

Product datasheet

Anti-ErbB2 / HER2 (phospho Y1248) + ErbB4 / HER4 (phospho Y1284) antibody [EPR19547] ab201013

Recombinant RabMAb

8 Images

Overview

Product name	Anti-ErbB2 / HER2 (phospho Y1248) + ErbB4 / HER4 (phospho Y1284) antibody [EPR19547]
Description	Rabbit monoclonal [EPR19547] to ErbB2 / HER2 (phospho Y1248) + ErbB4 / HER4 (phospho Y1284)
Host species	Rabbit
Tested applications	Suitable for: WB, ICC/IF, IP, Flow Cyt (Intra), Dot blot
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, SK-BR-3 and A431 whole cell lysates starved 4 hours, then treated with 200 ng/ml EGF for 15 minutes. ICC/IF: HeLa cells treated with EGF (200ng/ml; 30min). Flow Cyt (intra): HeLa cells. IP: HeLa and A431 whole cell lysates starved 4 hours, then treated with 200 ng/ml EGF for 15 minutes. Dot blot: ErbB4 non-phospho peptide, ErbB4 Y1284 phospho peptide, ErbB2 non-phospho peptide, ErbB2 Y1248 phospho peptide
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

Properties

Form	Liquid
Storage instructions	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.
Storage buffer	<p>pH: 7.2</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA</p>

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19547
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab201013 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

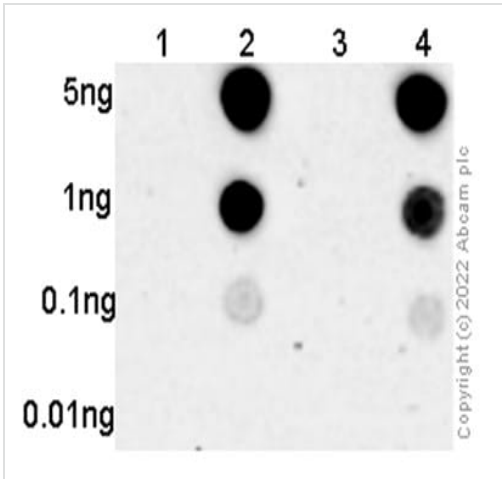
Application	Abreviews	Notes
WB		1/1000 - 1/5000. Detects a band of approximately 180 kDa (predicted molecular weight: 138, 147 kDa).
ICC/IF		1/100.
IP		1/30.
Flow Cyt (Intra)		1/50.
Dot blot		Use at an assay dependent concentration.

Target

Cellular localization

ErbB 2: Cytoplasm. Nucleus and Cell membrane. Cytoplasm, perinuclear region. Nucleus. Translocation to the nucleus requires endocytosis, probably endosomal sorting and is mediated by importin beta-1/KPNB1. ErbB 4: Membrane and Nucleus. Following proteolytical processing E4ICD (E4ICD1 or E4ICD2 generated from the respective isoforms) is translocated to the nucleus. Significantly more E4ICD2 than E4ICD1 is found in the nucleus. E4ICD2 colocalizes with YAP1 in the nucleus.

Images



Dot Blot - Anti-ErbB2 / HER2 (phospho Y1248) + ErbB4 / HER4 (phospho Y1284) antibody [EPR19547] (ab201013)

Dot blot analysis using 1/1000 dilution ab201013 and Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) secondary at 1/100000 dilution.

Blocking and diluting buffer: 5% NFDm/TBST

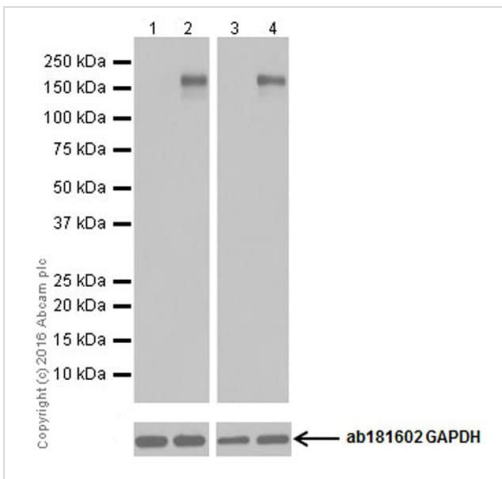
Lane 1: ErbB4 non-phospho peptide

Lane 2: ErbB4 Y1284 phospho peptide

Lane 3: ErbB2 non-phospho peptide

Lane 4: ErbB2 Y1248 phospho peptide

Exposure time: 3 minutes



Western blot - Anti-ErbB2 / HER2 (phospho Y1248) + ErbB4 / HER4 (phospho Y1284) antibody [EPR19547] (ab201013)

All lanes : Anti-ErbB2 / HER2 (phospho Y1248) + ErbB4 / HER4 (phospho Y1284) antibody [EPR19547] (ab201013) at 1/1000 dilution

