Anti-Phospho-HER2 / ErbB2 (Y1139) Antibody [JE60-90] HA722201

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 138 kDa
Clone number:	JE60-90

Description: HER-2 (also called HER-2/neu, c-erbB2, ERBB2 or neu) is a transmembrane receptor tyrosine kinase. HER-2 is a proto-oncogene, i.e. its activation causes malignant transformation and increases the malignant potential (cell proliferation, invasiveness etc.) of the cells. Amplification of HER-2 gene invariably leads to over-expression of its protein product. The magnitude of over- expression is usually 10-100 folds or even more. Over-expressed HER-2 protein disturbs the HER-receptor family signalling networks, i.e. signalling mediated via EGFR receptor, HER-3 and HER-4. In tumours, HER-2 is over-expressed in 15-25% of primary breast cancers. Metastases usually have the same amplification status as the primary tumours. HER-2 amplification and over-expression are typical features of hormone receptor negative, rapidly growing histologic grade 2-3 tumours. Of the histologic types, Paget's disease is almost invariably HER-2 positive, whereas only a small minority of lobular and tubular carcinomas shows HER-2 amplification. HER-2 amplification and over-expression can also be found in intestinal type gastric and gastroesophageal carcinomas, ovarian carcinomas, high grade endometrial carcinomas and some salivary duct tumours. Low-level copy number increases have been found also in rare cases of lung tumours. Because of its central importance in breast cancer therapy selection, standardization of HER-2 IHC assays and slide interpretation are of outmost clinical and economical importance.

Immunogen: Synthetic phosphopeptide corresponding to residues surrounding Tyr1139 of Human HER2.

Positive control: A431 cell lysate, A431 treated with 100ng/mL EGF for 10 minutes cell lysate, SK-Br-3 cell lysate, SK-Br-3 starved for 4 hours add 200ng/mL EGF for 15 minutes cell lysate, A431 cells treated with 100ng/mL EGF for 10 minutes.

Subcellular location: Cell membrane, Cell projection, ruffle membrane; Early endosome, Cytoplasm, perinuclear region, Nucleus.

Database links: SwissProt: P04626 Human

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:5,000
FC	1:10,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

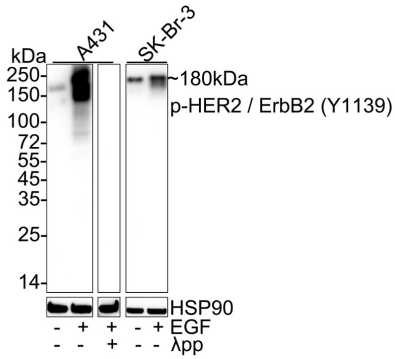

Fig1: Western blot analysis of Phospho-HER2 / ErbB2 (Y1139) on different lysates with Rabbit anti-Phospho-HER2 / ErbB2 (Y1139) antibody (HA722201) at 1/1,000 dilution.

Lane 1: A431 cell lysate
Lane 2: A431 treated with 100ng/mL EGF for 10 minutes cell lysate
Lane 3: A431 treated with 100ng/mL EGF for 10 minutes cell lysate, then the membrane treated with λpp for 1 hour
Lane 4: SK-Br-3 cell lysate
Lane 5: SK-Br-3 starved for 4 hours add 200ng/mL EGF for 15 minutes cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 138 kDa
Observed band size: 180 kDa

Exposure time: 10 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722201) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

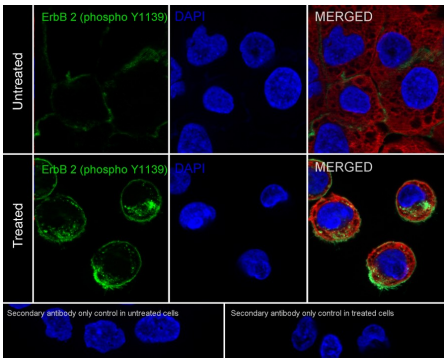

Fig2: Immunocytochemistry analysis of A431 cells treated with or without 100ng/mL EGF for 10 minutes labeling Phospho-HER2 / ErbB2 (Y1139) with Rabbit anti-Phospho-HER2 / ErbB2 (Y1139) antibody (HA722201) at 1/5,000 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-HER2 / ErbB2 (Y1139) antibody (HA722201) at 1/5,000 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

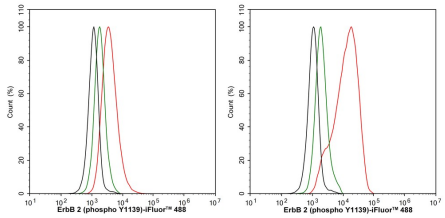

Fig3: Flow cytometric analysis of A431 cells (left) and A431 cells treated with 100ng/mL EGF for 10 minutes (right) labeling Phospho-HER2 / ErbB2 (Y1139).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722201, 1/10,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Fernandez AI et al. Examination of Low ERBB2 Protein Expression in Breast Cancer Tissue. JAMA Oncol. 2022 Apr
2. Augustin JE et al. Targeting the complexity of ERBB2 biology in gastroesophageal carcinoma. Ann Oncol. 2022 Nov