Product datasheet

# Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] ab68476

リコンビナント RabMAb

3 Abreviews 40 References 画像数 12

## 製品の概要

| | |
|---|---|
| **製品名** | Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] |
| **製品の詳細** | Rabbit monoclonal [EPR933Y] to GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) |
| **由来種** | Rabbit |
| **特異性** | This antibody detects both GSK alpha phosphorylated on tyrosine 279 and GSK3 beta phosphorylated on tyrosine 216. |
| **アプリケーション** | **適用あり:** WB, IP, IHC-P, Dot blot<br>**適用なし:** Flow Cyt or ICC/IF |
| **種交差性** | **交差種:** Mouse, Rat, Human, Zebrafish |
| **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** | WB: HEK-293 cell lysate. Cell lysate from untreated, nerve growth factor-beta treated PC-12 and SH-SY5Y cells, Zebrafish lysates and mouse brain lysates. IHC: Human brain tissue, glioma, thyroid carcinoma and ovarian carcinoma. |
| **特記事項** | This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**.<br>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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle. |
| **バッファー** | pH: 7.2<br>Preservative: 0.01% Sodium azide<br>Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS |

| | |
|---|---|
| **精製度** | Protein A purified |
| **ポリ/モノ** | モノクローナル |
| **クローン名** | EPR933Y |
| **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ab68476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。**

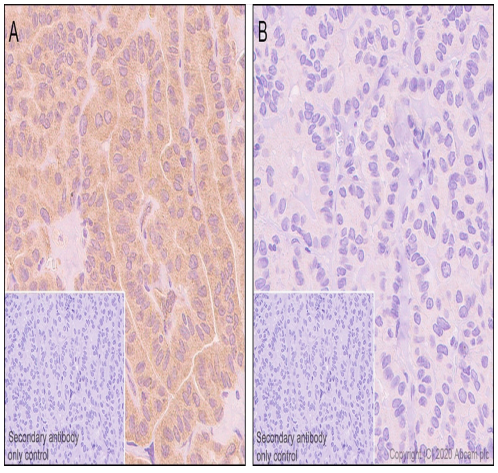
| アプリケーション | Abreviews | 特記事項 |
|---|---|---|
| **WB** | | 1/500 - 1/2000. Detects a band of approximately 47-52 kDa (predicted molecular weight: 47-52 kDa). |
| **IP** | | 1/40. |
| **IHC-P** | | 1/50. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.<br>This antibody may not be suitable for IHC with mouse, rat or zebrafish samples.<br>See **IHC antigen retrieval protocols**.<br>**For unpurified use at 1/100 - 1/250.** |
| **Dot blot** | | Use at an assay dependent concentration. |

**追加情報** Is unsuitable for Flow Cyt or ICC/IF.

## ターゲット情報

**細胞内局在** 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.

## 画像

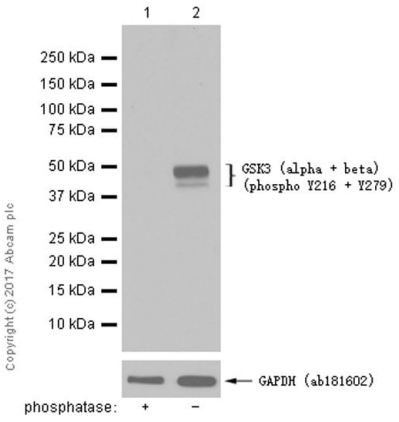



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid carcinoma tissue labelling GSK3 (alpha + beta) with purified ab68476 at 1/2000. Heat mediated antigen retrieval was performed using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. **ab209101**, a Rabbit specific IHC polymer detection kit HRP/DAB was used. Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

Positive staining on human thyroid carcinoma without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B).

The section was incubated with ab68476 for 30 minutes at room temperature. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument.




Western blot - Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476)

**All lanes :** Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476) at 1/1000 dilution (purified)

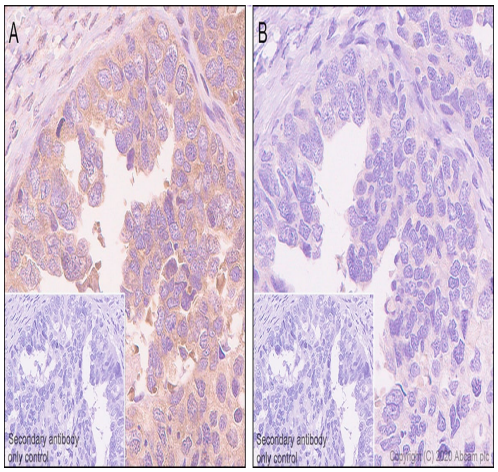
**Lane 1 :** Zebrafish lysates
Then the membrane was incubated with phosphatase
**Lane 2 :** Untreated Zebrafish lysates

Lysates/proteins at 15 µg per lane.