Lane 1 : Untreated HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate starved 4 hours, then treated with 200 ng/ml EGF for 15 minutes

Lane 3 : Untreated A431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 4 : A431 (Human epidermoid carcinoma cell line) whole cell lysate starved 4 hours, then treated with 200 ng/ml EGF for 15 minutes

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 138, 147 kDa

Observed band size: 180 kDa

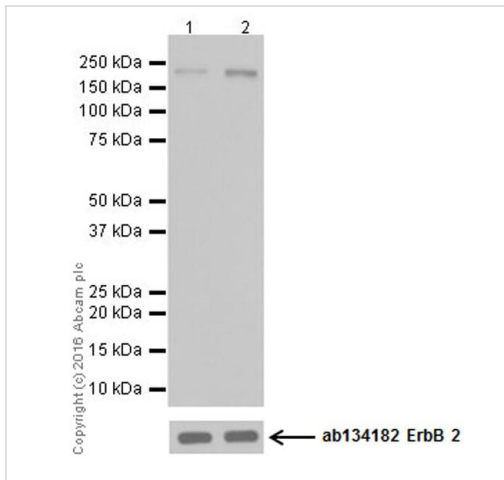
Blocking/Dilution buffer: 5% NFDm/TBST.

Exposure time: Lane 1 and 2: 5 seconds; Lane 3 and 4: 10

seconds.

This target could be induced to increase by EGF as proved by literatures (PMID: 18945363; PMID: 17030621).

The MW is also consistent with some literatures (PMID: 18180459).



Western blot - Anti-ErbB2 / HER2 (phospho Y1248)
+ ErbB4 / HER4 (phospho Y1284) antibody
[EPR19547] (ab201013)

All lanes : Anti-ErbB2 / HER2 (phospho Y1248) + ErbB4 / HER4 (phospho Y1284) antibody [EPR19547] (ab201013) at 1/5000 dilution

Lane 1 : Untreated SK-BR-3 (human mammary gland adenocarcinoma) whole cell lysate

Lane 2 : SK-BR-3(human mammary gland adenocarcinoma) whole cell lysate starved 4 hours, then treated with 200 ng/ml EGF for 15 minutes

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 138, 147 kDa

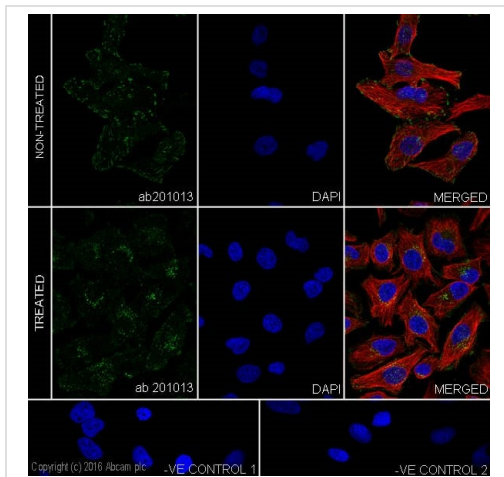
Observed band size: 180 kDa

Exposure time: 30 seconds

Blocking/Diluting buffer 5% NFDm/TBST.

This target could be induced to increase by EGF that had be proved by literatures(PMID: 18945363; PMID: 17030621).

The MW is also consist with some literatures showed(PMID: 18180459)



Immunocytochemistry/ Immunofluorescence - Anti-ErbB2 / HER2 (phospho Y1248) + ErbB4 / HER4 (phospho Y1284) antibody [EPR19547] (ab201013)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling ERBB2 (phospho Y1248) + ERBB4 (phospho Y1284) with ab201013 at 1/100 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing increased cytoplasmic staining after EGF treatment (200ng/ml; 30min).

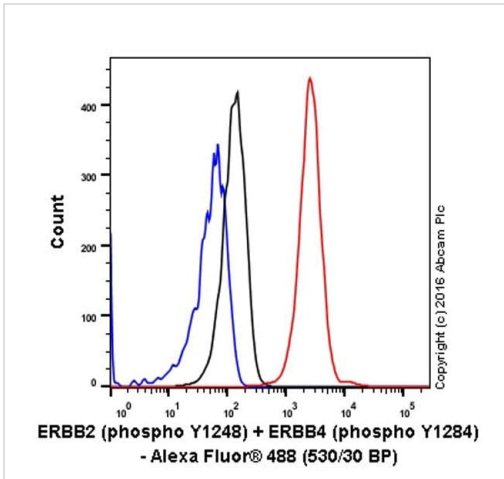
The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) at 1/1000 dilution (red).

