

Product datasheet

Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] ab185141

KO VALIDATED

Recombinant

RabMAb

[1 References](#) [11 Images](#)

Overview

Product name	Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102]
Description	Rabbit monoclonal [EPR18814-102] to GSK3 beta + GSK3 alpha
Host species	Rabbit
Specificity	Unsuitable for human IHC-P.
Tested applications	Suitable for: IHC-P, IP, ICC/IF, WB, 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: His-tagged mouse GSK3 beta (aa218-420) recombinant protein; His-tagged mouse GSK3 alpha (aa330-490) recombinant protein; Human fetal brain, fetal heart and fetal kidney lysates; NIH/3T3, HeLa, A549, RAW 264.7 and PC-12 whole cell lysates; Mouse and rat heart lysates. IHC-P: Mouse and rat testis tissues. ICC/IF: HeLa and NIH/3T3 cells. Flow Cyt (intra): NIH/3T3 cells. IP: NIH/3T3 whole cell lysate.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide

	Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18814-102
Isotype	IgG

Applications

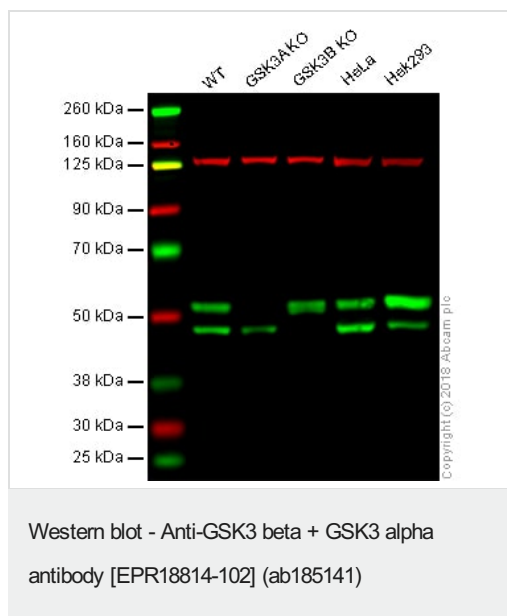
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab185141 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		1/4000. 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.
IP		1/30.
ICC/IF		1/150.
WB		1/5000. Detects a band of approximately 47, 52 kDa (predicted molecular weight: 47, 52 kDa).
Flow Cyt (Intra)		1/60.

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 phosphorylated form to the cell membrane.

Images



Lane 1: Wild-type HAP1 whole cell lysate (20 µg)

Lane 2: GSK3 alpha knockout HAP1 whole cell lysate (20 µg)

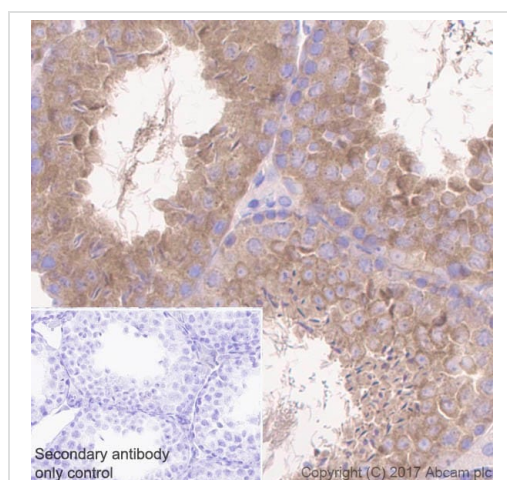
Lane 3: GSK3 beta whole cell lysate (20 µg)

Lane 4: HeLa whole cell lysate (20 µg)

Lane 5: Hek293 whole cell lysate (20 µg)

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

ab185141 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. ab185141 and **ab18058** (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 IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

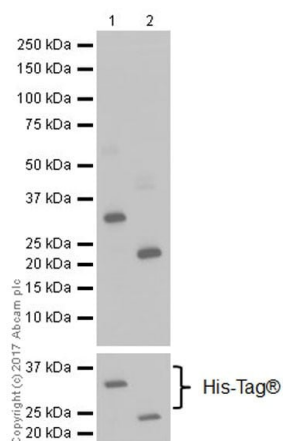


Immunohistochemical analysis of paraffin-embedded mouse testis tissue labeling GSK3 beta + GSK3 alpha with ab185141 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on mouse testis (PMID: 22792253). Counter stained with hematoxylin.

