abcam

Product datasheet

Anti-EGFR (phospho Y845) antibody [EPR2149Y] ab97613

ועלשעבע RabMAb

4 References 画像数6

製品の概要

製品名 Anti-EGFR (phospho Y845) antibody [EPR2149Y]

製品の詳細 Rabbit monoclonal [EPR2149Y] to EGFR (phospho Y845)

由来種 Rabbit

アプリケーション 適用あり: Flow Cyt (Intra), Dot blot, WB, ICC/IF

適用なし: IHC-P

種交差性 交差種: Human

免疫原 Synthetic peptide within Human EGFR (phospho S845). The exact sequence is proprietary.

ポジティブ・コントロール A431 cells and cell lysates.

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 +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: 7.20

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

EPR2149Y

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ab97613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 | |
|------------------|-----------|---|--|
| Flow Cyt (Intra) | | 1/400. | |
| Dot blot | | Use at an assay dependent concentration. | |
| WB | | 1/500. Detects a band of approximately 175 kDa (predicted molecular weight: 134 kDa). | |
| ICC/IF | | 1/100 - 1/250. | |

追加情報

Is unsuitable for IHC-P.

ターゲット情報

組織特異性

機能

Receptor tyrosine kinase binding ligands of the EGF family and activating several signaling cascades to convert extracellular cues into appropriate cellular responses. Known ligands include EGF, TGFA/TGF-alpha, amphiregulin, epigen/EPGN, BTC/betacellulin, epiregulin/EREG and HBEGF/heparin-binding EGF. Ligand binding triggers receptor homo- and/or heterodimerization and autophosphorylation on key cytoplasmic residues. The phosphorylated receptor recruits adapter proteins like GRB2 which in turn activates complex downstream signaling cascades. Activates at least 4 major downstream signaling cascades including the RAS-RAF-MEK-ERK, PI3 kinase-AKT, PLCgamma-PKC and STATs modules. May also activate the NF-kappa-B signaling cascade. Also directly phosphorylates other proteins like RGS16, activating its GTPase activity and probably coupling the EGF receptor signaling to the G protein-coupled receptor signaling. Also phosphorylates MUC1 and increases its interaction with SRC and CTNNB1/betacatenin.

Isoform 2 may act as an antagonist of EGF action.

Ubiquitously expressed. Isoform 2 is also expressed in ovarian cancers.

関連疾患 Lung cancer

Inflammatory skin and bowel disease, neonatal, 2

配列類似性 Belongs to the protein kinase superfamily. Tyr protein kinase family. EGF receptor subfamily.

Contains 1 protein kinase domain.

翻訳後修飾 Phosphorylation at Ser-695 is partial and occurs only if Thr-693 is phosphorylated.

Phosphorylation at Thr-678 and Thr-693 by PRKD1 inhibits EGF-induced MAPK8/JNK1 activation. Dephosphorylation by PTPRJ prevents endocytosis and stabilizes the receptor at the plasma membrane. Autophosphorylation at Tyr-1197 is stimulated by methylation at Arg-1199 and enhances interaction with PTPN6. Autophosphorylation at Tyr-1092 and/or Tyr-1110 recruits

STAT3. Dephosphorylated by PTPN1 and PTPN2.

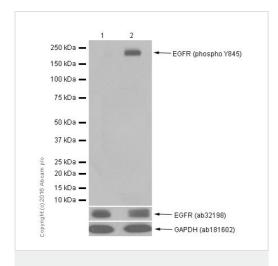
Monoubiquitinated and polyubiquitinated upon EGF stimulation; which does not affect tyrosine

kinase activity or signaling capacity but may play a role in lysosomal targeting. Polyubiquitin linkage is mainly through 'Lys-63', but linkage through 'Lys-48', 'Lys-11' and 'Lys-29' also occurs. Deubiquitination by OTUD7B prevents degradation. Ubiquitinated by RNF115 and RNF126. Methylated. Methylation at Arg-1199 by PRMT5 stimulates phosphorylation at Tyr-1197.

細胞内局在

Secreted and Cell membrane. Endoplasmic reticulum membrane. Golgi apparatus membrane. Nucleus membrane. Endosome. Endosome membrane. Nucleus. In response to EGF, translocated from the cell membrane to the nucleus via Golgi and ER. Endocytosed upon activation by ligand. Colocalized with GPER1 in the nucleus of estrogen agonist-induced cancer-associated fibroblasts (CAF).

