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ProductInformation

MONOCLONAL ANTI-CHK2 (hCDS1)
CLONE DCS-270
Purified Mouse Immunogobulin

Product Number **C 9233**

Product Description

Monoclonal Anti-Chk2 (hCds1) (mouse IgG2a isotype) is derived from the DCS-270 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a recombinant human Chk2.[1,2] The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Chk2 (hCds1) recognizes human Chk2, also known as hCds1.[1-4] The epitope recognized by the antibody resides within the SQ-rich N-terminal regulatory domain of the Chk2 molecule.[2] The product is useful in immunoblotting[1-4] (61 kDa), immunoprecipitation,[1] immunocytochemistry,[2,3] and immunohistochemistry [2,3] (formalin-fixed paraffin-embedded tissues, microwave unmasking[2]). Cross-reactivity has been observed with mouse Chk2.[2]

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.[5] When the DNA of a cell is damaged, a network of checkpoint proteins tells 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 G_1, S, or G_2 phases of the cell cycle after damage to DNA. The first is the transient arrest seen at G_1, S, or G_2 (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, G_1 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 unreplicated 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-G_2 phases of the cell cycle, while Chk2, the human homologue of *S. cerevisiae* Rad53 and *S. pombe* Cds1, is found expressed throughout the cell cycle.[2]

Arrest in G_2 is mainly 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 G_2M DNA checkpoint mechanism involves inactivation and translocation of Cdc25C into the cytoplasm. This is at least partially mediated by phosphorylation of Cdc25C on Ser^{216} and its consequent binding with the 14-3-3 proteins. Chk1 and Chk2 have been shown to phosphorylate Cdc25C at Ser^{216}.[11,12] This modification is thought to maintain Cdc25C phosphorylation in cells arrested at G_2M in response to DNA damage.[5] It has also been shown that Chk1 can phosphorylate Wee1, a negative regulator of G_2 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 Ser^{988} 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.[1] Finally, Chk2 stabilizes the tumor suppressor protein p53, a key player in regulating the prolonged G_1 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) G_1 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 Ser^{15} and Ser^{20} of p53 is involved in response to DNA damage. Chk2 is the kinase that phosphorylates Ser^{20} of p53, leading to its stabilization, which results in cell cycle arrest in G_1.[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
Monoclonal Anti-CHK2 (hCDS1) is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Precautions and Disclaimer
Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS 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 is not recommended. Storage in "frost-free" freezers is also 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
A working concentration of 1-2 µg/ml is determined by immunoblotting, 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.

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