# abcam

### Product datasheet

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

יולצעבע RabMAb

★★★★ 10 Abreviews 100 References 画像数7

#### 製品の概要

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

製品の詳細 Rabbit monoclonal [EPR2286Y] to GSK3 beta (phospho S9)

由来種 Rabbit

アプリケーション 適用あり: Dot blot, WB, IHC-P, ICC/IF

適用なし: Flow Cyt or IP

種交差性 交差種: Human

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 293T cell lysates, untreated or treated with Calyculin A and human ovarian carcinoma tissue.

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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

#### 製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.

バッファー

Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue

culture supernatant

精製度 Protein A purified

ポリモノ モノクローナル

クローン名

EPR2286Y

アイソタイプ

lgG

#### アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab75814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
Dot blot		Use at an assay dependent concentration.
WB	<b>★★★★</b>	1/10000 - 1/20000. Detects a band of approximately 47 kDa (predicted molecular weight: 47 kDa).
IHC-P	**** (1)	1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ICC/IF	<b>★★★★★</b> (2)	1/100 - 1/250.

追加情報

Is unsuitable for Flow Cyt or IP.

#### 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

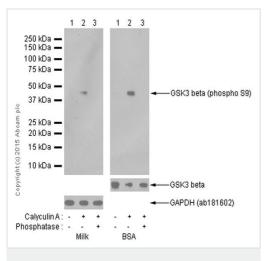
Phosphorylated by AKT1 and ILK1. Activated by phosphorylation at Tyr-216.

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 (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 lgG H&L (HRP) (ab97051) at 1/20000 dilution (Goat anti-rabbit lgG H&L (HRP))

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

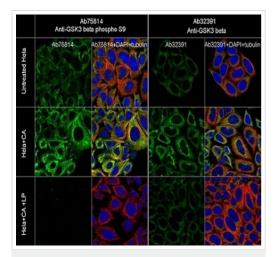
Exposure time: 5 seconds

Blocking buffer: 5% NFDM/TBST 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.



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

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - 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

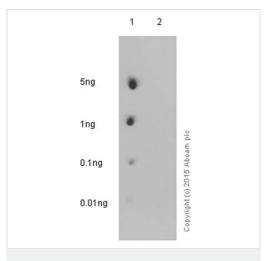
Nuclear counter stain: DAPI

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.

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.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

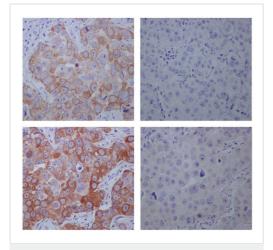


Dot Blot - Anti-GSK3 beta (phospho S9) antibody [EPR2286Y] (ab75814)

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 lgG (H+L)) was used as the secondary antibody at a dilution of 1/100000.

Blocking and dilution buffer: 5% NFDM/TBST.

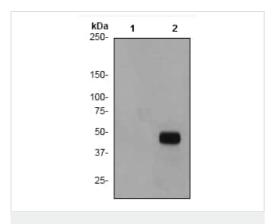
Exposure time: 3 minutes.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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 <u>ab32391</u> (bottom-left) at a dilution of 1/1000 and alkaline phophatase treated <u>ab32391</u> (bottom-right) at a dilution of 1/1000. Ab97051 was used as secondary antibody at a dilution of 1/500 and counterstained with hematoxylin.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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

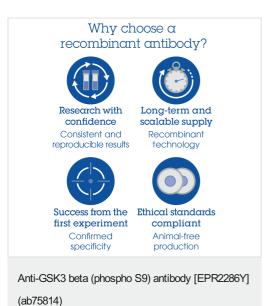
Lane 2: 293T cell lysates, treated with Calyculin A

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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