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

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

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] (ab185141)



Western blot - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] (ab185141)

All lanes : Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] (ab185141) at 1/1000 dilution

Lane 1 : His-tagged mouse GSK3 beta (aa218-420) recombinant protein

Lane 2 : His-tagged mouse GSK3 alpha (aa330-490) recombinant protein

Lysates/proteins at 0.01 µg per lane.

Secondary

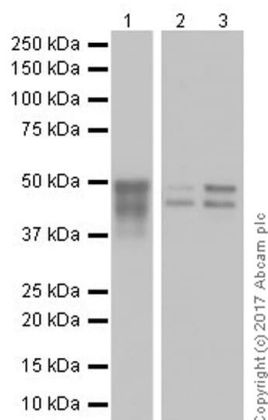
All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 47, 52 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% NFDm/TBST.



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Western blot - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] (ab185141)

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

Lane 1 : Human fetal brain lysate

Lane 2 : Human fetal heart lysate

Lane 3 : Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at 1/4000 dilution

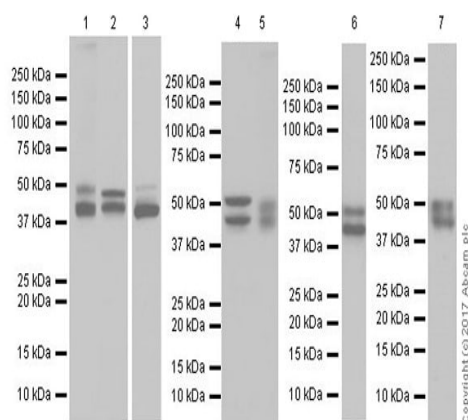
Developed using the ECL technique.

Predicted band size: 47, 52 kDa

Observed band size: 47,52 kDa

Exposure time : Lane 1: 30 seconds; Lanes 2 and 3: 3 minutes.

Blocking/Dilution buffer: 5% NFDM/TBST.



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Western blot - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] (ab185141)

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

Lane 1 : NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate

Lane 2 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 3 : A549 (human lung carcinoma cell line) whole cell lysate

Lane 4 : RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate

Lane 5 : PC-12 (rat adrenal gland pheochromocytoma cell line) whole cell lysate

Lane 6 : Mouse heart lysate

Lane 7 : Rat heart lysate

Lysates/proteins at 20 µg per lane.

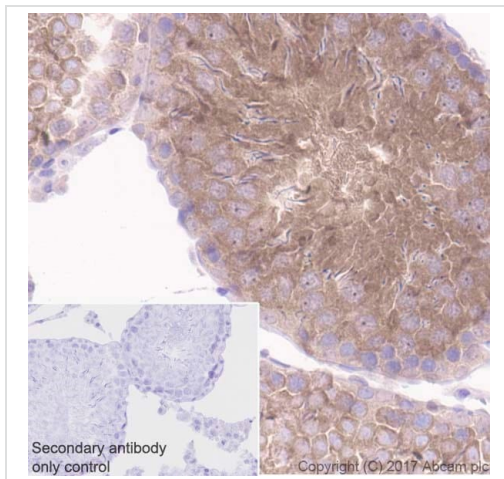
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 47, 52 kDa

Observed band size: 47,52 kDa

Exposure time : Lanes 1 and 2:10 seconds; Lane 3: 1 minute;
Lanes 4 and 5: 5 seconds; Lane 6:3 seconds; Lane 7:10 seconds.
Blocking/Dilution buffer: 5% NFDM/TBST.

