Revision 1

Store at -20C

#2669

# Phospho-Chk2 (Ser516) Antibody

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**For Research Use Only. Not for Use in Diagnostic Procedures.**

| Applications: | Reactivity: | Sensitivity: | MW (kDa): | Source/Isotype: | UniProt ID: | Entrez-Gene Id: |
|---|---|---|---|---|---|---|
| W | H | Endogenous | 62 | Rabbit | #O96017 | 11200 |

## Product Usage Information

| Application | Dilution |
|---|---|
| Western Blotting | 1:1000 |

## 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 antibody.

## Specificity/Sensitivity

Phospho-Chk2 (Ser516) Antibody detects endogenous levels of Chk2 only when phosphorylated at serine 516. The antibody does not cross-react with Chk2 phosphorylated at other sites.

## Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser516 of human Chk2. Antibodies are purified by protein A and peptide affinity chromatography.

## Background

Chk2 is the mammalian orthologue of the budding yeast Rad53 and fission yeast Cds1 checkpoint kinases (1-3). The amino-terminal domain of Chk2 contains a series of seven serine or threonine residues (Ser19, Thr26, Ser28, Ser33, Ser35, Ser50, and Thr68) each followed by glutamine (SQ or TQ motif). These are known to be preferred sites for phosphorylation by ATM/ATR kinases (4,5). After DNA damage by ionizing radiation (IR), UV irradiation, or hydroxyurea treatment, Thr68 and other sites in this region become phosphorylated by ATM/ATR (5-7). The SQ/TQ cluster domain, therefore, seems to have a regulatory function. Phosphorylation at Thr68 is a prerequisite for the subsequent activation step, which is attributable to autophosphorylation of Chk2 at residues Thr383 and Thr387 in the activation loop of the kinase domain (8).
Chk2 autophosphorylation at Ser516 is important for optimal Chk2 function, and a Ser516Ala mutant Chk2 is defective in IR-induced apoptosis (9).

## Background References

1. Allen, J.B. et al. (1994) *Genes Dev.* 8, 2401-2415.
2. Weinert, T.A. et al. (1994) *Genes Dev.* 8, 652-665.
3. Murakami, H. and Okayama, H. (1995) *Nature* 374, 817-819.
4. Kastan, M.B. and Lim, D.S. (2000) *Nat. Rev. Mol. Cell Biol.* 1, 179-186.
5. Matsuoka, S. et al. (2000) *Proc. Natl. Acad. Sci. USA* 97, 10389-10394.
6. Melchionna, R. et al. (2000) *Nat. Cell Biol.* 2, 762-765.
7. Ahn, J.Y. et al. (2000) *Cancer Res.* 60, 5934-5936.
8. Lee, C.H. and Chung, J.H. (2001) *J. Biol. Chem.* 276, 30537-30541.
9. Wu, X. and Chen, J. (2003) *J. Biol. Chem.* 278, 36163-36168.

## Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

## Western Blot Buffer

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

## Applications Key

**W:** Western Blotting

## Cross-Reactivity Key

**H:** Human

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