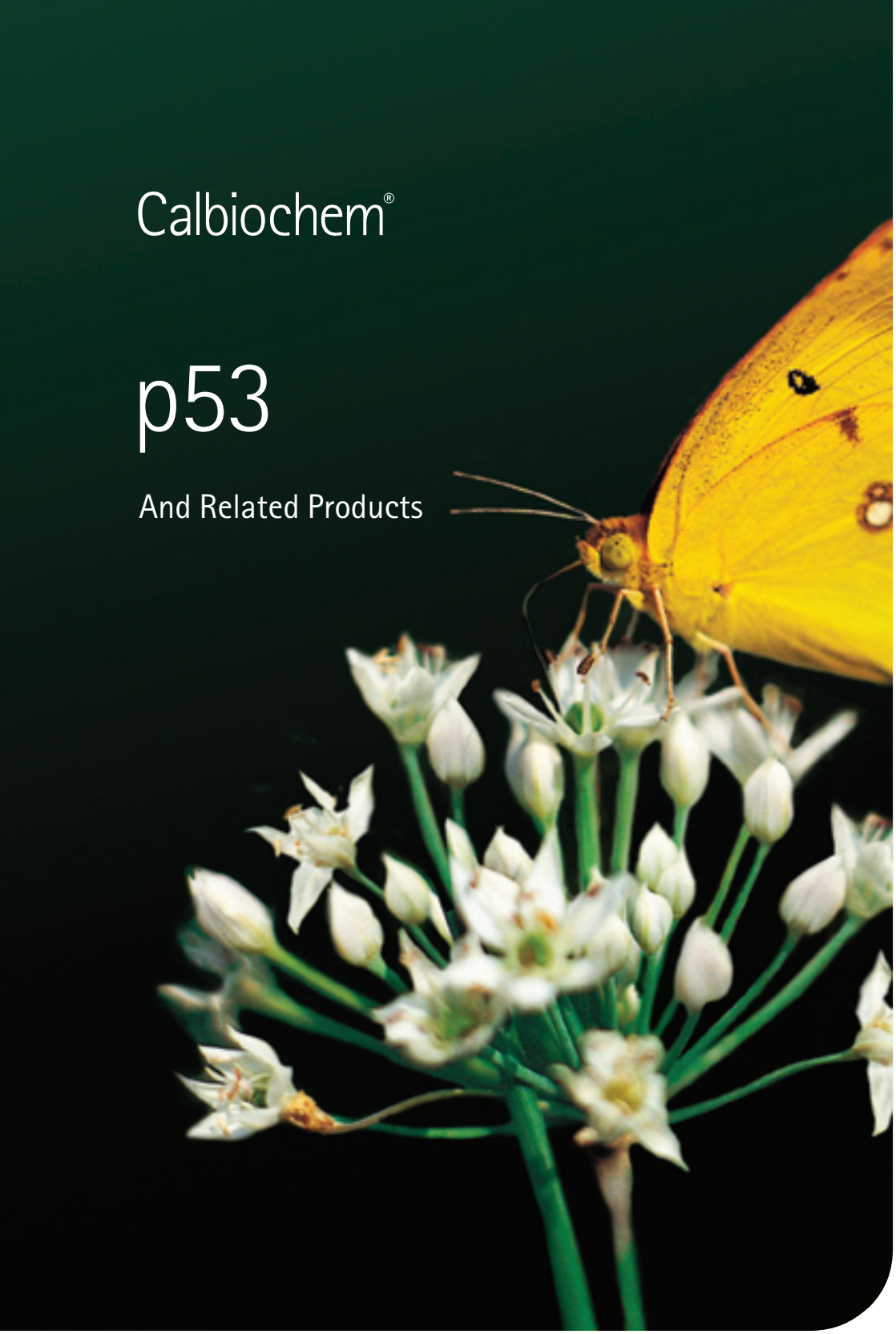
Calbiochem®

p53

And Related Products



Includes:

