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ProductInformation

MONOCLONAL ANTI-MDM2
CLONE MD-219
Purified Mouse Immunoglobulin

Product Number **M8558**

Product Description

Monoclonal Anti-MDM2 (mouse IgG1 isotype) is derived from the MD-219 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a recombinant mouse MDM2. The isotype is determined using Sigma ImmunoType™ Kit (Sigma ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma ISO-2).

Monoclonal Anti-MDM2 reacts specifically with mouse, but not with human MDM2 protein. The antibody may be used for ELISA, immunoblotting (doublets at 90 kDa and approx. 55 kDa), immunocytochemistry (predominantly nuclear and some cytoplasmic staining) and flow cytometry.

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 kD 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, which is 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 with the ability of MDM2 to target bound p53 for degradation. p53-MDM2 interaction may be regulated through two entirely separated and independent mechanisms which are regulated through distinct signaling pathways: changes in p53 due to covalent modification, and/or through noncovalent 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. It is well established that MDM2 is itself a transcriptional target of p53 that is induced after p53 becomes stabilized and activated; by interacting with MDM2 and inhibiting ARF expression, p53 levels are kept 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 cervix cancers, and in glial cells, and a range of hematological disorders. Monoclonal antibody reacting specifically with MDM2 is an essential tool in defining the interactions and

distributions of MDM2, its function in signaling pathways and in mutual regulation of p53.

Reagents
Monoclonal Anti-MDM2 is supplied as purified mouse immunoglobulin in 0.01M phosphate buffered saline pH 7.4, containing 15 mM sodium azide.

Antibody concentration is approximately 2mg/ml.

Precautions and Disclaimer
Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution.
Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability
For continuous use, store at 2-8°C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. 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
A working concentration of 0.5-2 µg/ml is determined by immunoblotting using a whole extract of transfected 293T (human embryonal kidney) cells, expressing mouse MDM2.

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

References

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lpg 3/00