abcam

Product datasheet

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





RabMAb

1 References 11 图像

概述

产品名称 Anti-GSK3 beta + GSK3 alpha抗体[EPR18814-102]

宿主 Rabbit

特异性 Unsuitable for human IHC-P.

经测试应用 适用于: IHC-P, IP, ICC/IF, WB, Flow Cyt (Intra)

种属反应性 与反应: Mouse, Rat, Human

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

阳性对照 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.

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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

存储溶液 pH: 7.2

Preservative: 0.01% Sodium azide

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

纯**度** Protein A purified

克隆 单克隆

克隆编号 EPR18814-102

同种型 IgG

应用

The Abpromise guarantee

Abpromise

承诺保证使用ab185141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
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.

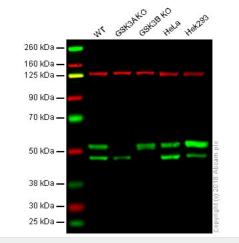
靶标

细胞定位 GSK3 beta: Cytoplasm. Nucleus. Cell membrane. The phosphorylated form shows localization to

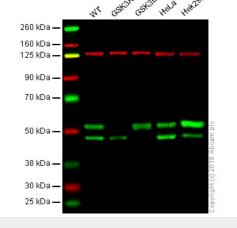
cytoplasm and cell membrane. The MEMO1-RHOA-DIAPH1 signaling pathway controls

localization of the phosophorylated form to the cell membrane.

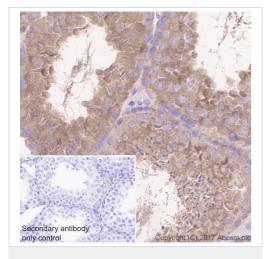
图片



antibody [EPR18814-102] (ab185141)



Western blot - Anti-GSK3 beta + GSK3 alpha



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

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)

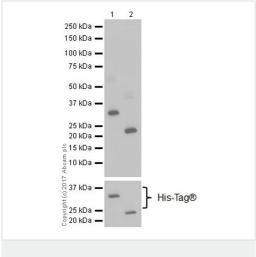
Lanes 1 - 5: Merged signal (red and green). Green - ab185141 observed at 47/52 kDa. Red - loading control, ab18058, observed at 130 kDa.

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 lgG 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.



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 lgG H&L (HRP) (<u>ab97051</u>) 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.



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) (<u>ab131366</u>) 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.

1 2 3 4 5 250 kDa -250 kDa -250 kDa — 150 kDa — 250 kDa -150 kDa -150 kDa -150 kDa -100 kDa -100 kDa -100 kDa -100 kDa -75 kDa -75 kDa -75 kDa -75 kDa -50 kDa -50 kDa -50 kDa -50 kDa -37 kDa -37 kDa -37 kDa • 37 kDa -25 kDa -25 kDa -25 kDa -20 kDa -25 kDa -20 kDa -20 kDa -20 kDa -15 kDa -15 kDa -15 kDa -15 kDa -10 kDa -10 kDa -10 kDa -10 kDa •

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 lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

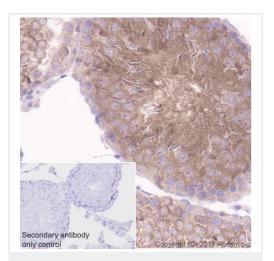
Predicted band size: 47, 52 kDa **Observed band size:** 47,52 kDa

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.

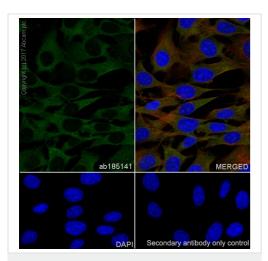
Immunohistochemical analysis of paraffin-embedded rat testis tissue labeling GSK3 beta + GSK3 alpha with ab185141 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on rat testis (PMID: 22792253). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG 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 paraffinembedded sections) - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] (ab185141)

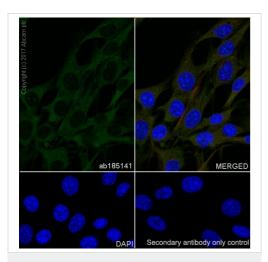


Immunocytochemistry/ Immunofluorescence - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] (ab185141)

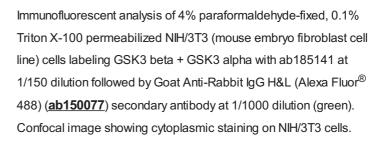
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling GSK3 beta + GSK3 alpha with ab185141 at 1/150 dilution followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody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

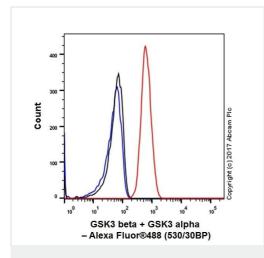


Immunocytochemistry/ Immunofluorescence - Anti-GSK3 beta + GSK3 alpha antibody [EPR18814-102] (ab185141)



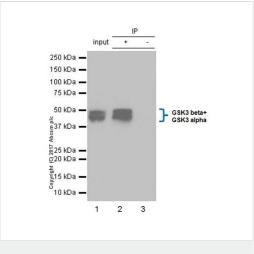
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) (**ab195889**) (red) at 1/200 dilution.

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.



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 lgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit lgG 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) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

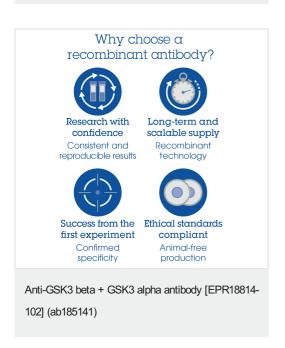
Lane 1: NIH/3T3 whole cell lysate 10 µg (Input).

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

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab185141 in NIH/3T3 whole cell lysate.

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

Exposure time: 3 seconds.



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