MERCK

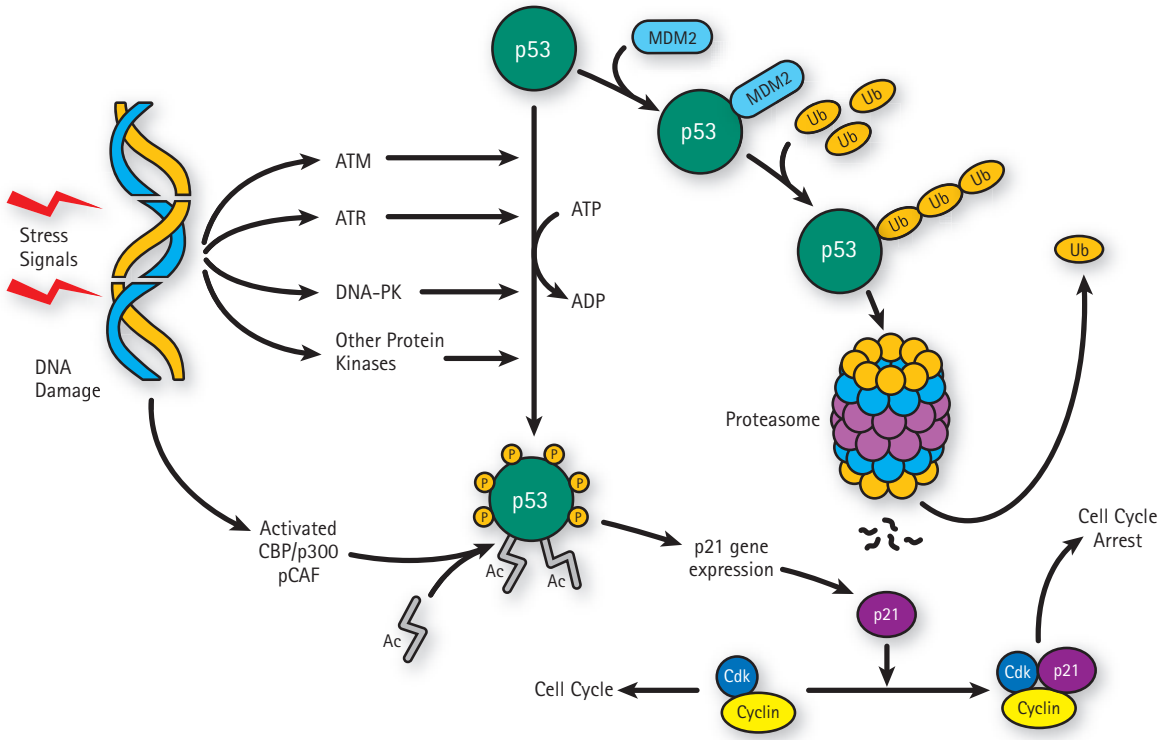
p53: Choice of Response: Repair or Death

Chandra Mohan, Ph.D., EMD Biosciences, San Diego, California

p53, a well-conserved phosphoprotein, is one of the best known tumor suppressors. Human p53 consists of 393 amino acids assembled into four structurally and functionally different domains: an acidic N-terminal region that contains the 42 amino acid transactivation domain followed by a hydrophobic proline-rich region (amino acids 64–92), a central sequence-specific DNA-binding domain (amino acids 102–292), a tetramerization domain (amino acids 324–355), and a highly basic C-terminal region regulatory domain (amino acids 363–393). p53 is a sequence-specific nuclear transcription factor that binds to defined consensus sites within DNA as a tetramer and represses transcription of a set of genes involved in cell growth stimulation, while activating a different set of genes involved in cell cycle control. It causes growth arrest before either DNA replication in the G_1 phase or mitosis in the G_2 phase. This provides a window for DNA repair or elimination of cells with severely damaged DNA strands. Hence, p53 is considered as an important regulator of DNA repair that ensures genomic integrity. Agents that damage DNA induce p53 to become very stable by a post-translational mechanism, allowing its concentration in the nucleus to increase dramatically.

In unstressed cells, p53 is latent and is maintained at low levels by targeted degradation mediated by MDM2. Through its binding to p53, MDM2 can shuttle p53 out of the nucleus into the cytoplasm for degradation. When normal mammalian cells are subjected to stress signals, such as hypoxia, radiation, or chemotherapeutic drugs, p53 is phosphorylated at multiple sites, including those involved in its binding to MDM2. This leads to its activation and blockage of its ubiquitin-dependent degradation. Activation of p53 can result in cell cycle arrest, presumably to allow for DNA repair before replication or mitosis. In some cell types, however, p53 activation results in apoptosis as means of eliminating severely damaged cells. The final outcome of p53 activation depends on many factors, and is mediated largely through the action of downstream effector genes transactivated by p53.

