

Product Information

Anti-Chk2 antibody, Mouse monoclonal
clone DCS-273, purified from hybridoma cell culture

Product Number **C9108**

Product Description

Anti-Chk2 antibody, Mouse monoclonal (mouse IgG1 isotype) is derived from the DCS-273 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a recombinant human Chk2.¹ The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Product Number ISO2.

Monoclonal Anti-Chk2 (hCds1) recognizes human Chk2, also known as hCds1.^{1,2} The epitope recognized by the antibody resides within the protein-protein interaction FHA domain of the Chk2 molecule.¹ The product is useful in immunoblotting^{1,2} (61 kDa) and immunohistochemistry^{1,2} (formalin-fixed, paraffin-embedded tissues, microwave unmasking¹). Cross-reactivity has been observed with mouse Chk2.¹

The survival of organisms depends on the accurate transmission of genetic information from one cell to its daughters. Such faithful transmission requires the ability to survive spontaneous and induced DNA damage while minimizing the number of heritable mutations. To achieve this fidelity, cells have evolved surveillance mechanisms that monitor the structure of chromosomes and coordinate repair and cell-cycle progression.³⁻⁵ When the DNA of a cell is damaged, a network of checkpoint proteins tell the cell to stop at the nearest cell cycle checkpoint, so that the DNA repair machinery can set about shoring up the damage, allowing time for repair prior to proliferation.^{6,7} Several checkpoint genes are essential for cell and organism survival, implying that these pathways are not only surveyors of occasional damage, but are firmly integrated components of cellular physiology.^{5,7} There are two manifestations of the delays that cells experience in G₁, S, or G₂ phases of the cell cycle after damage to DNA. The first is the transient arrest seen at G₁, S, or G₂ (independent of the key tumor suppressor protein p53) that gives the DNA repair machinery time to shore up the damage before division continues. The second (dependent on p53) is apoptosis or prolonged, probably permanent, G₁ delay that results in removal of

damaged cells from the population. A failure to halt at these checkpoints leads to genomic instability and an increased likelihood that the cell will become cancerous. Studies in yeast have identified a network of DNA integrity checkpoint proteins (including four conserved kinases) that regulate the cell's entry into and exit from these cell cycle checkpoints. Mammalian homologs of the four yeast checkpoint kinases have been identified, suggesting that organisms from yeast to human have similar protein pathways for regulating these checkpoints.^{6,8} The DNA damage response network of interacting pathways are signal transduction agents consisting of sensors, transducers, and effectors. The signal transducers are composed of sets of conserved proteins with recognizable motifs. One class is composed of phospho-inositide kinase (PIK)-related proteins which include ATM and ATM-Rad3-related (ATR) in mammals and their homologues in budding and fission yeast. These proteins are central to the entire DNA damage response. Downstream of these proteins are two families of checkpoint kinases (CHK), the Chk1 (54 kDa) and Chk2 (61 kDa, also known as hCds1) kinases, and their homologues.^{9,10}

Studies have established that mammalian Chk1 is structurally unrelated to Chk2, but performs an analogous role in propagating signals from damaged or unrepllicated DNA.⁵ Chk2 contains a C-terminal kinase domain, an N-terminal regulatory region that is rich in TQ and SQ pairs, and a forked head-associated domain (FHA) which is found in other cell cycle kinases. Chk1 is expressed and active only in S-G₂ phases of the cell cycle, while Chk2, the human homologue of *S. cerevisiae* Rad53 and *S. pombe* Cds1, is expressed throughout the cell cycle.¹

Arrest in G₂ is primarily regulated by the maintenance of inhibitory phosphorylation of Cdc2. Cdc2 dephosphorylation and activation is catalyzed by the dual specificity phosphatase Cdc25. Evidence indicates that part of the G₂M DNA checkpoint mechanism involves inactivation and translocation of Cdc25C into the cytoplasm. This is at least partially mediated by phosphorylation of Cdc25C on Ser²¹⁶ and its consequent binding with 14-3-3 proteins.

Chk1 and Chk2 have been shown to phosphorylate Cdc25C at Ser²¹⁶.^{11,12} This modification is thought to maintain Cdc25C phosphorylation in cells arrested at G₂M in response to DNA damage.⁵ It has also been shown that Chk1 can phosphorylate Wee1, a negative regulator of G₂ to M transition.

Also, among the targets for Chk2/hCds1 is the BRCA1 protein.¹³ Chk2/hCds1 and BRCA1 normally interact and localize to discrete foci within the nucleus. Following DNA damage, Chk2/hCds1 phosphorylates BRCA1 on Ser⁹⁸⁸ causing complex dissociation and release of BRCA1.¹³ It has also been found that the ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis.³ Finally, Chk2 stabilizes the tumor suppressor protein p53, a key player in regulating the prolonged G₁ arrest checkpoint.⁶ In undamaged cells that are dividing normally, p53 is highly unstable, with a half-life measured in minutes. Following DNA damage (induced by e.g. ionizing radiation), p53 accumulates, resulting in a prolonged (possibly irreversible) G₁ arrest or apoptosis. The instability of p53 depends on Mdm2, which binds to its amino terminus and targets it for ubiquitination and degradation. Preventing the interaction of p53 with Mdm2 is sufficient to promote its stabilization. Phosphorylation of amino acids Ser¹⁵ and Ser²⁰ of p53 is involved in response to DNA damage. Chk2 is the kinase that phosphorylates Ser²⁰ of p53, leading to its stabilization, which results in cell cycle arrest in G₁.^{14,15} The formation of this complex requires an intact FHA domain of Chk2, and the tetramerization domain of p53.⁴

Chk2 has also been identified as the gene implicated in a small number of families with the cancer predisposition syndrome Li-Fraumeni, who do not have germ line mutations in *p53*.¹⁶ Monoclonal antibody reacting specifically with Chk2 (hCds1) is a useful tool to study the role and importance of Chk2 as a checkpoint kinase in preventing genomic instability.

Reagent

Supplied at ~2 mg/ml in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at -20 °C. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a working concentration of 2-4 µg/ml is determined using a whole extract of 293T (human embryonal kidney) cells.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilutions by titration test.

References

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