abcam

Product datasheet

Anti-ErbB4 / HER4 (phospho Y1162) antibody [EP2270Y] ab68478

Recombinant RabMAb

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Overview

Product name	Anti-ErbB4 / HER4 (phospho Y1162) antibody [EP2270Y]
Description	Rabbit monoclonal [EP2270Y] to ErbB4 / HER4 (phospho Y1162)
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP, IHC-P **Unsuitable for:** Flow Cyt or ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: A431 treated with 100 ng/ml EGF. IHC-P: Human breast carcinoma tissue. IP: A431 cells.
General notes	This product has switched from a hybridoma to recombinant production method on 31st May 2023. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EP2270Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab68478 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		1/5000. Predicted molecular weight: 200 kDa.
IP		1/20.
IHC-P	★★★★☆ (1)	1/1000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Application notes Is unsuitable for Flow Cyt or ICC/IF.

Target

Function Specifically binds and is activated by neuregulins, NRG-2, NRG-3, heparin-binding EGF-like growth factor, betacellulin and NTAK. Interaction with these factors induces cell differentiation. Not activated by EGF, TGF-A, and amphiregulin. The C-terminal fragment (CTF) of isoform JMA-A CYT-2 (containing E4ICD2) can stimulate transcription in the presence of YAP1. ERBB4 intracellular domain is involved in the regulation of cell growth. Conflicting reports are likely due at least in part to the opposing effects of the isoform-specific and nuclear-translocated ERBB4 intracellular domains (E4ICD1 and E4ICD2). Overexpression studies in epithelium show growth inhibition using E4ICD1 and increased proliferation using E4ICD2. E4ICD2 has greater in vitro kinase activity than E4ICD1. The kinase activity is required for the nuclear translocation of E4ICD2.

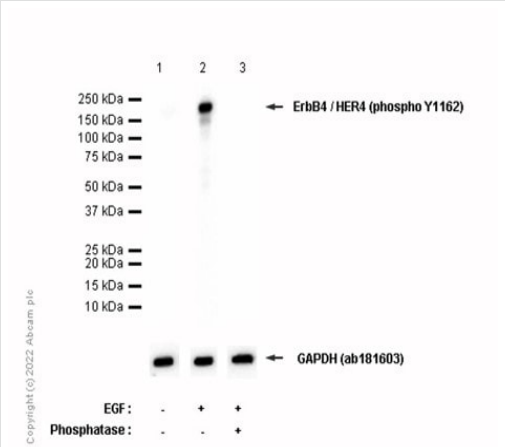
Tissue specificity Expressed at highest levels in brain, heart, kidney, in addition to skeletal muscle, parathyroid, cerebellum, pituitary, spleen, testis and breast. Lower levels in thymus, lung, salivary gland, and pancreas. Isoform JM-A CYT-1 and isoform JM-B CYT-1 are expressed in cerebellum, but only the isoform JM-B is expressed in the heart.

Sequence similarities Belongs to the protein kinase superfamily. Tyr protein kinase family. EGF receptor subfamily. Contains 1 protein kinase domain.

Post-translational modifications Isoform JM-A CYT-1 and isoform JM-A CYT-2 but not isoform JM-B CYT-1 and isoform JM-B CYT-2 are processed by ADAM17. Proteolytic processing in response to ligand or 12-O-tetradecanoylphorbol-13-acetate stimulation results in the production of 120 kDa soluble receptor forms and intermediate membrane-anchored 80 kDa fragments (m80HER4), which are further processed by a presenilin-dependent gamma-secretase to release the respective cytoplasmic intracellular domain E4ICD (either E4ICD1/s80Cyt1 or E4ICD2/s80Cyt2). Membrane-anchored 80 kDa fragments of the processed isoform JM-A CYT-1 are more readily degraded by the proteasome than fragments of isoform JM-A CYT-2 suggesting a prevalence of E4ICD2 over E4ICD1.
Ligand-binding increases phosphorylation on tyrosine residues. Isoform JM-A CYT-2 is constitutively phosphorylated on tyrosine residues in a ligand-independent manner. E4ICD2 but not E4ICD1 is phosphorylated on tyrosine residues.

	Ubiquitinated. The ERBB4 intracellular domain is ubiquitinated and targeted to proteosomal degradation during mitosis mediated by the APC/C complex. Isoform JM-A CYT-1 and isoform JM-B CYT-1 are ubiquitinated by WWP1. The ERBB4 intracellular domain (E4ICD1) is ubiquitinated, and this involves NEDD4.
Cellular localization	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



Western blot - Anti-ErbB4 / HER4 (phospho Y1162) antibody [EP2270Y] (ab68478)

All lanes : Anti-ErbB4 / HER4 (phospho Y1162) antibody [EP2270Y] (ab68478) at 1/5000 dilution

Lane 1 : A431 (Human epidermoid carcinoma epithelial cell) whole cell lysate
Lane 2 : A431 (Human epidermoid carcinoma epithelial cell) treated with 100 ng/ml EGF for 30 min whole cell lysate
Lane 3 : A431 (Human epidermoid carcinoma epithelial cell) treated with 100 ng/ml EGF for 30 min whole cell lysate, then membrane treated with Alkaline phosphatase

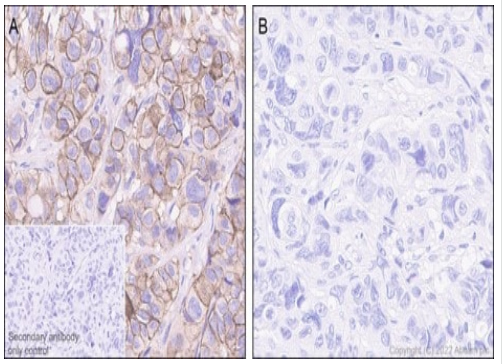
Lysates/proteins at 15 µg per lane.

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

Predicted band size: 200 kDa
Observed band size: 200 kDa

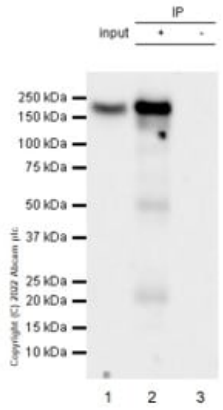
Exposure time: 10 seconds

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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ErbB4 / HER4 (phospho Y1162) antibody [EP2270Y] (ab68478)

Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labelling ErbB4 / HER4 with ab68478 at 1/1000 dilution, followed by **ab209101** Rabbit specific IHC polymer detection kit HRP/DAB. Positive staining on Human breast carcinoma without alkaline phosphatase treatment (A) compared to no signal detected when treated with alkaline phosphatase (B). The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, epitope retrieval Solution2) for 30 mins. Counterstained with hematoxylin.



Immunoprecipitation - Anti-ErbB4 / HER4 (phospho Y1162) antibody [EP2270Y] (ab68478)

ErbB4 / HER4 (phospho Y1162) was immunoprecipitated from 0.35 mg of A431 treated with EGF (100ng/ml 30min) whole cell lysate with ab68478 at 1/20 dilution. Western blot was performed from the immunoprecipitate using ab68478 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used as secondary antibody at 1/5000 dilution.

Lane 1: A431 treated with EGF (100ng/ml 30min) whole cell lysate.
Lane 2: ab68478 IP in A431 treated with EGF (100ng/ml 30min) whole cell lysate.
Lane 3: Isotype control instead of ab68478 in A431 treated with EGF (100ng/ml 30min) whole cell lysate.

Anti-ErbB4 / HER4 (phospho Y1162) antibody [EP2270Y] (ab68478)

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