Human p53 is phosphorylated at least at 23 different sites by stress-activated protein kinases, DNA protein kinase (DNA-PK), casein kinase I and II, and cyclin-dependent kinases. Although the exact functions of specific phosphorylation at various sites is still controversial, evidence indicates that phosphorylation of p53 provides stability by promoting its dissociation from MDM2 and enhancing its transcriptional activity. Most of the p53 phosphorylation sites are clustered within the 40 amino acids at its N-terminus. ATM and ATR kinases promote phosphorylation of human p53 at Ser^{15} and Ser^{20}, which are essential for the activation of p53 following DNA damage. DNA-PK phosphorylates Ser^{15} within the critical N-terminal region of p53, which controls the interaction of p53 with the transcriptional apparatus and with the MDM2 protein. DNA-PK also phosphorylates Ser^{9} and Thr^{18}; however, phosphorylation at these sites is dependent upon the presence of full-length p53, but is independent of phosphorylation at other sites. Phosphorylation at Thr^{18} alters the structure of the amphipathic α-helix with which MDM2 interacts. Studies have shown that when p53 co-localizes with



DNA-PK and ssDNA, there is a 10-fold enhancement of p53 phosphorylation. Casein kinase I can also phosphorylate Ser[9] and Thr[18], however, these phosphorylations are dependent upon prior phosphorylation of Ser[6] and Ser[15]. All types of tumor cells exhibit higher levels of p53 phosphorylation when compared to normal non-transformed cells. These phosphorylations offer greater stability to p53 regardless of p53 mutations.

In spite of extensive studies on p53 phosphorylation, it is now known that phosphorylation is not the only mechanism that regulates activation of p53. Following cellular stress, p53 is shown to be acetylated by CBP/p300 at multiple lysine residues (Lys[370, 372, 373, 381, and 382]) and by pCAF at Lys[320]. The physiological relevance of p53 acetylation is still controversial, although acetylation does correlate well with increased cellular stress. Additional support for the role of acetylation comes from studies that show that increasing the level of p53 acetylation with deacetylase inhibitors prevents p53 degradation. Over-expression of MDM2 is also shown to effectively reduce p300-dependent p53 acetylation.

p53 is shown to be either non-functional or mutated in most human cancers. The most common anomaly of p53 in human cancers is mutation of the *p53* gene. A large number of mutations are caused by single base substitutions and about 30% of these mutations are reported to occur in hotspot codons. Functional p53 provides a protective mechanism against tumor growth and a loss of p53 function is a key step in the neoplastic cascade. In addition, the function of p53 is critical to the success of many cancer treatments since radiation and chemotherapy act in part by triggering cell suicide in response to DNA damage. A successful response to therapy is greatly reduced in tumors where mutant p53 is present, and these tumors are often very difficult to treat. The p53 network in normal, non-activated situations is non-functional, but is activated in cells as a response to various signals that take place in the carcinogenic process. Carcinogen-induced DNA damage, abnormal proliferative signals, hypoxia, and loss of cell adhesion are some of the most common signals that activate p53.

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Technical Support
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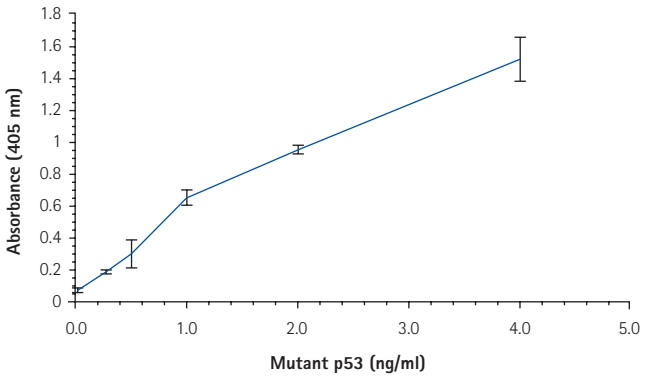
p53 ELISA Kit (Mutant Selective)

A 96-well ELISA kit suitable for assay of mutant p53 protein in cell extracts, serum, plasma, and other fluids. The antibodies utilized in this assay kit react with an epitope exposed on human and most mammalian mutant p53 proteins, but not on wild-type p53, thus making the assay mutant-selective. Suitable for use with most mammalian species. Also reacts with denatured wild-type p53.

