abcam

Product datasheet

Anti-GSK3 beta antibody [Y174] ab32391

KO 評価済 リコンビナント RabMAb

2 Abreviews 184 References 画像数 12

製品の概要

製品名	Anti-GSK3 beta antibody [Y174]
製品の詳細	Rabbit monoclonal [Y174] to GSK3 beta
由来種	Rabbit
特異性	This antibody is specific for human GSK3 beta. It may also detect the splice isoform 2 based on sequence homology. The immunogen used for this antibody is GSK3 beta phospho S9. This antibody shows partially phospho specificity to phospho S9 under certain conditions, for example, under low peptide concentration in ELISA assay, it has dominant reactivity with phospho S9 peptide.
アプリケーション	**適用あり:** ICC/IF, Flow Cyt (Intra), WB, IHC-P, ELISA
種交差性	**交差種:** Human, Recombinant fragment **交差が予測される動物種:** Mouse
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	A431 cell lysate. This antibody gave a positive result when used in the following formaldehyde fixed cell lines: DU145. IHC-P: Human breast adenocarcinoma FFPE tissue sections. ICC/IF: HeLa whole cell lysate (**ab150035**)
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
バッファー	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	Y174
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab32391の使用に適用されます**

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

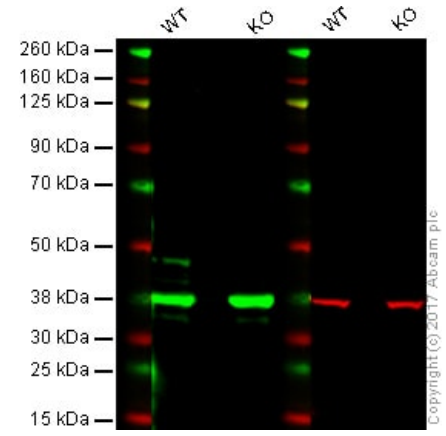
アプリケーション	Abreviews	特記事項
ICC/IF		1/100 - 1/500.
Flow Cyt (Intra)		1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (2)	1/5000 - 1/10000. Predicted molecular weight: 46 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ELISA		Use at an assay dependent concentration. Unit Type: 0 - 1000 ng/ml

ターゲット情報

機能	Participates in the Wnt signaling pathway. Implicated in the hormonal control of several regulatory proteins including glycogen synthase, MYB and the transcription factor JUN. Phosphorylates JUN at sites proximal to its DNA-binding domain, thereby reducing its affinity for DNA. Phosphorylates MUC1 in breast cancer cells, and decreases the interaction of MUC1 with CTNNB1/beta-catenin. Phosphorylates CTNNB1/beta-catenin. Phosphorylates SNAI1. Plays an important role in ERBB2-dependent stabilization of microtubules at the cell cortex. Prevents the phosphorylation of APC and CLASP2, allowing its association with the cell membrane. In turn, membrane-bound APC allows the localization of MACF1 to the cell membrane, which is required for microtubule capture and stabilization. Phosphorylates MACF1 and this phosphorylation inhibits the binding of MACF1 to microtubules which is critical for its role in bulge stem cell migration and skin wound repair.
組織特異性	Expressed in testis, thymus, prostate and ovary and weakly expressed in lung, brain and kidney.
配列類似性	Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. GSK-3 subfamily. Contains 1 protein kinase domain.
翻訳後修飾	Phosphorylated by AKT1 and ILK1. Activated by phosphorylation at Tyr-216.
細胞内局在	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.

