Human Phospho-ErbB2/Her2 (Y1248)

Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG

Catalog Number: AF1768

**DESCRIPTION**

<table>
<thead>
<tr>
<th>Species Reactivity</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>Detects human ErbB2 when phosphorylated at Y1248.</td>
</tr>
<tr>
<td>Source</td>
<td>Polyclonal Rabbit IgG</td>
</tr>
<tr>
<td>Purification</td>
<td>Antigen Affinity-purified</td>
</tr>
<tr>
<td>Immunogen</td>
<td>Phosphopeptide containing human ErbB2 Y1248 site</td>
</tr>
<tr>
<td>Formulation</td>
<td>Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.</td>
</tr>
</tbody>
</table>

*Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

**APPLICATIONS**

Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

<table>
<thead>
<tr>
<th>Recommended Concentration</th>
<th>Sample</th>
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</thead>
<tbody>
<tr>
<td>Western Blot</td>
<td>0.25 µg/mL See Below</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>0.3-15 µg/mL See Below</td>
</tr>
<tr>
<td>Simple Western</td>
<td>5 µg/mL See Below</td>
</tr>
</tbody>
</table>

**DATA**

**Western Blot**

Detection of Human Phospho-ErbB2/Her2 (Y1248) by Western Blot. Western blot shows lysates of MDA-MB-468 human breast cancer cell line and A431 human epidermoid carcinoma cell line untreated (-) or treated (+) with 1 mM Pervanadate (PV) for 10 minutes or with 10 ng/mL Recombinant Human EGF (Catalog # 236-EG) for 5 minutes. PVDF membrane was probed with 0.25 µg/mL of Rabbit Anti-Human Phospho-ErbB2/Her2 (Y1248) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1768) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for Phospho-ErbB2/Her2 (Y1248) at approximately 170 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

**Simple Western**

Detection of Human Phospho-ErbB2/Her2 (Y1248) by Simple Western™. Simple Western lane view shows lysates of A431 human epidermoid carcinoma cell line untreated (-) or treated (+) with 10 ng/mL Recombinant Human EGF (Catalog # 236-EG) for 5 minutes, loaded at 0.2 µg/mL. A specific band was detected for Phospho-ErbB2/Her2 (Y1248) at approximately 265 kDa (as indicated) using 5 µg/mL of Rabbit Anti-Human Phospho-ErbB2/Her2 (Y1248) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1768). This experiment was conducted under reducing conditions and using the 66-440 kDa separation system.

**Immunohistochemistry**

ErbB2/Her2 in Human Breast Cancer Tissue. ErbB2/Her2 was detected in immersion fixed paraffin-embedded sections of human breast cancer tissue using Rabbit Anti-Human Phospho-ErbB2/Her2 (Y1248) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1768) at 0.3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # V0003). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent- Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue).

Specific staining was localized to plasma membrane. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.
**PREPARATION AND STORAGE**

**Reconstitution**
Reconstitute at 0.2 mg/mL in sterile PBS.

**Shipping**
The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
*Small pack size (SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C.

**Stability & Storage**
Use a manual defrost freezer and avoid repeated freeze-thaw cycles.
- 12 months from date of receipt, -20 to -70 °C as supplied.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 6 months, -20 to -70 °C under sterile conditions after reconstitution.

**BACKGROUND**

ErbB2, also called Neu and Her2 (human epidermal growth factor receptor 2), is a type I membrane glycoprotein that is a member of the ErbB family of tyrosine kinase receptors. ErbB family members serve as receptors for the epidermal growth factor (EGF) family of growth factors. ErbB2 is widely expressed in epithelial cells and has also been found to be over-expressed in a large number of breast carcinomas. Among ErbB family members, ErbB2 is unique in that it has no identified ligands. Rather, ErbB2 heterodimerizes with the other members of the ErbB family (ErbB1 (EGFR), ErbB3, ErbB4) to form higher affinity signaling complexes. Because ErbB3 contains a defective kinase domain, the kinase domain of ErbB2 is responsible for initiating the tyrosine phosphorylation signal through the heterodimeric receptor. It has been found that a discrete three amino acid signal in the ErbB3 cytoplasmic domain is critical for transactivation of ErbB2. Interestingly, this same three amino acid signal has also been found in ErbB1 and ErbB4. Phosphoinositide 3-kinase has been shown to play a role in ErbB2 signal transduction. The cytoplasmic domain of ErbB2 has been shown to associate with beta-catenin and plakoglobin. Human ErbB2 consists of 1255 amino acids (aa) with a 21 aa signal sequence, a 631 aa extracellular domain, a 23 aa transmembrane region, and a 580 aa cytoplasmic domain. ErbB2 can be shed from the cell surface by proteolytic cleavage by an unidentified protease. ErbB2 appears to play roles in development, cancer, communication at the neuromuscular junction and regulation of cell growth and differentiation (1-10).

**REFERENCES**