Product datasheet

Anti-ErbB 4 antibody [HFR-1] ab19391

Overview

Product name
Anti-ErbB 4 antibody [HFR-1]

Description
Mouse monoclonal [HFR-1] to ErbB 4

Host species
Mouse

Tested applications
Suitable for: Flow Cyt, ICC/IF, WB, IP, IHC-P, IHC-Fr

Species reactivity
Reacts with: Mouse, Human
Predicted to work with: Rat, Chicken

Immunogen
Synthetic peptide:
(R)RSTLQHPDYLQEYST
, corresponding to amino acids 1250-1264 of HER4.

Positive control
Human A431 cell and mouse NIH3T3/HER4 cell extracts (western blot). NIH3T3 cells (IHC-P).

Properties

Form
Liquid

Storage instructions
Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer
Preservative: 0.05% Sodium azide
Constituents: PBS, 0.1% BSA

Purity
Protein A purified

Clonality
Monoclonal

Clone number
HFR-1

Isotype
IgG2b

Applications

Our Abpromise guarantee covers the use of ab19391 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
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.

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.

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

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

<table>
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<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tr>
<td>Flow Cyt</td>
<td>ab170192</td>
<td>Use 2µg for 10^6 cells. Mouse monoclonal IgG2b is suitable for use as an isotype control with this antibody.</td>
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<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐</td>
<td>Use a concentration of 1 - 5 µg/ml.</td>
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<tr>
<td>WB</td>
<td></td>
<td>Use a concentration of 3 µg/ml. Detects a band of approximately 185 kDa (predicted molecular weight: 147 kDa).</td>
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<tr>
<td>IP</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IHC-P</td>
<td></td>
<td>Use a concentration of 6 - 8 µg/ml.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
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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**

Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized Human Breast carcinoma tissue. To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:20 with a mouse monoclonal antibody recognizing ErbB4 (ab19391) or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP, followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.

ICC/IF image of ab19391 stained HepG2 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab19391, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.
Overlay histogram showing HEK293 cells stained with ab19391 (red line). The cells were fixed with methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab19391, 2μg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (ab91366, 2μg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HEK293 cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.

Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized human Heart tissue. To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:200 with a mouse monoclonal antibody recognizing ErbB4 (ab19391) or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP, followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.

Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized Human Pancreas tissue. To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:200 with a mouse monoclonal antibody recognizing ErbB4 (ab193911) or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP, followed by colorimetric detection using DAB. Tissues were
counterstained with hematoxylin and prepped for mounting.

ab19391 staining ErbB 4 in Mouse brain tissue by Immunohistochemistry (Frozen sections). The sections were fixed with paraformaldehyde. The primary antibody was used neat and incubated with the sample for 12 hours at 4°C. A Biotin-conjugated Rabbit anti-Mouse polyclonal was used as the secondary antibody.

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