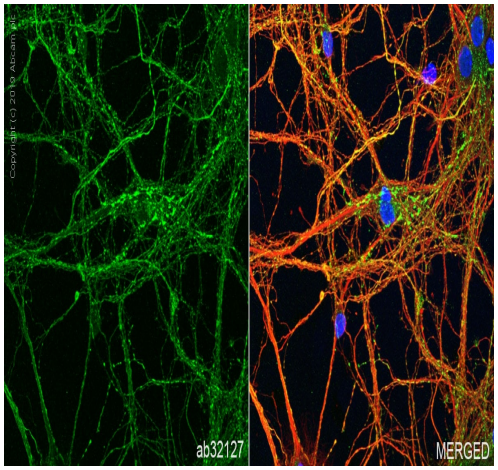
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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

Target

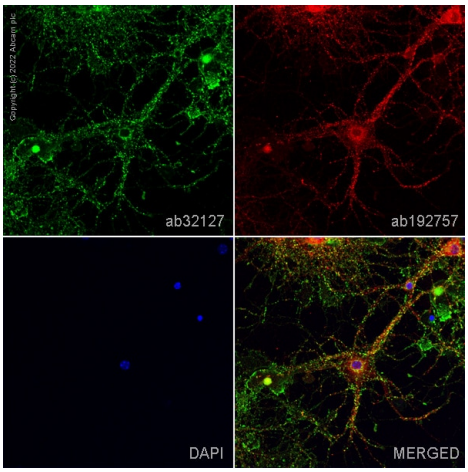
Function	Possibly involved in structural functions as organizing other membrane components or in targeting the vesicles to the plasma membrane. Involved in the regulation of short-term and long-term synaptic plasticity.
Tissue specificity	Characteristic of a type of small (30-80 nm) neurosecretory vesicles, including presynaptic vesicles, but also vesicles of various neuroendocrine cells of both neuronal and epithelial phenotype.
Involvement in disease	Mental retardation, X-linked, SYP-related
Sequence similarities	Belongs to the synaptophysin/synaptobrevin family. Contains 1 MARVEL domain.
Domain	The calcium-binding activity is thought to be localized in the cytoplasmic tail of the protein.
Post-translational modifications	Ubiquitinated; mediated by SIAH1 or SIAH2 and leading to its subsequent proteasomal degradation.
Cellular localization	Cytoplasmic vesicle, secretory vesicle, synaptic vesicle membrane. Cell junction, synapse, synaptosome.

Images



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

Immunocytochemistry/ Immunofluorescence analysis of mouse primary neuron cells labeling Synaptophysin with purified ab32127 at 1/100 (2.7µg/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® 594) 1/200 (2.5 µg/mL). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/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.

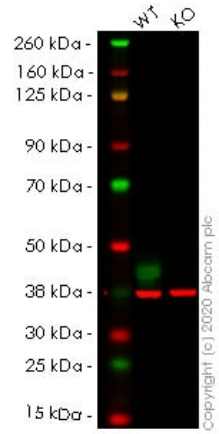


Immunocytochemistry/ Immunofluorescence - Anti-Synaptophysin antibody [YE269] (ab32127)

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 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 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

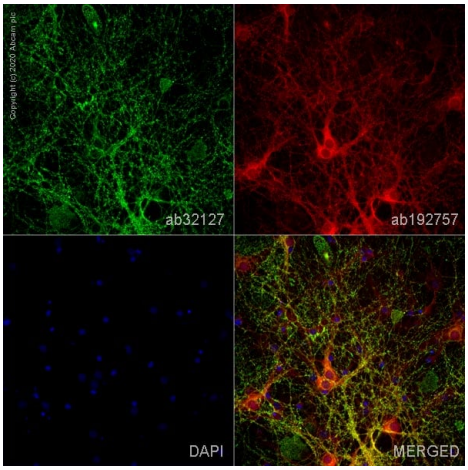
Lysates/proteins at 20 µg per lane.

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 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.

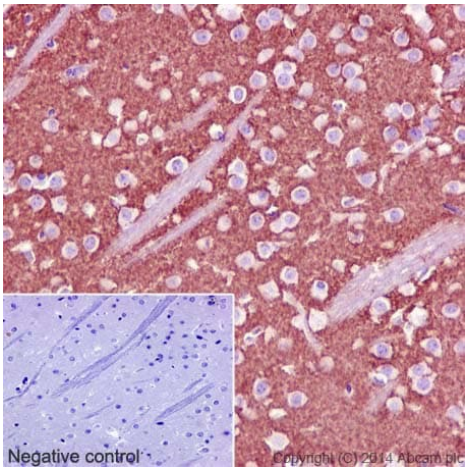


Immunocytochemistry/ Immunofluorescence - Anti-Synaptophysin antibody [YE269] (ab32127)

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 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.

