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Product Information

Anti-MDM2 antibody, Mouse monoclonal
clone HDM2-323, purified from hybridoma cell culture

Product Number **M7815**

Product Description

Monoclonal Anti-MDM2 (mouse IgG2a isotype) is derived from the HDM2-323 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide corresponding to amino acids 389-402 of human MDM2, conjugated to keyhole limpet hemocyanin (KLH). The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Number ISO2).

Monoclonal Anti-MDM2 recognizes human and mouse MDM2. The epitope recognized by the antibody is localized in the C-terminal region of MDM2 (amino acids 389-402 of human MDM2). The product can be used in ELISA and immunoblotting (~90 kDa one band or a doublet).

Inactivation of tumor-suppressor gene p53 leads to deregulated cell proliferation and is a key factor in human tumorigenesis. The ability of p53 to restrain a cell must be reigned in under normal conditions. Several cellular genes have been identified whose expression is activated by p53. One of these, the *mdm2* gene, originally found on *m*ouse *d*ouble *m*inute chromosomes, encodes a protein that interacts directly with p53 and negatively regulates p53 transcriptional activity. p53 and MDM2 (the human homologue of Mdm2, a 90 kDa molecule), function in mutual regulation; activation of p53 results in expression of MDM2, which consequently inhibits p53 transcriptional function.[1] MDM2 accomplishes this inhibition in two ways: as a result of its physical interaction with p53, MDM2 both represses p53 transcriptional activity and mediates the degradation of p53.[2]

Overexpression of MDM2 results in reduced quantities of coexpressed p53, and disruption of the p53-MDM2 interaction by mutation results in both activation and accumulation of p53.[3] The region on p53 with which MDM2 interacts (residues 17-27) is one of the segments of p53 that is highly conserved not only among different species, but even among some p53-related family members, such as p73.

In addition, this portion of p53 is located within the transcriptional activation region required for the interaction of p53 with components of the general transcriptional machinery, such as TAFs.

A number of phosphorylation sites have been identified in the vicinity of this region, that are highly likely to be involved in regulating p53. Indeed, MDM2 needs to bind to the p300 transcriptional coactivator/histone acetylase in order to mediate degradation of p53. It has also been shown that MDM2 shuttles p53 from the nucleus to the cytoplasm where it is then degraded.[4] One way to stabilize and activate p53 in cells is by interfering either with the interaction between MDM2 and p53 or by interfering with the ability of MDM2 to target bound p53 for degradation.

p53-MDM2 interaction may be regulated through two entirely separate and independent mechanisms that are regulated through distinct signaling pathways: changes in p53 due to covalent modification, and/or through non-covalent regulators of the p53-MDM2 association. Thus, DNA damage-induced phosphorylation of p53 can attenuate the p53-MDM2 interaction, and the product of the alternate reading frame (ARF) located within the $p16^{INK4A}$ locus (murine $p19^{ARF}$, human $p14^{ARF}$) can bind to MDM2 and prevent its destruction of p53.

MDM2, a transcriptional target of p53, is induced when p53 is stabilized and activated. p53 interacting with MDM2 inhibits ARF expression, keeping p53 levels low during normal conditions. After stress, modification of the p53 protein prevents or disrupts the p53-MDM2 interaction, while as a result of oncogene imbalance, ARF is induced and MDM2 is prevented from destabilizing p53. The outcome in both cases though is increased levels and activation of p53 protein

In addition to its relationships with p53, MDM2 protein has been shown to activate cell proliferation by stimulating the S-phase-inducing transcription factors, E2F1/DP1.[5] The amplification of the MDM2 gene has been demonstrated in various human neoplasms.[6]

MDM2 is frequently overexpressed in the nuclei of soft-tissue sarcomas, and to a much lesser extent in a wide variety of malignancies, such as bladder, breast, testes, and cervical cancers, and in glial cells, and a range of hematological disorders. A monoclonal antibody reacting specifically with MDM2 is an essential tool in defining the interactions and distribution of MDM2, its function in signaling pathways, and in its regulation of p53.

Reagent
Monoclonal Anti-MDM2 is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, and 15 mM sodium azide.

Antibody concentration: ~2 mg/ml

Precautions and Disclaimer
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 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 dilutions should be discarded if not used within 12 hours.

Product Profile
For immunoblotting, a minimum working antibody concentration of 1–2 μg/ml is recommended using a whole extract of cultured 293T cells transfected with human MDM2.

Note: In order to obtain the best results in various techniques and preparations, it is recommended to determine the optimal working dilution by titration.

References
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