ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Human Her-2/neu(erbB-2) protein Antibodies

□ **Cat #** HER21-M	Mouse Monoclonal anti-human Her-2/neu(erbB-2) protein IgG, aff pure	**SIZE**: 100 ug
□ **Cat #** HER21-C	Recombinant human Her-2/neu(erbB-2)-Fc protein control for WB	**SIZE**: 100 ul

HER2/neu (also known as ErbB-2, ERBB2) is a protein (protein accession # P04626; 1255 aa, ~185 kda, chromosome 17q21.1) highly expressed in breast cancers. It is a cell membrane surface-bound receptor tyrosine kinase and is normally involved in the signal transduction pathways leading to cell growth and differentiation. The oncogene neu is so-named because it was derived from a neuroglioblastoma cell line in rat. ErbB2 was named for its similarity to ErbB (avian erythroblastosis oncogene B). Herstatin, as the product of alternative HER-2 transcript, retains intron 8. The herstatin mRNA is expressed in normal human fetal kidney and liver, but is at reduced levels relative to p185HER-2 mRNA in carcinoma cells that contain an amplified HER-2 gene. Herstatin appears to be an inhibitor of p185HER-2, because it disrupts dimers, reduces tyrosine phosphorylation of p185, and inhibits the anchorage-independent growth of transformed cells that overexpress HER-2

ERBB2 overexpression confers resistance to taxol-induced apoptosis by inhibiting p34(CDC2) activation. One mechanism is via ERBB2-mediated upregulation of p21(CIP1), or CDKN1A, which inhibits CDC2. Overexpression also occurs in other cancer such as ovarian cancer and stomach cancer. Clinically, HER2/neu is important as the target of the monoclonal antibody trastuzumab (marketed as Herceptin). Trastuzumab is only effective in breast cancer where the HER2/neu receptor is overexpressed. One of the mechanisms of how traztuzumab works after it binds to HER2 is by increasing p27, a protein that halts cell proliferation. Another monoclonal antibody, pertuzumab, which inhibits dimerization of HER2 and HER3 receptors, is in advanced clinical trials.

Protein name Receptor tyrosine-protein kinase erbB-2 [Precursor] Synonyms EC 2.7.10.1, p185erbB2, C-erbB-2, NEU proto-oncogene, Tyrosine kinase-type cell surface receptor HER2, MLN 19, CD340 antigen HER2, NEU, NGL
Gene name Name: ERBB2

Sources of antigen and antibodies

Antigen	Recombinant purified human ErbB2 extracellular domain 23-652 aa, protein accession #P04626)
Ab Host/type	Mouse, monoclonal IgG2b, Aff pure IgG (**cat # HER21-M**)
2-ab	**Goat Anti-mouse IgG-HRP conjugate** Cat # 40320 (AP, biotin, FITC conjugates also available)
-ve control IgG	Cat # 20008-1, Mouse (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control

Human Erbb2.neu protein (23-652aa) was expressed in NSO cells as human IgG-Fc (100-330aa) his-tag fusion protein and purified (>95%). Recombinant Erbb2-Fc chimeric protein (#HER21-C) is ~130 kda under reducing conditions. For Western blot +ve control (**Cat # HER21-C**) is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of **HER21**-C for good visibility with antibody Cat # **HER21**-M. Store at –20oC in suitable size aliquots. SDS may crystallize in cold conditions. It should redissolve by warming before taking it from the stock. It should be heated once prior to loading on gels. If the product has been stored for several weeks, then it may be preferable to add 5 ul of fresh 2x sample buffer per 10 ul of the **HER21**-C solution prior to heating and loading on gels. This preparation is not biologically active. It is not suitable for ELISA or other applications where native protein is required. Do not freeze, thaw, or heat repeatedly

Form & Storage of Peptide and Antibodies

Affinity pure IgG
□ 100 ug/100ul □ solution □ lyophilized powder in PBS pH 7.4 +5% Trehalose;
Reconstitute powder in PBS at 1 mg/ml

Storage
Short-term: Liquid , unopened, undiluted vials for less than a week at 4oC and powder up to several months at 4oC.
Long-term: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20oC or below.
Shipping: 4oC for solutions and room temp for lyophilized items.

Recommended Usage

Western blot: Optimal dilution must be determined by user. We suggest initial testing of antibody at 1-2 ug/ml using ECL. Full length Acrp30 is ~30 kDa. However, recombinant Acrp30 has given a mol wt of ~37 kDa.

ELISA (1:10-50K; 10-100 ng of control peptide/well).

Immunohistochemistry: We suggest testing of aff pure IgG at 5-15 ug/ml using paraffin embedded sections.

Flow cytometry – use 5-10 ug per sample (~1-3 x10^5 cells) in ~200 ul.

Specificity and crossreactivity

Antibody # HER21-M is specific for human ErbB2 with no reaction with human EGFR, Erbb3 or ErbB4. Other species not tested. Human recombinant ErbB2-Fc chimeric protein (#HER21-C) can be used as positive control.

General References: (1) Yamamoto T (1986) Nature 319, 230-234; Semba K (1985) PNAS 82, 6497-6501; Akiyama T (1986) Science 232, 1644-1646; Bargmann CI (1986) Nature 319, 226-230; Coussens L (1985) Science 230, 1132-1139; Doherty JK (1999) PNAS 96, 10689-10874

This product is for In vitro research use only.

Related items
Anti-ErbB1-4 and recombinant proteins

HER21-M-C 80325A

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