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

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

Blocking and diluting buffer: 5% NFDM/TBST

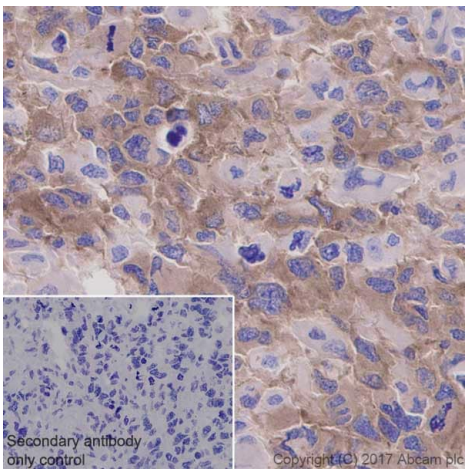



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human ovarian carcinoma tissue labelling GSK3 (alpha + beta) with purified ab68476 at 1/2000. Heat mediated antigen retrieval was performed using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. **ab209101**, a Rabbit specific IHC polymer detection kit HRP/DAB was used. Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

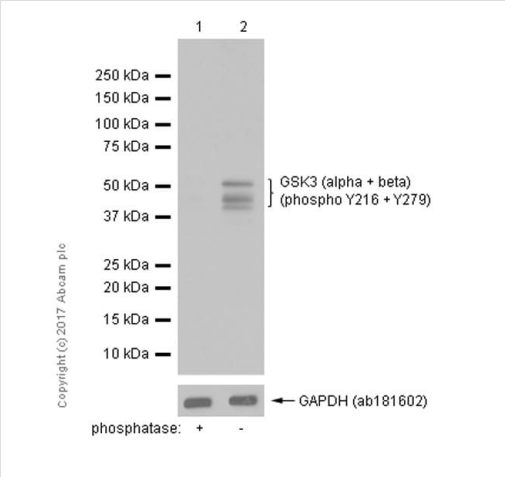
Positive staining on human ovarian carcinoma without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B).

The section was incubated with ab68476 for 30 minutes at room temperature. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument.




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human glioma tissue sections labeling GSK3 (alpha + beta) with Purified ab68476 at 1:50 dilution (5.3 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.




Western blot - Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476)

**All lanes :** Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476) at 1/1000 dilution (purified)

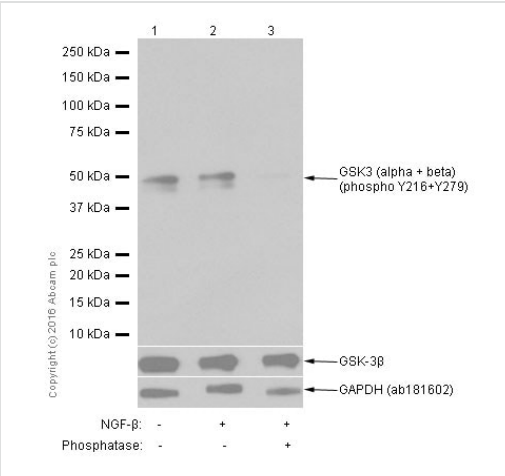
**Lane 1 :** Mouse brain lysates.
Then the membrane was incubated with phosphatase
**Lane 2 :** Untreated Mouse brain lysates

Lysates/proteins at 15 µg per lane.

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

**Predicted band size:** 47-52 kDa

Blocking and diluting buffer: 5% NFDM/TBST




Western blot - Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476)

**All lanes :** Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476) at 1/500 dilution (unpurified)

**Lane 1 :** SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysates
**Lane 2 :** SH-SY5Y (Human neuroblastoma epithelial cell) treated with nerve growth factor at 100ng/ml for 5 minutes. Whole cell lysates.
**Lane 3 :** SH-SY5Y (Human neuroblastoma epithelial cell) treated with nerve growth factor at 100ng/ml for 5 minutes. Whole cell lysates. Then the membrane was incubated with phosphatase.

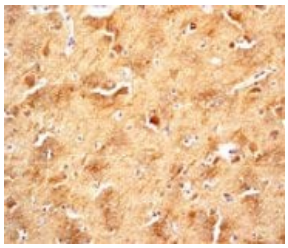
Lysates/proteins at 15 µg per lane.

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

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

**Exposure time:** 10 seconds

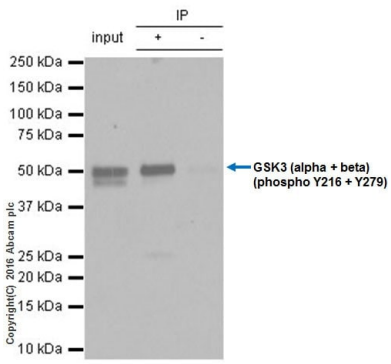
Blocking and diluting buffer and concentration: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476)

Human brain tissue stained with unpurified ab68476 at 1/100 dilution.

Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.