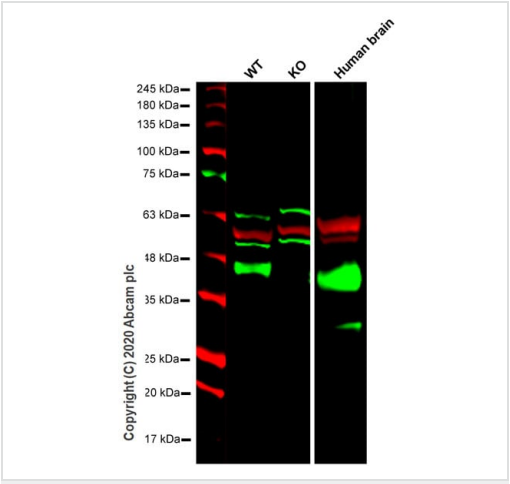


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Synaptophysin antibody [YE269] (ab32127)

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 perfomed 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

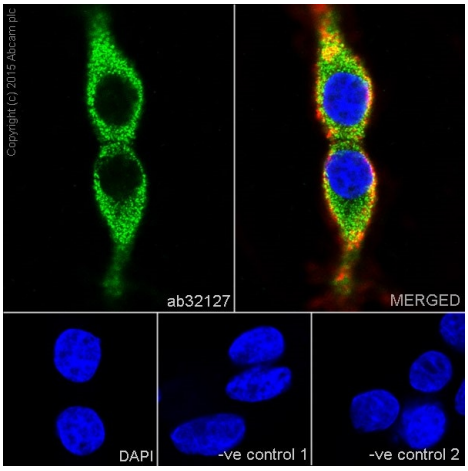
Lane 1 : Wild-type HEK-293T cell lysate at 20 µg
Lane 2 : SYP knockout HEK-293T cell lysate at 20 µg
Lane 3 : Human brain tissue lysate

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 **ab267272** (knockout cell lysate **ab257060**) was used. Wild-type and Synaptophysin knockout samples were subjected to SDS-PAGE. ab32127 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) 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 (**ab216773**) and Goat anti- Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.

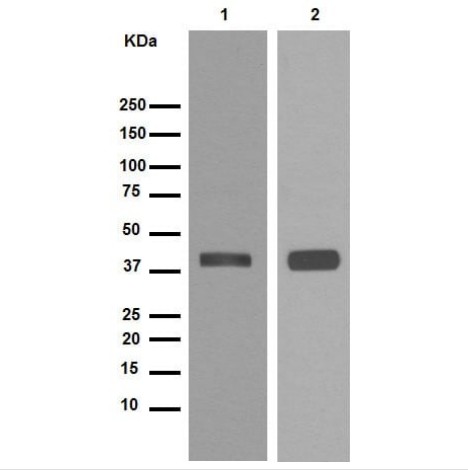


Immunocytochemistry/ Immunofluorescence - Anti-Synaptophysin antibody [YE269] (ab32127)

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® 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® 594 goat anti-mouse was used at a dilution of 1/500.



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

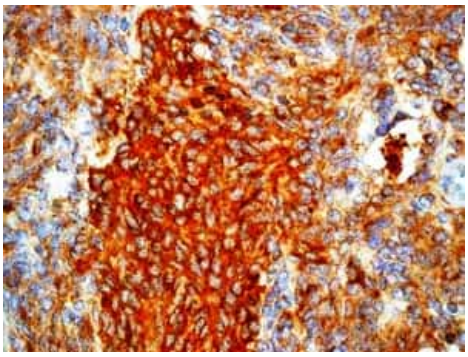
All lanes : Anti-Synaptophysin antibody [YE269] (ab32127) at 1/100000 dilution (purified)

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

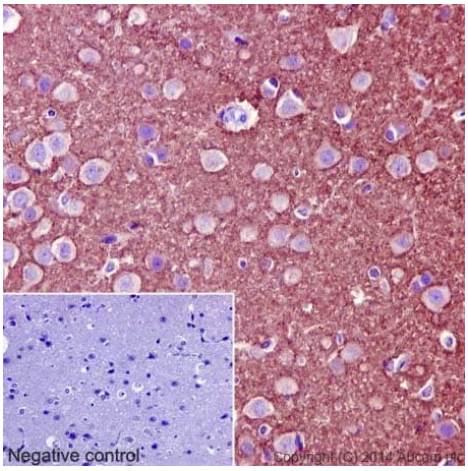
Blocking/Dilution buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded 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.

