# Store at -20C

# Phospho-Chk1/2 Antibody Sampler Kit



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1 Kit (9 x 20 microliters)

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

| Product Includes   | Product # | Quantity | Mol. Wt | Isotype/Source |
|--|-----------|----------|---------|----------------|
| Phospho-Chk1 (Ser317) (D12H3) XP <sup>®</sup> Rabbit mAb | 12302     | 20 µl    | 56 kDa  | Rabbit IgG     |
| Phospho-Chk1 (Ser345) (133D3) Rabbit mAb                 | 2348      | 20 µl    | 56 kDa  | Rabbit IgG     |
| Phospho-Chk1 (Ser296) Antibody                           | 2349      | 20 µl    | 56 kDa  | Rabbit         |
| Chk1 (2G1D5) Mouse mAb                                   | 2360      | 20 µl    | 56 kDa  | Mouse IgG1     |
| Chk2 (D9C6) Rabbit mAb                                   | 6334      | 20 µl    | 62 kDa  | Rabbit IgG     |
| Phospho-Chk2 (Ser19) Antibody                            | 2666      | 20 µl    | 62 kDa  | Rabbit         |
| Phospho-Chk2 (Ser33/35) Antibody                         | 2665      | 20 µl    | 62 kDa  | Rabbit         |
| Phospho-Chk2 (Thr68) (C13C1) Rabbit mAb                  | 2197      | 20 µl    | 62 kDa  | Rabbit IgG     |
| Phospho-Chk2 (Ser516) Antibody                           | 2669      | 20 µl    | 62 kDa  | Rabbit         |
| Anti-rabbit IgG, HRP-linked Antibody                     | 7074      | 100 µl   |         | Goat           |

Please visit cellsignal.com for individual component applications, species cross-reactivity, dilutions, protocols, and additional product information.

## Description

Storage

**Background** 

The Phospho-Chk1/2 Antibody Sampler Kit offers an economical means to evaluate the phosphorylation status of Chk1 and Chk2 on multiple residues. The kit contains enough primary and secondary antibodies to perform two Western blot experiments with each primary antibody.

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

Chk1 kinase acts downstream of ATM/ATR kinase and plays an important role in DNA damage checkpoint control, embryonic development, and tumor suppression (1). Activation of Chk1 involves phosphorylation at Ser317 and Ser345 by ATM/ATR, followed by autophosphorylation of Ser296. Activation occurs in response to blocked DNA replication and certain forms of genotoxic stress (2). While phosphorylation at Ser345 serves to localize Chk1 to the nucleus following checkpoint activation (3), phosphorylation at Ser317 along with site-specific phosphorylation of PTEN allows for re-entry into the cell cycle following stalled DNA replication (4). Chk1 exerts its checkpoint mechanism on the cell cycle, in part, by regulating the cdc25 family of phosphatases. Chk1 phosphorylation of cdc25A targets it for proteolysis and inhibits its activity through 14-3-3 binding (5). Activated Chk1 can inactivate cdc25C via phosphorylation at Ser216, blocking the activation of cdc2 and transition into mitosis (6). Centrosomal Chk1 has been shown to phosphorylate cdc25B and inhibit its activation of CDK1-cyclin B1, thereby abrogating mitotic spindle formation and chromatin condensation (7). Furthermore, Chk1 plays a role in spindle checkpoint function through regulation of aurora B and BubR1 (8). Research studies have implicated Chk1 as a drug target for cancer therapy as its inhibition leads to cell death in many cancer cell lines (9).

Chk2 is the mammalian homologue of the budding yeast Rad53 and fission yeast Cds1 checkpoint kinases (5-7). The amino-terminal domain of Chk2 contains a series of seven serine or threonine residues (Ser19, Thr26, Ser28, Ser33, Ser35, Ser50 and Thr68) followed by glutamine (SQ or TQ motif). These are known to be preferred sites for phosphorylation by ATM/ATR kinases (8). Indeed, after DNA damage by ionizing radiation (IR), UV irradiation and DNA replication blocked by hydroxyurea, Thr68 and other sites in this region become phosphorylated by ATM/ATR (9-11). 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 on residues Thr383 and Thr387 in the activation loop of the kinase domain (12).

### **Background References**

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