The negative controls are as follows:-

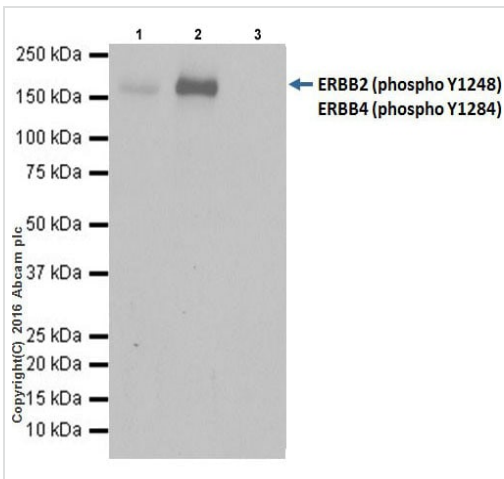
-ve control 1: ab201013 at 1/100 dilution followed by **ab150120** at 1/1000 dilution.

-ve control 2: **ab7291** at 1/1000 dilution followed by **ab150077** at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-ErbB2 / HER2 (phospho Y1248) + ErbB4 / HER4 (phospho Y1284) antibody [EPR19547] (ab201013)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling ERBB2 (phospho Y1248) + ERBB4 (phospho Y1284) with ab201013 at 1/50 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A]-isotype control (**ab172730**) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit IgG (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-ErbB2 / HER2 (phospho Y1248) + ErbB4 / HER4 (phospho Y1284) antibody [EPR19547] (ab201013)

ERBB2 (phospho Y1248) + ERBB4 (phospho Y1284) were immunoprecipitated from 0.35 mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate, starved 4 hours, then treated with 200 ng/ml EGF for 15 minutes, with ab201013 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab201013 at 1/500 dilution.

VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

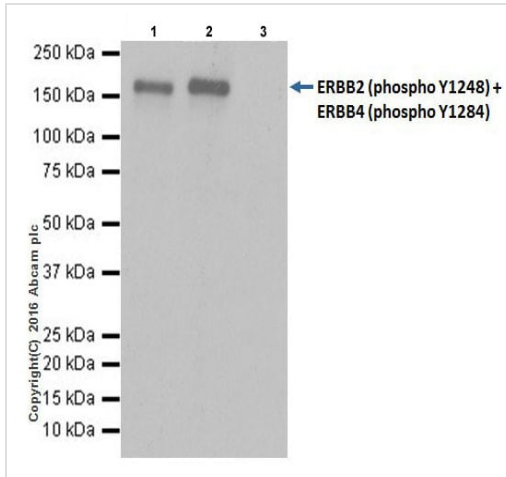
Lane 1: HeLa whole cell lysate starved 4 hours, then treated with 200 ng/ml EGF for 15 minutes 10µg (Input).

Lane 2: ab201013 IP in HeLa whole cell lysate starved 4 hours, then treated with 200 ng/ml EGF for 15 minutes.

Lane 3: Rabbit IgG, monoclonal [EPR25A]- Isotype Control (**ab172730**) instead of ab201013 in HeLa whole cell lysate starved 4 hours, then treated with 200 ng/ml EGF for 15 minutes.

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

Exposure time: 1 second.



Immunoprecipitation - Anti-ErbB2 / HER2 (phospho Y1248) + ErbB4 / HER4 (phospho Y1284) antibody [EPR19547] (ab201013)

ERBB2 (phospho Y1248) + ERBB4 (phospho Y1284) were immunoprecipitated from 0.35 mg of A431 (Human epidermoid carcinoma cell line) whole cell lysate, starved 4 hours, then treated with 200 ng/ml EGF for 15 minutes, with ab201013 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab201013 at 1/500 dilution.

VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

Lane 1: A431 whole cell lysate starved 4 hours, then treated with 200 ng/ml EGF for 15 minutes 10 μ g (Input).

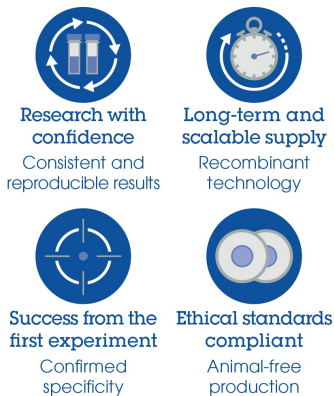
Lane 2: ab201013 IP in A431 whole cell lysate starved 4 hours, then treated with 200 ng/ml EGF for 15 minutes.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab201013 in A431 whole cell lysate starved 4 hours, then treated with 200 ng/ml EGF for 15 minutes.

Blocking and dilution buffer and concentration: 5% NFDm/TBST.

Exposure time: 1 second.

Why choose a recombinant antibody?



Anti-ErbB2 / HER2 (phospho Y1248) + ErbB4 / HER4 (phospho Y1284) antibody [EPR19547] (ab201013)

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