画像

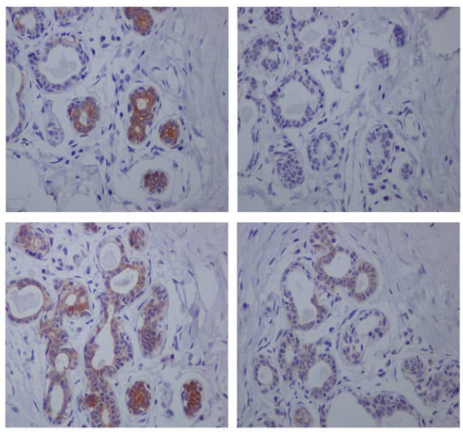


Western blot - Anti-GSK3 beta antibody [Y174] (ab32391)

Lane 1 & 3: Wild type HAP1 whole cell lysate (20 µg)
Lane 2 & 4: GSK3B knockout HAP1 whole cell lysate (20 µg)

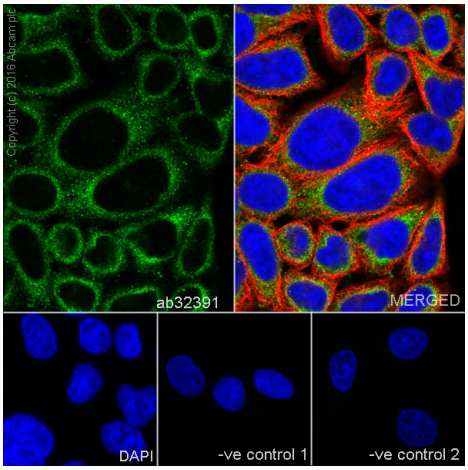
Lanes 1 - 4: Green - ab32391 observed at 46 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab32391 was shown to recognize GSK3B in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when GSK3B knockout samples were examined. Wild-type and GSK3B knockout samples were subjected to SDS-PAGE. Ab32391 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/2000 dilution and 1/10,000 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 1/10,000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GSK3 beta antibody [Y174] (ab32391)

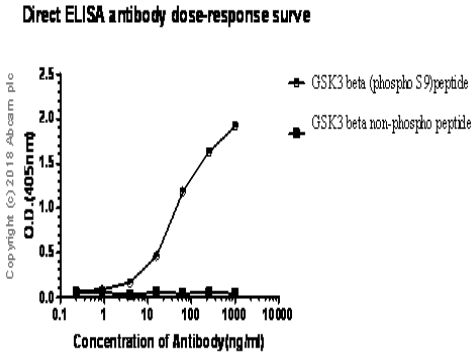
Immunohistochemical analysis of paraffin-embedded human breast tissue labelled with untreated **ab75814** (phospho) (top-left) at a dilution of 1/1000, alkaline phosphatase treated **ab75814** (phospho) (top-right) at a dilution of 1/1000, untreated ab32391 (bottom-left) at a dilution of 1/1000 and alkaline phophatase treated ab32391 (bottom-right) at a dilution of 1/1000. Ab97051 was used as secondary antibody at a dilution of 1/500 and counterstained with hematoxylin.



Immunocytochemistry/ Immunofluorescence - Anti-GSK3 beta antibody [Y174] (ab32391)

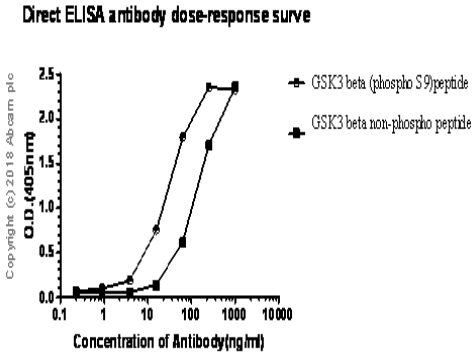
Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling GSK3 beta with purified ab32391 at 1/500. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% tritonX-100. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (**ab150077**) at 1/1000 dilution was used as the secondary antibody. Cells were co-stained with **ab7291**, a mouse anti-tubulin antibody (1/1000) using **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) as the secondary antibody. Nuclei were counterstained with DAPI (blue).

For negative control 1, rabbit primary antibody was used followed by anti-mouse secondary antibody (**ab150120**).
For negative control 2, mouse primary antibody (**ab7291**) and anti-rabbit secondary antibody (**ab150077**) were used.



