abcam

Product datasheet

Anti-EGFR antibody [ICR10] ab231

★★★★★ 5 Abreviews   15 References   4 Images

Overview

Product name	Anti-EGFR antibody [ICR10]
Description	Rat monoclonal [ICR10] to EGFR
Host species	Rat
Tested applications	**Suitable for:** Inhibition Assay, Flow Cyt, ELISA, ICC/IF, IP, IHC-Fr, ICC **Unsuitable for:** WB
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Extracellular domain of Human EGF-receptor from head and neck carcinoma
Epitope	ICR10 binds to epitope B, and has an affinity of 6.7×10^{-9} M.
Positive control	FACS: A431 cells IHC: Breast Carcinoma tissue WB: HN5 cell lysate

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.09% Sodium azide Constituent: PBS
Purity	Ion Exchange Chromatography
Purification notes	Purified IgG prepared by ion exchange chromatography.
Clonality	Monoclonal
Clone number	ICR10
Myeloma	unknown
Isotype	IgG2a
Light chain type	unknown

Applications

Our Abpromise guarantee covers the use of **ab231** 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
Inhibition Assay		Use at an assay dependent concentration. See ELISA Abreview.
Flow Cyt	★★★★★	1/50 - 1/100. ab18450 - Rat monoclonal IgG2a, is suitable for use as an isotype control with this antibody.
ELISA	★★★★☆	Use at an assay dependent concentration.
ICC/IF		Use a concentration of 5 µg/ml.
IP		Use at an assay dependent concentration.
IHC-Fr	★★★★★	Use at an assay dependent concentration.
ICC		Use at an assay dependent concentration.

Application notes		Is unsuitable for WB.

Target

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Function	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/beta-catenin. Isoform 2 may act as an antagonist of EGF action.
Tissue specificity	Ubiquitously expressed. Isoform 2 is also expressed in ovarian cancers.
Involvement in disease	Lung cancer Inflammatory skin and bowel disease, neonatal, 2
Sequence similarities	Belongs to the protein kinase superfamily. Tyr protein kinase family. EGF receptor subfamily. Contains 1 protein kinase domain.
Post-translational modifications	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.

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	Deubiquitination by OTUD7B prevents degradation. Ubiquitinated by RNF115 and RNF126. Methylated. Methylation at Arg-1199 by PRMT5 stimulates phosphorylation at Tyr-1197.
Cellular localization	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).

Images
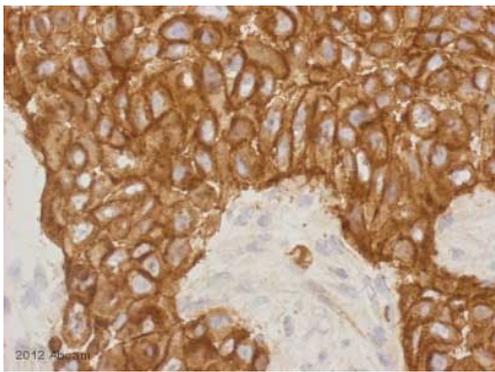
Immunohistochemistry (Frozen sections) - Anti-EGFR antibody [ICR10] (ab231)

Image courtesy of an anonymous Abreview.

ab231 staining EGFR in human tumor cell line xenograft in mouse by Immunohistochemistry (Frozen sections).
Tissue was fixed in acetone. Samples incubated with ab231 at a 1/100 dilution for 1 hour at 23°C. The secondary used was an undiluted HRP conjugated goat anti-rat polyclonal.
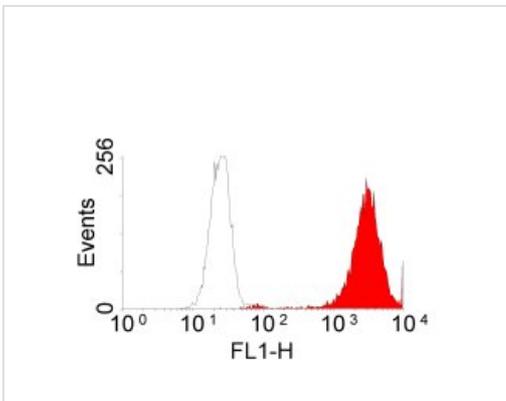

Flow Cytometry - Anti-EGFR antibody [ICR10] (ab231)

Staining of A431 cells with ab231 visualised with F(ab')2 rabbit anti-rat FITC conjugated secondary.
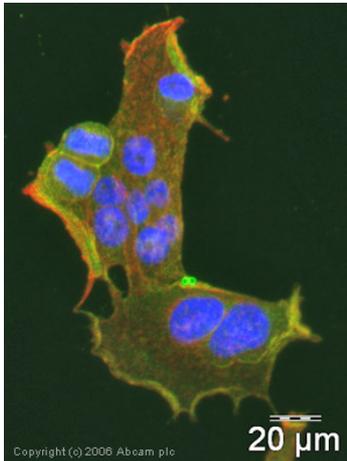
ICC/IF image of ab231 stained human HeLa cells. The cells were methanol fixed (5 min) and incubated with the antibody (ab231, 5µg/ml) for 1h at room temperature. The secondary antibody (green) was Alexa Fluor® 488 donkey anti-rat IgG (H+L) used at a 1/1000 dilution for 1h. Image-iT^{TM} FX Signal Enhancer was used as the primary blocking agent, 5% BSA (in TBS-T) was used for all other blocking steps. DAPI was used to stain the cell nuclei (blue). Alexa Fluor® 594 WGA was used to label plasma membranes (red).

Immunocytochemistry/ Immunofluorescence - Anti-EGFR antibody [ICR10] (ab231)
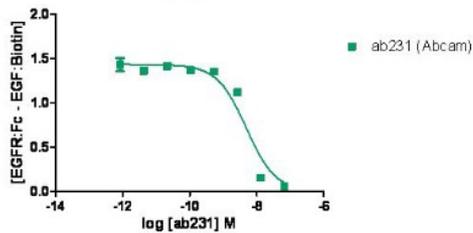
ab231 used undiluted in inhibition assay against human recombinant protein EGFR1:Fc to inhibit binding to EGF:Biotin. The primary incubated with sample for 1 hour. The antibody inhibits the binding of biotinylated EGF to coated EGFR:Fc with an IC50 ~ 5 nM.

Inhibition Assay - Anti-EGFR antibody [ICR10] (ab231)

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