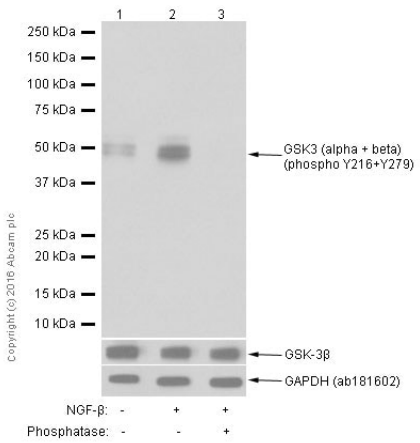



Immunoprecipitation - Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476)

GSK3 (alpha + beta) (phospho Y216 + Y279) was immunoprecipitated from 10µg HeLa (human cervix adenocarcinoma) whole cell lysate with ab68476 at 1/50 dilution (2µg in 0.35mg lysates). Western blot was performed from the immunoprecipitate using ab68476 at 1/200 dilution (9 µg/ml). VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

**Lane 1 (Input):** HeLa (human cervix adenocarcinoma) treated with 1uM staurosporine for 4h whole cell lysate 10µg
**Lane 2 (+):** HeLa (human cervix adenocarcinoma) treated with 1uM staurosporine for 4h whole cell lysate
**Lane 3:** Rabbit monoclonal IgG (**ab172730**) instead of ab68476 in HeLa (human cervix adenocarcinoma) treated with 1uM staurosporine for 4h whole cell lysate

Exposure Time: 30 seconds
Blocking and diluting buffer and concentration: 5% NFDM/TBST.




Western blot - Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476)

**All lanes :** Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476) (unpurified)

**Lane 1 :** PC12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate
**Lane 2 :** Whole cell lysate from PC12 (Rat adrenal gland pheochromocytoma cell line) cells treated with nerve growth factor-ß at 100ng/ml for 5 minutes.
**Lane 3 :** Whole cell lysate from PC12 (Rat adrenal gland pheochromocytoma cell line) cells ) treated with nerve growth factor-ß at 100ng/ml for 5 minutes. Membrane incubated with phosphatase

Lysates/proteins at 15 µg per lane.

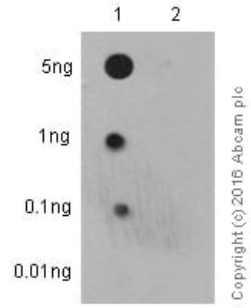
**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution (Goat Anti-Rabbit IgG H+L (HRP))

Developed using the ECL technique.

**Predicted band size:** 47-52 kDa

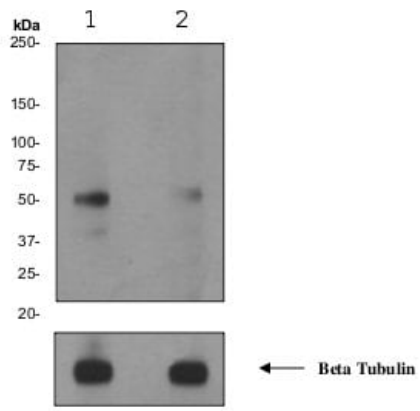
**Exposure time:** 3 seconds

Blocking and dilution buffer: 5% NFDM/TBST




Dot Blot - Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476)

Dot Blot analysis of Lane 1: Human GSK3 (alpha + beta) (pY216 + pY279) phospho peptide and Lane 2: Human GSK3 (alpha + beta) non-phospho peptide labeling GSK3 (alpha + beta) (phospho Y216 + Y279) with ab68476 at 1/1000 dilution. 5% NFDM/TBST was used as the diluting and blocking buffer. **ab97051** Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated was used as the secondary antibody at 1/100000 dilution. Exposure time: 3 minutes.




Western blot - Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476)

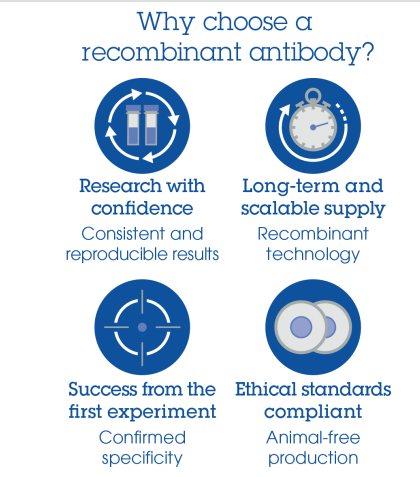
**All lanes :** Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476) at 1/2000 dilution (unpurified)

**Lane 1 :** HEK-293 (Human epithelial cell line from embryonic kidney) cell lysate, cells untreated.
**Lane 2 :** HEK-293 cell lysate, cells treated with AP.

Lysates/proteins at 10 µg per lane.

**Secondary**
**All lanes :** HRP conjugated Goat anti-rabbit at 1/2000 dilution

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




Anti-GSK3 beta (phospho Y216) + GSK3 alpha (phospho Y279) antibody [EPR933Y] (ab68476)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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