

Anti-HER2 / ErbB2 Antibody [PD00-53]

HA721178



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	IHC-P, WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 138 kDa
Clone number:	PD00-53

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, Page'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: Recombinant protein within Human ErbB2/ HER2 aa 500-650.

Positive control: Human breast carcinoma tissue, SK-Br-3 cell lysates, MCF-7, SK-Br-3.

Subcellular location: Cell membrane, Nucleus, Cytoplasm.

Database links: SwissProt P04626 Human

Recommended Dilutions:

IHC-P	1:2,000
WB	1:1,000
IF-Cell	1:50
FC	1:1,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% SodiumAzide.

Storage Instruction: Store at +4°C after thawing. Aliquot store at -20°C. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

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Images

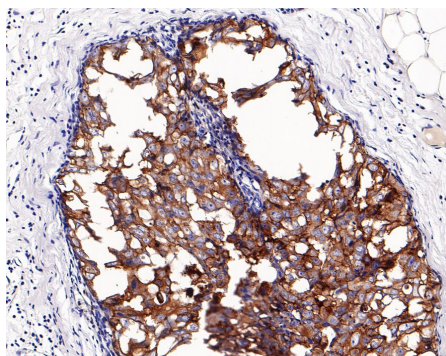


Fig1: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-HER2 / ErbB2 antibody (HA721178) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA721178) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

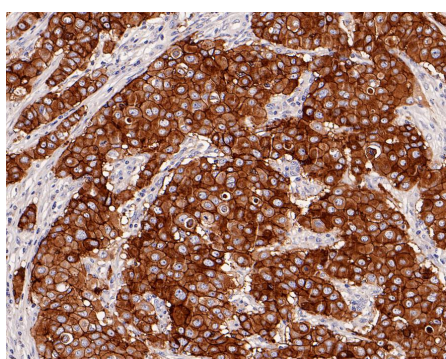
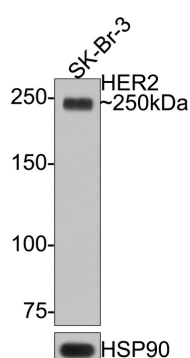


Fig2: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-HER2 / ErbB2 antibody (HA721178) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA721178) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Fig3: Western blot analysis of HER2 / ErbB2 on SK-Br-3 cell lysates with Rabbit anti-HER2 / ErbB2 antibody (HA721178) at 1/1,000 dilution.



Lysates/proteins at 10 µg/Lane.

Predicted band size: 138 kDa
Observed band size: 250 kDa

Exposure time: 2 minutes;

6% 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 (HA721178) at 1/1,000 dilution was used in 5% NFDm/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

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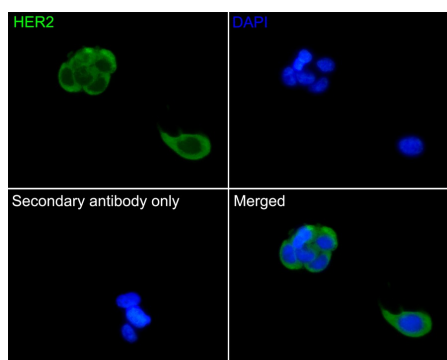


Fig4: Immunocytochemistry analysis of MCF-7 cells labeling HER2 / ErbB2 with Rabbit anti-HER2 / ErbB2 antibody (HA721178) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 °C, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-HER2 / ErbB2 antibody (HA721178) at 1/50 dilution in 2% negative goat serum overnight at 4 °C. 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.

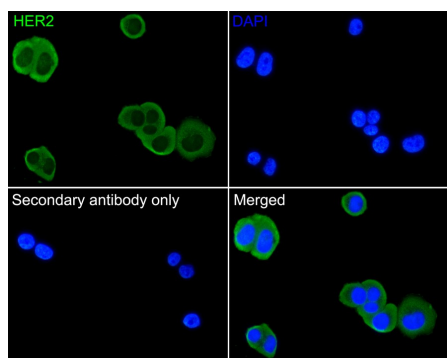


Fig5: Immunocytochemistry analysis of SK-Br-3 cells labeling HER2 / ErbB2 with Rabbit anti-HER2 / ErbB2 antibody (HA721178) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 °C, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-HER2 / ErbB2 antibody (HA721178) at 1/50 dilution in 2% negative goat serum overnight at 4 °C. 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.

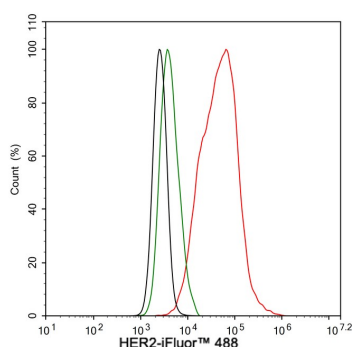


Fig6: Flow cytometric analysis of SK-Br-3 cells labeling HER2 / ErbB2.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721178, 1µg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C 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°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are “FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE”.

Background References

1. Ellis CM, Dyson MJ, Stephenson TJ, Maltby EL. HER2 amplification status in breast cancer: a comparison between immunohistochemical staining and fluorescence in situ hybridisation using manual and automated quantitative image analysis scoring techniques. *J Clin Pathol.* 2005 Jul;58(7):710-4.
2. Ganberg D, Jarvinen T, di Leo A, Rouas G, Cardoso F, Paesmans M, Verhest A, Piccart MJ, Isola J, Larsimont D. Evaluation of HER-2/NEU protein expression in breast cancer by immunohistochemistry: an interlaboratory study assessing the reproducibility of HER-2/NEU testing. *Breast Cancer Res Treat.* 2002 Jul;74(2):113-20.

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