Assay range: 250–4000 pg/ml

Cat. No. QIA03 **1 kit**

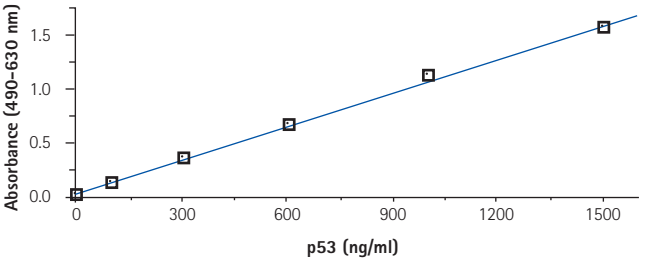


p53 ELISA Kit (Pantropic)

A 96-well ELISA kit suitable for assay of mutant and wild-type p53 in human cell extracts, tissue extracts, conditioned media, serum, and urine samples.

Assay range: 93.75–1500 pg/ml **Sensitivity:** 10 pg/ml

Cat. No. QIA07 **1 kit**



PhosphoDetect™ p53 (pSer[15]) ELISA Kit

A 96-well solid phase sandwich ELISA kit for the detection of p53 phosphorylated on Ser[15] in human cell lysates.

Assay range: 1–100 units/ml

Cat. No. CBA009 **1 kit**

ELISA: Abs 450 nm	0.156	0.265	0.336	0.433	0.625	0.993	1.57	2.72
Phospho-p53 (Ser[15]) units/test	0	0.156	0.313	0.625	1.25	2.5	5	10

p53 Activators

Name	Cat. No.	Comments	Size	Price
p53 Activator, Cell-Permeable	506131	A synthetic cell-permeable peptide corresponding to the C-terminal amino acids 361–382 of p53. Binds mutant p53 and restores the growth suppressor functions of p53 protein in human tumor cells.	500 µg	
p53 Activator II, Cell-Permeable	506144	A cell-permeable, proteolytically stable p53-activating peptide that displays antitumor properties.	500 µg	
p53 Activator III, RITA	506149	A cell-permeable, p53-targeting, tricyclic thiophene derivative that blocks p53-MDM2 interaction and p53 ubiquitination and induces p53-dependent apoptosis in tumor cells expressing wild-type p53.	1 mg	
p53, MDM-2 Binding Domain, N-Terminal 17-26, Cell-Permeable	506136	A decapeptide derived from the N-terminal MDM-2 binding domain of p53, residues 17–26, and linked to the antennapedia leader sequence at the C-terminal. Directly binds to MDM-2 and induces non-apoptotic cell-death (IC_{50} = 40 µg/ml in TUC-3 cells)	500 µg	
PRIMA-1	530050	A cell-permeable quinuclidinone analog that restores biochemical and biological function to mutant p53 and induces p53-dependent apoptosis *in vitro* and *in vivo*.	10 mg	

p53 Inhibitors

Name	Cat. No.	Comments	Size	Price
Pifithrin-α	506132	A cell-permeable chemical inhibitor of p53. Reversibly inhibits p53-dependent transactivation of p53-responsive genes and reversibly blocks p53-mediated apoptosis.	5 mg 10 mg	
Pifithrin-α, Cyclic-	506134	A cell-permeable, very stable analog of Pifithrin-α (Cat. No. 506132), with similar biological function, but with reduced cytotoxicity.	10 mg	
Pifithrin-α, *p*-Nitro	506152	A cell-permeable p53 inhibitor that serves as the prodrug form of Pifithrin-α, *p*-Nitro, Cyclic (Cat. No. 506154).	5 mg	
Pifithrin-α, *p*-Nitro, Cyclic	506154	A cell-permeable p53 inhibitor with 10-fold higher potency (ED_{50} = 30 nM in protecting etoposide-induced cortical neuron death) and 50% longer half-life than Pifithrin-α (Cat. No. 506132).	5 mg	
Pifithrin-µ	506155	A cell-permeable blocker of p53 interaction with Bcl-xL and Bcl-2 proteins. Selectively inhibits p53 translocation to mitochondria without affecting the transactivation function of p53. Targets only the mitochondrial p53 pathway without affecting the important transcriptional functions of p53.	10 mg	

