HER2/ErbB2 (29D8) Rabbit mAb

Applications: W, IP, IF-IC, IHC-P, F
Endogenous

Species Cross-Reactivity
H, M, (R)

Molecular Wt. 185 kDa
Isotype Rabbit IgG

Background: The ErbB2 (HER2) proto-oncogene encodes a 185 kDa transmembrane, receptor-like glycoprotein with intrinsic tyrosine kinase activity (1). While ErbB2 lacks an identified ligand, ErbB2 kinase activity can be activated in the absence of a ligand when overexpressed and through heteromeric associations with other ErbB family members (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 ErbB2 at Tyr1112 leads to ErbB2 poly-ubiquitination and enhances degradation of this kinase (4). ErbB2 is a key therapeutic target in the treatment of breast cancer and other carcinomas and targeting the regulation of ErbB2 degradation by the c-Cbl-regulated proteolytic pathway is one potential therapeutic strategy. Phosphorylation of the kinase domain residue Tyr877 of ErbB2 (homologous to Tyr416 of pp60c-Src) may be involved in regulating ErbB2 biological activity. The major autophosphorylation sites in ErbB2 are Tyr1248 and Tyr1221/1222; phosphorylation of these sites couples ErbB2 to the Ras-Raf-MAP kinase signal transduction pathway (1,5).

Specificity/Sensitivity: HER2/ErbB2 (29D8) Rabbit mAb detects endogenous levels of total ErbB2 protein. This antibody does not cross-react with related kinases.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding tyrosine 1248 of human ErbB2 protein.

Background References:

Recommended Antibody Dilutions:
Western blotting 1:1000
Immunoprecipitation 1:100
Immunohistochemistry (Paraffin) 1:200-1:800
Optimal IHC dilutions determined using SignalStain
Boost IHC Detection Reagent.
Unmasking buffer: EDTA
Antibody diluent: SignalStain Antibody Diluent #8112
Detection reagent: SignalStain Boost (HRP, Rabbit) #8114
Immunofluorescence (IF-IC) 1:100-1:400
IF Protocol: Methanol Fixation Required
Flow Cytometry 1:200-1:400

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

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

Western blot analysis of cell extracts from various cell lines, using HER2/ErbB2 (29D8) Rabbit mAb.

Confocal immunofluorescent analysis of MDA-MB-453 cells (upper) and MDA-MB-231 cells (lower), using HER2/ErbB2 (29D8) Rabbit mAb (green). Blue pseudocolor = DRAQ5 (fluorescent DNA dye).

Storage: Supplied 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.

Species cross-reactivity is determined by western blot.

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

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Immunohistochemical analysis of paraffin-embedded SKBR3 (high HER2) (left), MDA-MB-453 (moderate HER2) (middle) and MDA-MB-468 (low HER2) (right), using HER2/ErbB2 (29D8) Rabbit mAb.

Immunohistochemical analysis of paraffin-embedded human breast carcinoma using HER2/ErbB2 (29D8) Rabbit mAb in the presence of control peptide (left) or HER2/ErbB2 Blocking Peptide #1059 (right).

Flow cytometric analysis of MDA-MB-468 cells (blue) and SKBR3 cells (green) using HER2/ErbB2 (29D8) Rabbit mAb (solid lines) or a concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (dashed lines). Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.