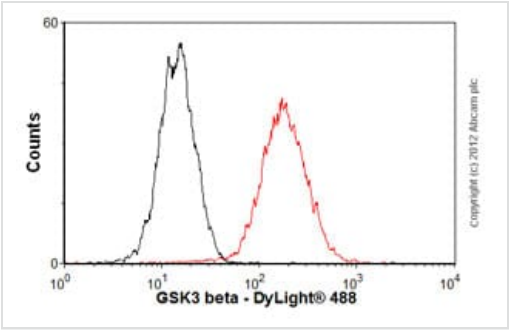
ELISA - Anti-GSK3 beta antibody [Y174] (ab32391)

ELISA of GSK3 beta (phospho S9) peptide and GSK3 beta non-phospho peptide at 10 ng/ml. Detected with ab32391 at 0~1000 ng/ml. Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/2500 was used as a secondary antibody.



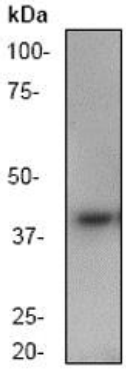
ELISA - Anti-GSK3 beta antibody [Y174] (ab32391)

ELISA of GSK3 beta (phospho S9) peptide and GSK3 beta non-phospho peptide at 1000 ng/ml. Detected with ab32391 at 0~1000 ng/ml. Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/2500 was used as a secondary antibody.



Flow Cytometry (Intracellular) - Anti-GSK3 beta antibody [Y174] (ab32391)

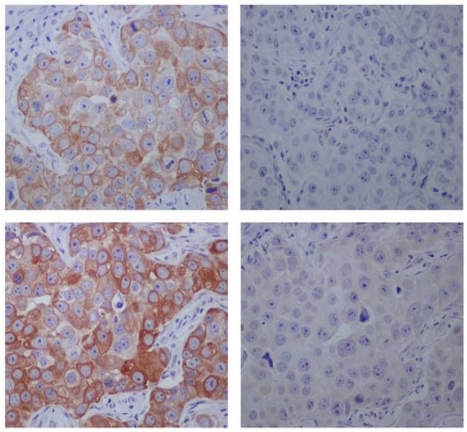
Overlay histogram showing HeLa cells stained with ab32391 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32391, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



Western blot - Anti-GSK3 beta antibody [Y174] (ab32391)

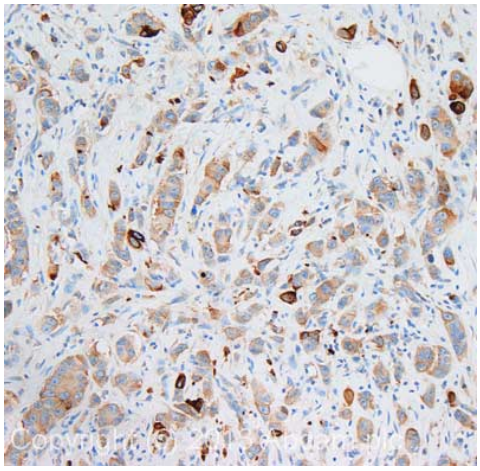
Anti-GSK3 beta antibody [Y174] (ab32391) at 1/10000 dilution + A431 cell lysate.

Predicted band size: 46 kDa
Observed band size: 46 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GSK3 beta antibody [Y174] (ab32391)