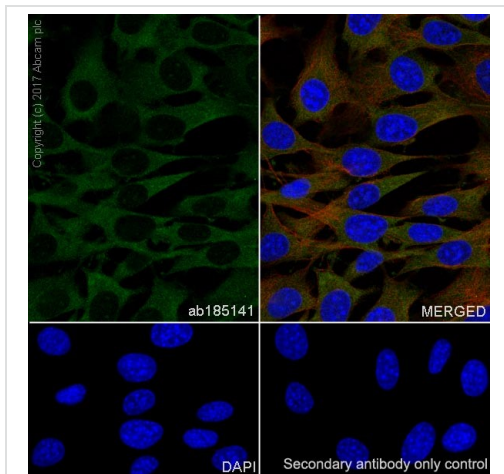


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] (ab185141)

Immunohistochemical analysis of paraffin-embedded rat 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 rat 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.

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

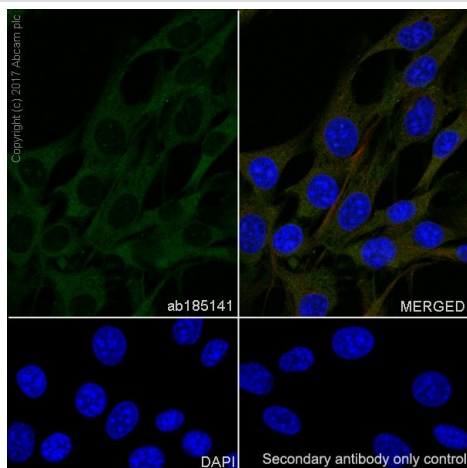


Immunocytochemistry/ Immunofluorescence - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] (ab185141)

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 ab185141 at 1/150 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) ([ab150077](#)) 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 IgG H&L (Alexa Fluor® 488) ([ab150077](#)) secondary antibody at 1/1000 dilution.

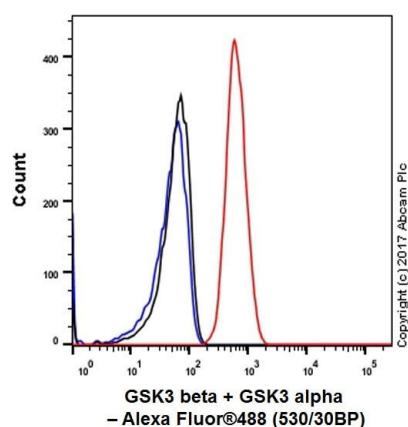


Immunocytochemistry/ Immunofluorescence - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] (ab185141)

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 ab185141 at 1/150 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) ([ab150077](#)) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on NIH/3T3 cells.

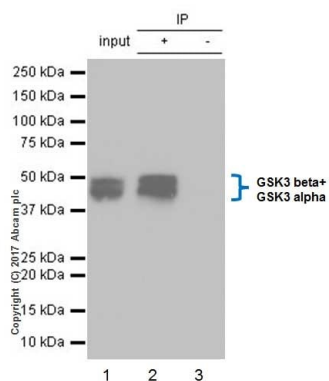
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) ([ab195889](#)) (red) at 1/200 dilution.

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



Flow Cytometry (Intracellular) - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] (ab185141)

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 ab185141 at 1/60 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control ([ab172730](#)) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) ([ab150077](#)) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] (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 1/10000 dilution.

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

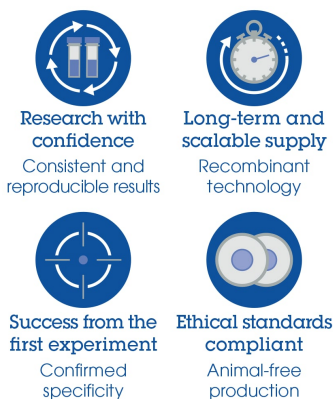
Lane 2: ab185141 IP in NIH/3T3 whole cell lysate.

Lane 3: Rabbit monoclonal IgG ([ab172730](#)) instead of ab185141 in NIH/3T3 whole cell lysate.

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

Exposure time: 3 seconds.

Why choose a recombinant antibody?



Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] (ab185141)

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