

Product datasheet

Anti-GSK3 beta (phospho S9) antibody [EPR2286Y] ab75814

Recombinant RabMAb

★★★★☆ 6 Abreviews 37 References 6 Images

Overview

Product name	Anti-GSK3 beta (phospho S9) antibody [EPR2286Y]
Description	Rabbit monoclonal [EPR2286Y] to GSK3 beta (phospho S9)
Host species	Rabbit
Specificity	ab75814 only detects GSK3 beta phosphorylated on Serine 9.
Tested applications	Suitable for: Dot blot, WB, IHC-P, ICC/IF Unsuitable for: Flow Cyt or IP
Species reactivity	Reacts with: Human
Immunogen	A phospho-specific peptide corresponding to residues surrounding Serine 9 of human GSK3 beta
Positive control	293T cell lysates, untreated or treated with Calyculin A and human ovarian carcinoma tissue.
General notes	Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information. Our RabMAb [®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab[®] patents This product is a recombinant rabbit monoclonal antibody.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, 40% Glycerol, 9.85% Tris glycine, 50% Tissue culture supernatant
Purity	Tissue culture supernatant
Clonality	Monoclonal
Clone number	EPR2286Y

Isotype IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab75814** 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
Dot blot		Use at an assay dependent concentration.
WB	★★★★☆	1/10000 - 1/20000. Detects a band of approximately 47 kDa (predicted molecular weight: 47 kDa).
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ICC/IF	★★★★★	1/100 - 1/250.

Application notes Is unsuitable for Flow Cyt or IP.

Target

Function 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.

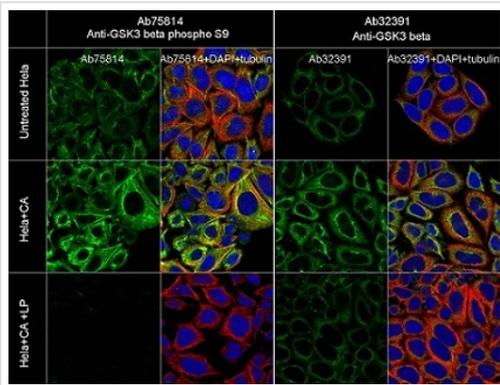
Tissue specificity Expressed in testis, thymus, prostate and ovary and weakly expressed in lung, brain and kidney.

Sequence similarities Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. GSK-3 subfamily. Contains 1 protein kinase domain.

Post-translational modifications Phosphorylated by AKT1 and ILK1. Activated by phosphorylation at Tyr-216.

Cellular localization 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



Immunocytochemistry/ Immunofluorescence - Anti-GSK3 beta (phospho S9) antibody [EPR2286Y] (ab75814)

Cell line: HeLa (human cervix adenocarcinoma)

Target: Ab75814 anti-GSK3 beta (phospho S9)

Secondary: ab150077 AlexaFluor®488 Goat anti-Rabbit

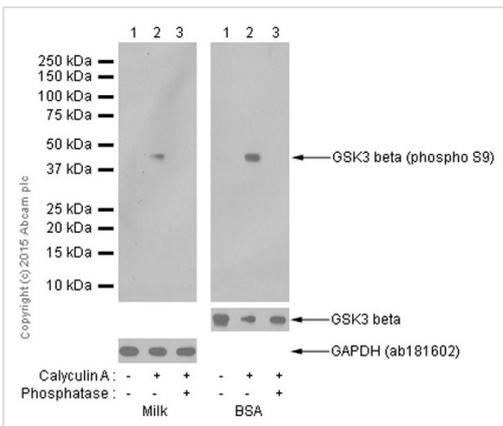
Fixative: 4% PFA

Permeabilization: 0.1% Triton-X

Nuclear counter stain: DAPI

Confocal image showed increased cytoplasmic staining after Calyculin A (CA) (ab141784 100ng/ml, 10min) treatment on HeLa cells. The LP treatment decreased the increased cytoplasmic staining caused by CA.

Ab32391 was used as a control for ab75814. The results showed cytoplasmic staining on untreated, CA and CA+LP treated HeLa cells.