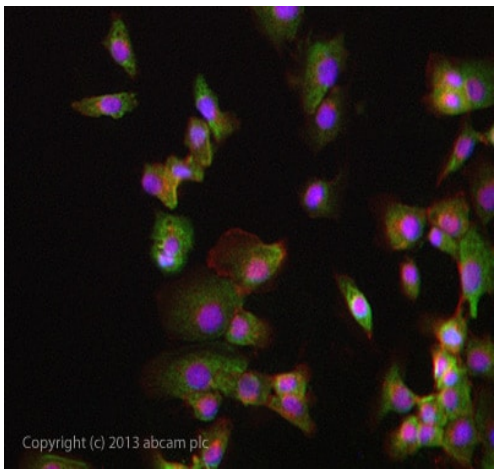
Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labelled with untreated **ab75814** (phospho) (top-left) at a dilution of 1/1000, alkaline phosphatase treated **ab75814** (phospho) (top-right) at a dilution of 1/1000, untreated ab32391 (bottom-left) at a dilution of 1/1000 and alkaline phophatase treated ab32391 (bottom-right) at a dilution of 1/1000. Ab97051 was used as secondary antibody at a dilution of 1/500 and counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GSK3 beta antibody [Y174] (ab32391)

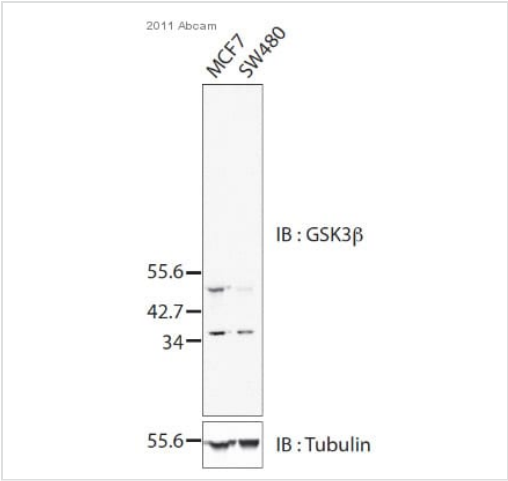
IHC image of GSK3 staining in human breast adenocarcinoma formalin fixed paraffin embedded tissue section, performed on a Leica Bond system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6, epitope retrieval solution 1) for 20 minutes. The section was then incubated with ab32391, 1/200 diution, for 15 minutes at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunocytochemistry/ Immunofluorescence - Anti-GSK3 beta antibody [Y174] (ab32391)

ICC/IF image of ab32391 stained DU145 cells. The cells were 4% formaldehyde fixed (10 minutes) and then incubated in 1 %BSA / 10 % normal goat serum / 0.3 M glycine in 0.1 % PBS-Tween for 1 hour to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab32391 at 1/200 dilution overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- rabbit (**ab96899**) IgG (H+L) used at a 1/250 dilution for 1 hour. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1 hour. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43 µM.



Western blot - Anti-GSK3 beta antibody [Y174] (ab32391)

Image courtesy of an anonymous Abreview.

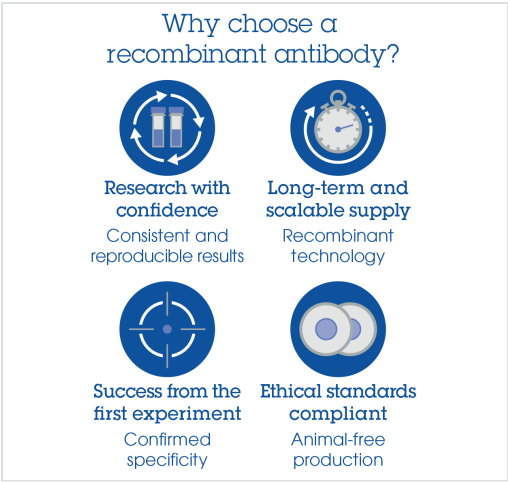
All lanes : Anti-GSK3 beta antibody [Y174] (ab32391) at 1/2500 dilution

Lane 1 : MCF7 (human breast adenocarcinoma cell line) cell lysate
Lane 2 : SW480 (human colorectal adenocarcinoma cell line) cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Donkey polyclonal IRDye 800CW at 1/15000 dilution

Predicted band size: 46 kDa
Observed band size: 46 kDa
Additional bands at: 37 kDa. We are unsure as to the identity of these extra bands.



Anti-GSK3 beta antibody [Y174] (ab32391)

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