

#9923 Store at -20°C

Phospho-HER2/ErbB2 Antibody Sampler Kit

1 Kit
 (3 x 40 µl)

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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-HER2/ErbB2 (Tyr1248) Antibody	2247	40 µl	185 kDa	Rabbit IgG
Phospho-HER2/ErbB2 (Tyr1221/1222) (6B12) Rabbit mAb	2243	40 µl	185 kDa	Rabbit IgG
HER2/ErbB2 (D8F12) XP® Rabbit mAb	4290	40 µl	185 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

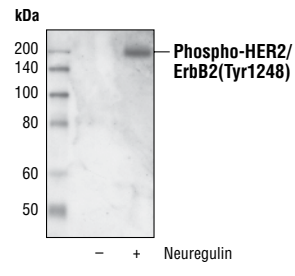
See www.cellsignaling.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Phospho-HER2/ErbB2 Antibody Sampler Kit provides an economical means to evaluate the activation status of HER2/ErbB2, including the phosphorylation of Tyr1248 and Tyr1221/1222. The control HER2/ErbB2 antibody is also included. The kit contains enough primary antibodies to perform four Western blot experiments per primary antibody.

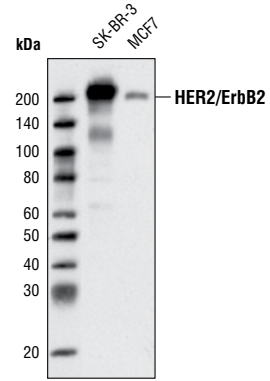
Background: The ErbB2 (HER2) proto-oncogene encodes a transmembrane receptor-like glycoprotein of 185 kDa with intrinsic tyrosine kinase activity (1). ErbB2 does not have any known ligand. However, the kinase activity of ErbB2 can be activated without ligand if it is overexpressed and by heteromeric association with other members of the ErbB family (2). Amplification of the ErbB2 gene and overexpression of its product are detected in almost 40% of human breast cancers (3). Binding of the c-Cbl ubiquitin ligase to Tyr1112 of ErbB2 leads to poly-ubiquitination of ErbB2 and enhances its degradation (4). ErbB2 is one of the major targets for the treatment of breast cancer and other carcinomas. Direction of ErbB2 to the c-Cbl-regulated proteolytic pathway may have therapeutic potential. Tyr877 of ErbB2 is homologous to Tyr416 of pp60c-Src, located in the kinase domain. Phosphorylation of this site may be involved in regulation of ErbB2 biological activity. Tyr1248 and Tyr1221/1222 are the major autophosphorylation sites in ErbB2. Phosphorylation of these sites couples ErbB2 to the Ras-Raf-MAP kinase signal transduction pathway (1,5).

Specificity/Sensitivity: Each phospho-HER2/ErbB2 antibody recognizes only the phosphorylated form of HER2/ErbB2 at the indicated sites. The control HER2/ErbB2 receptor antibody recognizes both phosphorylated and nonphosphorylated forms of HER2/ErbB2.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr1248. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding tyrosines 1221/1222 and residues near the amino terminus of human ErbB2 protein.



Western blot analysis of MCF-7 cells untreated or treated with Neuregulin (100ng/ml) for 5 minutes, using **Phospho-Her2 (Tyr 1248) #2247**.



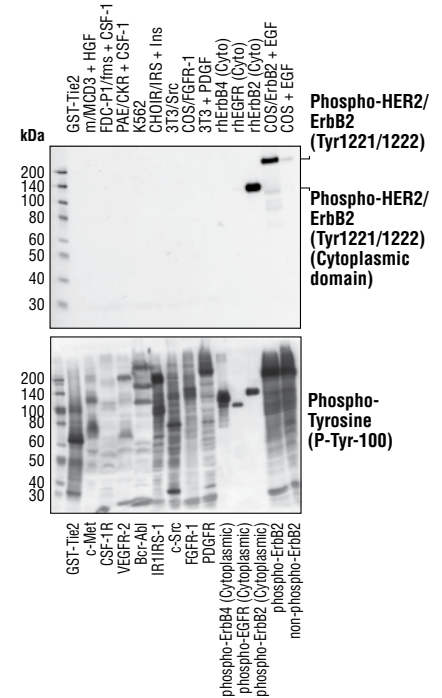
Western blot analysis of extracts from SK-BR-3 and MCF-7 cells using **HER2/ErbB2 (D8F12) XP® Rabbit mAb #4290**.

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

Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignaling.com for a complete listing of recommended companion products.

- Background References:**
- (1) Muthuswamy, S.K. et al. (1999) *Mol. Cell. Biol.* 19, 6845–6857.
 - (2) Qian, X. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91, 1500–1504.
 - (3) Dittadi, R. and Gion, M. (2000) *J. Natl. Cancer Inst.* 92, 1443–1444.
 - (4) Klapper, L.N. et al. (2000) *Cancer Res.* 60, 3384–3388.
 - (5) Kwon, Y.K. et al. (1997) *J. Neurosci.* 17, 8293–8299.



Western blot analysis of extracts from cells expressing different activated tyrosine kinase proteins, using **Phospho-HER2/ErbB2 (Tyr1221/1222) (6B12) Rabbit mAb #2243** (upper) or **Phospho-Tyrosine mAb (P-Tyr-100) #9411** (lower). Phospho-HER2/ErbB2 (Tyr1221/1222) (6B12) Rabbit mAb specifically detects phosphorylated HER2/ErbB2 but not other phosphorylated tyrosine kinases.

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Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
 Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

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.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.