abcam

Product datasheet

Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] -BSA and Azide free ab226169





1 References 8 Images

Overview

Product name Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] - BSA and Azide free

Rabbit monoclonal [EPR18814-102] to GSK3 beta + GSK3 alpha - BSA and Azide free **Description**

Host species Rabbit

Specificity Unsuitable for human IHC-P.

Tested applications Suitable for: WB, IHC-P, ICC/IF, 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 IHC-P: Mouse testis tissue.

General notes ab226169 is the carrier-free version of ab185141.

> 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[®] 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® 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. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal

Clone number EPR18814-102

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab226169 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		Use at an assay dependent concentration. Detects a band of approximately 47, 52 kDa (predicted molecular weight: 47, 52 kDa).
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. This antibody is not recommended for IHC in human.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.

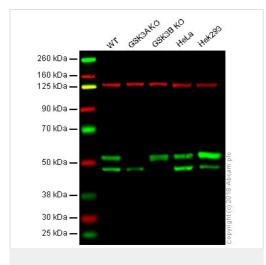
Target

Cellular localization GSK3 beta: Cytoplasm. Nucleus. Cell membrane. The phosphorylated form shows localization to

cytoplasm and cell membrane. The MEMO1-RHOA-DIAPH1 signaling pathway controls

localization of the phosophorylated form to the cell membrane.

Images



Western blot - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] - BSA and Azide free (ab226169)

All lanes : Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] (ab185141) at 1/5000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: GSK3 alpha knockout HAP1 whole cell lysate

Lane 3: GSK3 beta whole cell lysate

Lane 4 : HeLa whole cell lysate

Lane 5 : Hek293 whole cell lysate

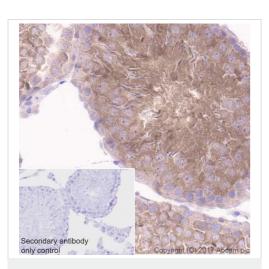
Lysates/proteins at 20 µg per lane.

Predicted band size: 47, 52 kDa

Lanes 1 - 5: Merged signal (red and green). Green - <u>ab185141</u> observed at 47/52 kDa. Red - loading control, <u>ab18058</u>, observed at 130 kDa.

<u>ab185141</u> was shown to specifically react with GSK3 alpha and GSK3 beta in wild-type HAP1 cells as signal was lost in GSK3 alpha and GSK3 beta knockout cells. Wild-type and GSK3 alpha and GSK3 beta knockout samples were subjected to SDS-PAGE. <u>ab185141</u> and <u>ab18058</u> (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/5000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye[®] 800CW) preabsorbed <u>ab216773</u> and Goat anti-Mouse lgG H&L (IRDye[®] 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185141).



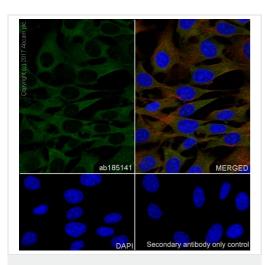
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] - BSA and Azide free (ab226169)

Immunohistochemical analysis of paraffin-embedded rat testis tissue labeling GSK3 beta + GSK3 alpha with <u>ab185141</u> at 1/4000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) Ready to use. Cytoplasmic staining on rat testis (PMID: 22792253). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab185141</u>).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



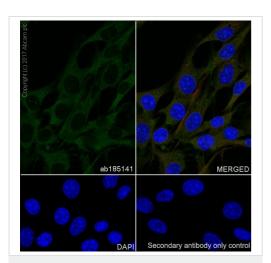
Immunocytochemistry/ Immunofluorescence - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] - BSA and Azide free (ab226169)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling GSK3 beta + GSK3 alpha with <u>ab185141</u> at 1/150 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on HeLa cells.

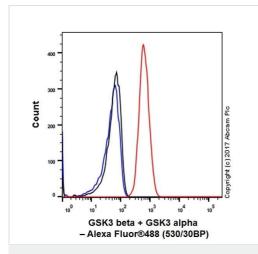
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab185141</u>).



Immunocytochemistry/ Immunofluorescence - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] - BSA and Azide free (ab226169)



Flow Cytometry (Intracellular) - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] - BSA and Azide free (ab226169)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embryo fibroblast cell line) cells labeling GSK3 beta + GSK3 alpha with <u>ab185141</u> at 1/150 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on NIH/3T3 cells.

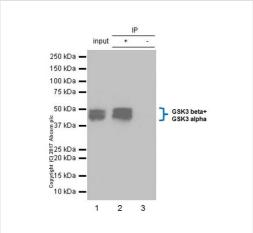
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.

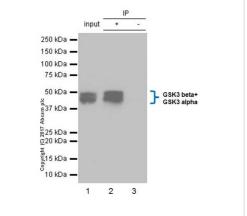
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab185141</u>).

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized NIH/3T3 (mouse embryo fibroblast cell line) cell line labeling GSK3 beta + GSK3 alpha with <u>ab185141</u> at 1/60 dilution (red) compared with a Rabbit lgG, monoclonal [EPR25A] - Isotype Control (<u>ab172730</u>) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185141).



Immunoprecipitation - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] - BSA and Azide free (ab226169)



Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 3 seconds.

NIH/3T3 whole cell lysate.

1/10000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185141).

GSK3 beta + GSK3 alpha was immunoprecipitated from 0.35 mg of NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate with ab185141 at 1/30 dilution. Western blot was performed from the

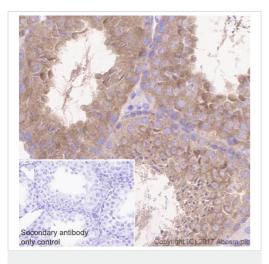
immunoprecipitate using ab185141 at 1/1000 dilution. VeriBlot for

IP Detection Reagent (HRP) (ab131366), was used for detection at

Lane 3: Rabbit monoclonal lgG (ab172730) instead of ab185141 in

Lane 1: NIH/3T3 whole cell lysate 10 µg (Input).

Lane 2: ab185141 IP in NIH/3T3 whole cell lysate.



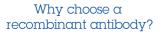
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] - BSA and Azide free (ab226169)

Immunohistochemical analysis of paraffin-embedded mouse testis tissue labeling GSK3 beta + GSK3 alpha with ab185141 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on mouse testis (PMID: 22792253). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185141).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.









technology





first experiment Confirmed specificity

compliant Animal-free production

Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-

102] - BSA and Azide free (ab226169)

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

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