画像



Western blot - Anti-EGFR (phospho Y845) antibody [EPR2149Y] (ab97613) **All lanes :** Anti-EGFR (phospho Y845) antibody [EPR2149Y] (ab97613) at 1/1500 dilution (purified)

Lane 1 : Untreated A431 (Human epidermoid carcinoma cell line) whole cell lysates

Lane 2: A431 (Human epidermoid carcinoma cell line) treated with 100 ng/ml EGF for 10 minutes whole cell lysates

Lysates/proteins at 15 µg per lane.

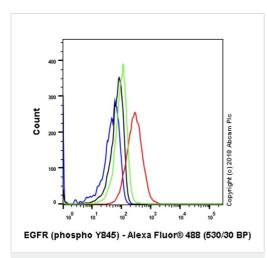
Secondary

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

Predicted band size: 134 kDa **Observed band size:** 175 kDa

Exposure time: 30 seconds

Blocking and dilution buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-EGFR (phospho Y845) antibody [EPR2149Y] (ab97613) Intracellular Flow Cytometry analysis of A431 (Human epidermoid carcinoma epithelial cell) treated with 100ng/ml EGF for 10 minutes cells labeling EGFR with purified ab97613 at 1/400 dilution (1µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit lgG (Alexa Fluorr® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue). Untreated A431 cells (Green).

| | Ab97613 Anti-EGFR (phospho Y845) | | Ab32077 Anti-EGFR | |
|-----------------|-------------------------------------|----------------------|----------------------|-------------------------------|
| Untreated A431 | Ab97613 | Ab97613+DAPI+tubulin | Ab32077 | Ab32077+DAP(+tubulin |
| A431 + EGF | | | | |
| A431 + EGF + LP | | | | Copyright (c) 2016 Abesim plo |

Immunocytochemistry/ Immunofluorescence - Anti-EGFR (phospho Y845) antibody [EPR2149Y] (ab97613)

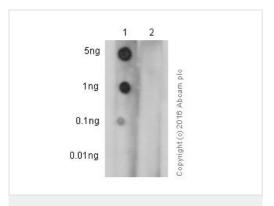
Immunofluorescent analysis of 4% paraformaldehyde-fixed,0.1% Triton X-100 permeabilized A431 (Human epidermoid carcinoma cell line) cell lines labeling EGFR with ab97613 (purified) at 1/50 dilution,followed by Goat anti-Rabbit IgG H & L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution

Cells were counterstained with $\underline{ab195889}$ (Anti-alpha Tubulin antibody [DM1A]) - Microtubule Marker (Alexa Fluor $^{\&}$ 594)

The nuclear counterstain is DAPI (blue).

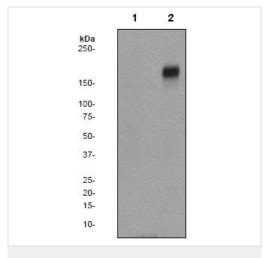
The green staining on the membrane was increased in the EGF (100ng/ml, 10min) treated A431 cells when compared with A431 cells without treatment. After LP treatment, the green signaling was obviously decreased.

For the pan antibody, there was no great difference after EGF (100ng/ml, 10min) or EGF (100ng/ml, 10min) + LP treatment. The data showed mostly membranous staining.



Dot Blot - Anti-EGFR (phospho Y845) antibody [EPR2149Y] (ab97613)

Dot Blot analysis of Lane 1: EGFR (pY845) phospho peptide and Lane 2: EGFR non-phospho peptide labeling EGFR (phospho Y845) with ab97613 (unpurified) 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-EGFR (phospho Y845) antibody [EPR2149Y] (ab97613)

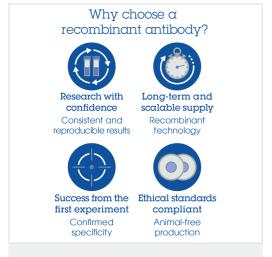
All lanes : Anti-EGFR (phospho Y845) antibody [EPR2149Y] (ab97613) at 1/500 dilution (unpurified)

Lane 1: A431 cell lysates, untreated

Lane 2: A431 cell lysates, treated with EGF

Lysates/proteins at 10 µg per lane.

Predicted band size: 134 kDa



Anti-EGFR (phospho Y845) antibody [EPR2149Y] (ab97613)

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