Western blot - Anti-GSK3 beta (phospho S9) antibody [EPR2286Y] (ab75814)

All lanes : Anti-GSK3 beta (phospho S9) antibody [EPR2286Y] (ab75814) at 1/20000 dilution

Lane 1 : Untreated HeLa cell lysate

Lane 2 : HeLa cells were starved for 3 hours then treated with Calyculin A (100nM) for 30 minutes.

Lane 3 : HeLa cells were starved for 3 hours then treated with Calyculin A (100nM) for 30 minutes. The membrane was treated with alkaline phosphatase.

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution (Goat anti-rabbit IgG H&L (HRP))

Predicted band size: 47 kDa

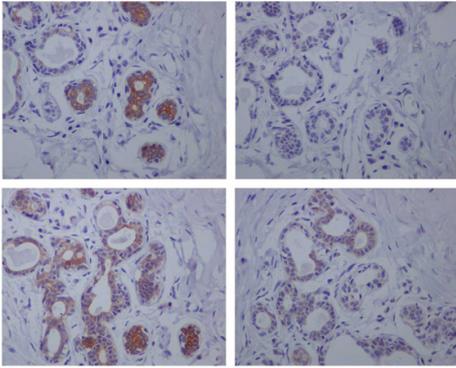
Observed band size: 47 kDa

Exposure time: 5 seconds

Blocking buffer: 5% NFD/MBST for the first 3 lanes, 2%

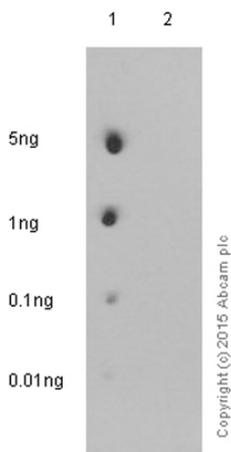
BSA/TBST for the second 3 lanes.

Dilution buffer: 5% NFDM/TBST for the first 3 lanes, 2% BSA/TBST for the second 3 lanes.



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 phosphatase 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 (phospho S9) antibody [EPR2286Y] (ab75814)

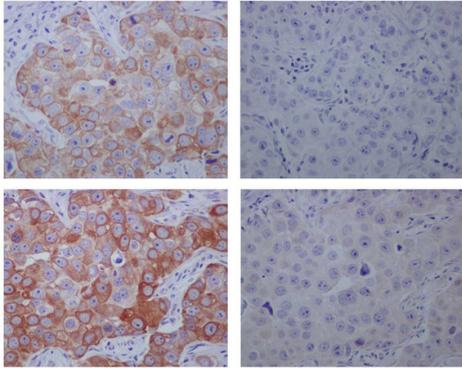


Dot blot analysis of GSK3 beta (pS9) peptide (Lane 1) and GSK3 beta non-phospho peptide (Lane 2) labelling GSK3 (pS9) with purified ab75814 at a dilution of 1/1000. ab97051 (Peroxidase conjugated goat anti-rabbit IgG (H+L)) was used as the secondary antibody at a dilution of 1/100000.

Blocking and dilution buffer: 5% NFDM/TBST.

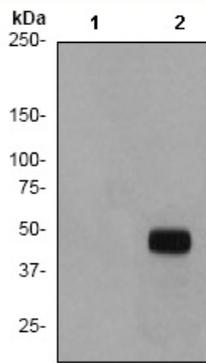
Exposure time: 3 minutes.

Dot Blot - Anti-GSK3 beta (phospho S9) antibody [EPR2286Y] (ab75814)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GSK3 beta (phospho S9) antibody [EPR2286Y] (ab75814)

Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labelled with untreated ab75814 (top-left) at a dilution of 1/1000, alkaline phosphatase treated ab75814 (top-right) at a dilution of 1/1000, untreated ab32391 (bottom-left) at a dilution of 1/1000 and alkaline phosphatase 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.



Western blot - Anti-GSK3 beta (phospho S9) antibody [EPR2286Y] (ab75814)

All lanes : Anti-GSK3 beta (phospho S9) antibody [EPR2286Y] (ab75814) at 1/20000 dilution

Lane 1 : 293T cell lysates, untreated

Lane 2 : 293T cell lysates, treated with Calyculin A

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : goat anti-rabbit HRP at 1/2000 dilution

Predicted band size: 47 kDa

Observed band size: 47 kDa

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