Antibodies for p53 Research

Name	Cat. No.	Comments	Size	Price
Anti-p53 (Ab-1) (Pantropic) Mouse mAb (PAb421)	OP03	Liquid, purified. Immunogen used was partially purified mouse p53 protein. Recognizes the ~53 kDa mammalian wild type and mutant p53 protein. Cross reacts with human, monkey, mouse, rabbit, and rat. **FC, FS, GS, IB, IF, IP**	20 µg 100 µg	
Anti-p53 (Ab-1) (Pantropic) Mouse mAb (PAb421)	OP03L	Lyophilized, purified. Immunogen used was partially purified mouse p53 protein. Recognizes the ~53 kDa mammalian wild type and mutant p53 protein. Cross reacts with human, monkey, mouse, rabbit, and rat. **FC, FS, GS, IB, IF, IP**	100 µg	
Anti-p53 (Ab-1) (Pantropic) Mouse mAb (PAb421)	OP03F	Liquid, purified antibody conjugated to fluorescein. Immunogen used was partially purified mouse p53 protein. Recognizes the ~53 kDa mammalian wild type and mutant p53 protein. Cross reacts with human, monkey, mouse, rabbit, and rat. **FC, FS, IF**	100 µg	
Anti-p53 (Ab-2) (Pantropic) Mouse mAb (PAb1801)	OP09	Liquid, purified. Immunogen used was a human p53 fusion protein. Recognizes the ~53 kDa wild-type and mutant p53 protein in A-431 cells and breast carcinoma tissue. **FS, GS, IB, IP, PS**	100 µg 200 µg	
Anti-p53 (Ab-3) (Mutant) Mouse mAb (PAb240)	OP29	Liquid, purified. Immunogen used was a recombinant protein consisting of amino acids 14-389 of p53 fused to β-galactosidase. Recognizes the mutant p53 protein under non-denaturing conditions and both mutant and wild-type p53 under denaturing conditions. Reacts with bovine, chicken, hamster, human, mouse, and rat. **FC, FS, GS, IB, IF, IP, PS**	20 µg 100 µg 200 µg	
Anti-p53 (Ab-3) (Mutant) Mouse mAb (PAb240)	OP29L	Lyophilized, purified. Immunogen used was a recombinant protein consisting of amino acids 14-389 of p53 fused to β-galactosidase. Recognizes the mutant p53 protein under non-denaturing conditions and both mutant and wild-type p53 under denaturing conditions. Reacts with bovine, chicken, hamster, human, mouse, and rat. **FC, FS, GS, IB, IF, IP, PS**	100 µg	
Anti-p53 (Ab-4) (Wild type) Mouse mAb (PAb246)	OP32	Liquid, purified. Immunogen used was BALB/c SVA31 E7 cells. Recognizes the ~53 kDa wild-type p53 protein in its native conformation. Does not recognize mutant or denatured p53 protein. Reacts with mouse and rat. **IC, IP, PS**	100 µg	
Anti-p53 (Ab-4) (Wild type) Mouse mAb (PAb246)	OP32L	Lyophilized, purified. Immunogen used was BALB/c SVA31 E7 cells. Recognizes the ~53 kDa wild-type p53 protein in its native conformation. Does not recognize mutant or denatured p53 protein. Reacts with mouse and rat. **IC, IP, PS**	100 µg	
Anti-p53 (Ab-5) (Wild type) Mouse mAb (PAb1620)	OP33	Liquid, purified. Immunogen used was vLM mouse tumor cells. Recognizes the ~53 kDa wild-type p53 protein in its native conformation in Hs27 cells and breast carcinoma tissue. Does not recognize mutant or denatured p53 protein. Reacts with bovine, human, mouse, primate, and rat. **FS, IF, IP, PS**	20 µg 100 µg	
Anti-p53 (Ab-6) (Pantropic) Mouse mAb (DO-1)	OP43	Liquid, purified. Immunogen used was recombinant human wild type p53. Recognizes the ~53 kDa wild-type and mutant p53 protein in A-431 cells and breast carcinoma tissue. Reacts with feline and human. **FS, IB, IC, IP, PS**	20 µg 100 µg	