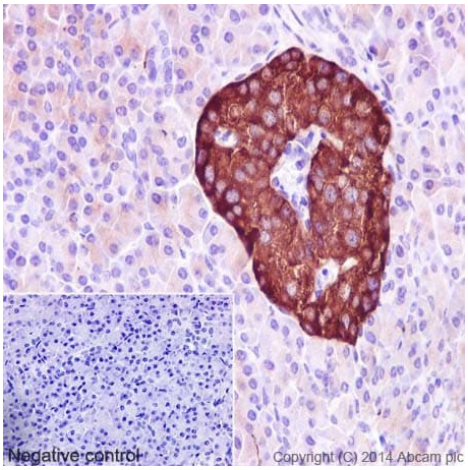


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Synaptophysin antibody [YE269] (ab32127)

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 perfomed 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.

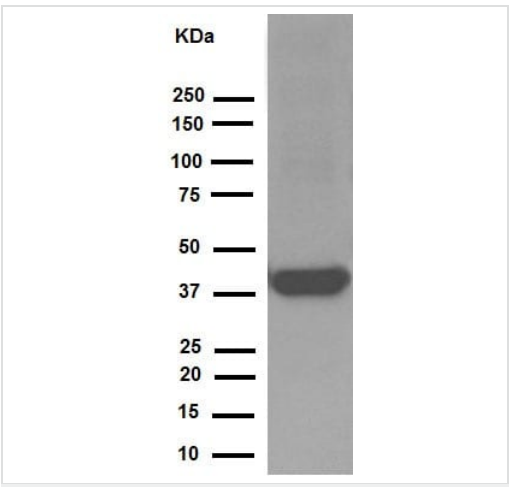


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Synaptophysin antibody [YE269] (ab32127)

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 perfomed 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)

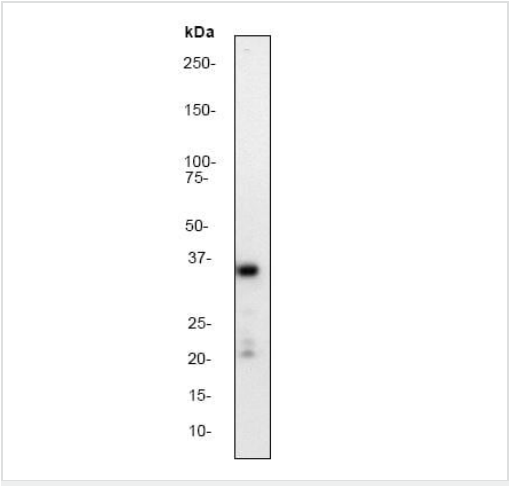
Anti-Synaptophysin antibody [YE269] (ab32127) at 1/100000 dilution (purified) + Human fetal brain at 20 µg

Secondary

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

Predicted band size: 34 kDa

Observed band size: 38 kDa



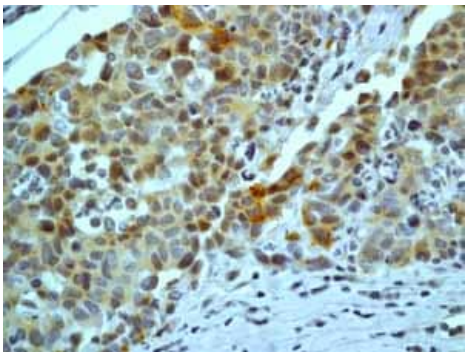
Western blot - Anti-Synaptophysin antibody [YE269] (ab32127)

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

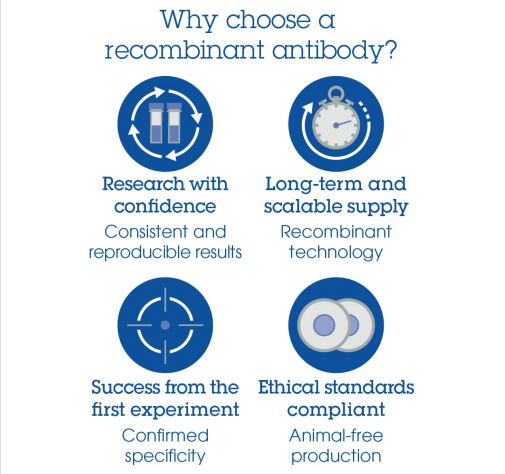
Additional bands at: 21 kDa, 22 kDa. We are unsure as to the identity of these extra bands.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded 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.



Anti-Synaptophysin antibody [YE269] (ab32127)

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