# abcam

# Product datasheet

# Anti-ErbB4 / HER4 antibody [E200] ab32375

Recombinant RabMAb

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Overview

**Product name** Anti-ErbB4 / HER4 antibody [E200]

**Description** Rabbit monoclonal [E200] to ErbB4 / HER4

**Host species** Rabbit

Specificity This antibody is specific to ErbB4 / HER4. It does not cross react with other EGF receptor family

members.

Suitable for: WB, IP **Tested applications** 

Unsuitable for: ICC/IF

Reacts with: Human Species reactivity

Predicted to work with: Mouse, Rat

**Immunogen** Synthetic peptide within Human ErbB4/ HER4 (C terminal). The exact sequence is proprietary.

Positive control WB: MCF7 and T47D cell lysate. IP: HEK-293 cell lysate.

**General notes** 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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

**Clonality** Monoclonal

Clone number E200

**Isotype** IgG

#### **Applications**

## The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab32375 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 - 1/10000. Predicted molecular weight: 147 kDa.
IP		1/100.

**Application notes** 

Is unsuitable for 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.

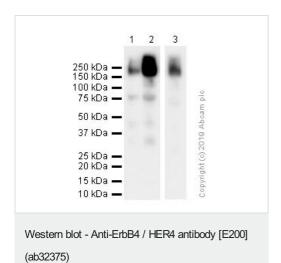
Ubiquitinated. The ERBB4 intracellular domain is ubiquitinated and targeted to proteosomal

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**



All lanes : Anti-ErbB4 / HER4 antibody [E200] (ab32375) at 1.09  $\mu g/ml$ 

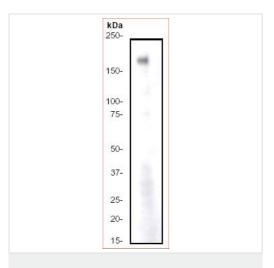
Lane 1: MCF7 (Human breast adenocarcinoma epithelial cell)
Lane 2: T47D (Human ductal breast epithelial tumor epithelial cell)
Lane 3: T47D (Human ductal breast epithelial tumor epithelial cell)
low exposure image of lane 2

Lysates/proteins at 20 µg per lane.

# Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) ( $\underline{ab97051}$ ) at 0.05  $\mu g/ml$ 

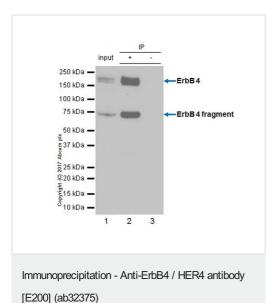
Predicted band size: 147 kDa



Western blot - Anti-ErbB4 / HER4 antibody [E200] (ab32375)

Anti-ErbB4 / HER4 antibody [E200] (ab32375) at 1/4000 dilution + MCF7 cell lysate

**Predicted band size:** 147 kDa **Observed band size:** 185 kDa



Lane 1 (input): HEK-293 (human embryonic kidney epithelial cell) whole cell lysate, 10µg

Lane 2 (+): HEK-293 whole cell lysate

Lane 3 (-): Rabbit monoclonal lgG (ab172730) instead of ab32375

in HEK-293 whole cell lysate

Ab32375 Immunoprecipitating ErbB 4 in HEK-293 whole cell lysate. Capture antibody was used at a 1:50 dilution (2µg in 0.35mg lysates). For western blotting, primary antibody used as ab32375 at 1:500 dilution. Ab131366 VeriBlot for IP Detection Reagent (HRP) was used for detection at 1:1000 dilution. The lower band at around 75kDa should be proteolysis fragment based on the literature.

(PMID: 9362517)

Blocking and diluting buffer: 5% NFDM/TBST



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