**Phospho-HER3/ErbB3 (Tyr1289) (D1B5) Rabbit mAb**

**Applications:** W, IP, IHC-P  
**Molecular Wt.** 185 kDa  
**Isotype** Rabbit IgG**

**Background:** HER3/ErbB3 is a member of the ErbB receptor protein tyrosine kinase family, but it lacks tyrosine kinase activity. Tyrosine phosphorylation of ErbB3 depends on its association with other ErbB tyrosine kinases. Upon ligand binding, heterodimers form between ErbB3 and other ErbB proteins, and ErbB3 is phosphorylated on tyrosine residues by the activated ErbB kinase (1,2). There are at least 9 potential tyrosine phosphorylation sites in the carboxy-terminal tail of ErbB3. These sites serve as consensus binding sites for signaling transducing proteins, including Src family members, Grb2, and the p85 subunit of PI3 kinase, which mediate ErbB downstream signaling (3). Both Tyr1222 and Tyr1289 of ErbB3 reside within a YXXM motif and participate in signaling to PI3K (4).

Investigators have found that ErbB3 is highly expressed in many cancer cells (5) and activation of the ErbB3/PI3K pathway is correlated with malignant phenotypes of adenocarcinomas (6). Research studies have demonstrated that in tumor development, ErbB3 may function as an oncogenic unit together with other ErbB members (e.g. ErbB2 requires ErbB3 to drive breast tumor cell proliferation) (7). Thus, investigators view inhibiting interaction between ErbB3 and ErbB tyrosine kinases as a novel strategy for anti-tumor therapy.

**Specificity/Sensitivity:** Phospho-HER3/ErbB3 (Tyr1289) (D1B5) Rabbit mAb detects endogenous levels of HER3/ErbB3 proteins only when phosphorylated at Tyr1289. This antibody may cross-react with overexpressed receptor tyrosine kinases.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr1289 of human HER3/ErbB3 protein.

**Recommended Antibody Dilutions:**
- Western blotting: 1:1000
- Immunoprecipitation: 1:100
- Immunohistochemistry: 1:1500

**Storage:** Store at 4°C for up to one year without significant loss in immunohistochemical activity. Store at -20°C. Do not aliquot the antibody.

**Species Cross-Reactivity**

<table>
<thead>
<tr>
<th>Applications</th>
<th>Species Cross-Reactivity*</th>
<th>Molecular Wt.</th>
<th>Isotype</th>
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<tbody>
<tr>
<td>W, IP, IHC-P</td>
<td>H, M, (R)</td>
<td>185 kDa</td>
<td>Rabbit IgG**</td>
</tr>
</tbody>
</table>

*Species cross-reactivity is determined by western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.**

**Recommended Antibody Dilutions:**
- Western blotting: 1:1000
- Immunoprecipitation: 1:100
- Immunohistochemistry: 1:1500

**Storage:** Store in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

**Recommended Antibody Dilutions:**
- Western blotting: 1:1000
- Immunoprecipitation: 1:100
- Immunohistochemistry (Paraffin): 1:1500

**Unmasking buffer:** EDTA

**Antibody diluent:** SignalStain® Antibody Diluent #8112

**Detection reagent:** SignalStain® Boost (HRP, Rabbit) #8114

**†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.**

**For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com**

**Background References:**
8. Tween® is a registered trademark of ICI America, Inc.

**Important:** For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

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Immunohistochemical analysis of paraffin-embedded HCC827 xenograft, untreated (left) or λ phosphatase-treated (right), using Phospho-HER3/ErbB3 (Tyr1289) (D1B5) Rabbit mAb.

Immunohistochemical analysis of paraffin-embedded human lung carcinoma using Phospho-HER3/ErbB3 (Tyr1289) (D1B5) Rabbit mAb.

Immunohistochemical analysis of paraffin-embedded MCF7 cell pellets, untreated (left) or treated with hNRG-1 #5218 (right), using Phospho-HER3/ErbB3 (Tyr1289) (D1B5) Rabbit mAb.

Immunohistochemical analysis of paraffin-embedded KYSE-450 cell pellets, untreated (left) or treated with Human Epidermal Growth Factor (hEGF) #8916 (right), using Phospho-EGF Receptor (Tyr1068) (D7A5) XP® Rabbit mAb #3777 (upper) or Phospho-HER3/ErbB3 (Tyr1289) (D1B5) Rabbit mAb (lower). Note lack of reactivity with Phospho-EGFR.

Immunohistochemical analysis of paraffin-embedded human lung carcinoma using Phospho-HER3/ErbB3 (Tyr1289) (D1B5) Rabbit mAb after retrieval with citrate (upper) or EDTA (lower).