Anti-p53 (Ab-6) (Pantropic) Mouse mAb (DO-1)	OP43L	Lyophilized, purified. Immunogen used was recombinant human wild type p53. Recognizes the ~53 kDa wild-type and mutant p53 protein in A-431 cells and breast carcinoma tissue. Reacts with feline and human. **FS, GS, IB, IC, IP, PS**	100 µg	
Anti-p53 (Ab-6) (Pantropic) Mouse mAb (DO-1) Agarose Conjugate	OP43A	Liquid slurry, purified antibody conjugated to agarose. Immunogen used was recombinant human wild type p53. Recognizes the ~53 kDa wild-type and mutant p53 protein in A-431 cells and breast carcinoma tissue. Reacts with feline and human. **AC, IP**	0.5 ml	
Anti-p53 (Ab-6) (Pantropic) Mouse mAb (DO-1) Fluorescein Conjugate	OP43F	Liquid, purified antibody conjugated to fluorescein. Immunogen used was recombinant human wild type p53. Recognizes the ~53 kDa wild-type and mutant p53 protein in A-431 cells. Reacts with feline and human. **FC, FS, IC, IF, PS**	100 µg	
Anti-p53 (Ab-7) (Pantropic) Sheep pAb	PC35	Diluted serum. Immunogen used was recombinant human p53 protein. Recognizes the ~53 kDa wild-type and mutant p53 protein in A-431 and SVT2 cells and breast carcinoma tissue. Reacts with human, mouse and rat. **FS, IB, IF, IP, PS**	1 each	
Anti-p53 (Ab-11) (Pantropic) Mouse mAb (PAb1802)	OP104L	Lyophilized, purified. Immunogen used was recombinant human p53 protein. Recognizes the ~53 kDa wild-type and mutant p53 protein in immunoblotting. Recognizes only the wild-type p53 protein by immunoprecipitation. Reacts with human and mouse. **IB, IP**	100 µg	
Anti-p53 (Ab-12) (Pantropic) Mouse mAb (DO-7)	OP140	Liquid, purified. Immunogen used was recombinant human p53 expressed in *E. coli*. Recognizes the ~53 kDa wild-type p53 and mutant p53 protein in SKBR-3 and MDA-231 cells as well as in colon carcinoma tissue. Reacts with bovine, human, and monkey. **FS, IB, IF, IP, PS**	100 µg	
Anti-p53 (Ab-8) (Pantropic) Mouse mAb (BP53.12)	OP73	Liquid, purified. Immunogen used was full-length human p53. Recognizes the ~53 kDa wild-type and mutant p53 protein in A-431 cells and breast carcinoma tissue. Reacts with human and other primates. **FS, IB, IP, PS**	100 µg	
PhosphoDetect™ Anti-p53 (pSer^{15}) (Ab-3) Rabbit pAb	PC386	Liquid, purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acids surrounding the Ser^{15} phosphorylation site of human p53, conjugated to KLH. Recognizes the ~53 kDa p53 protein phosphorylated at Ser^{15} in PC12 or HeLa cells treated with DNA damaging agents. Does not recognize unphosphorylated p53. Reacts with human, mouse, and rat. **FFS, IB, IC, IP, PS**	50 µl	
PhosphoDetect™ Anti-p53 (pSer^{15}) (Ab-6) Rabbit pAb	PC461	Undiluted serum. Immunogen used was a synthetic phosphopeptide corresponding to amino acids 10-20 surrounding the Ser^{15} phosphorylation site of human p53. Recognizes the ~53 kDa protein phosphorylated at Ser^{15}. Does not recognize unphosphorylated p53. Reacts with human. **IB, PS**	25 µl	

AC: affinity chromatography; **ELISA:** enzyme linked immunosorbent assay; **FC:** flow cytometry; **FFS:** free floating sections; **FS:** frozen sections; **GS:** gel shift; **IB:** immunoblotting; **IC:** immunocytochemistry; **IF:** immunofluorescence; **IP:** immunoprecipitation; **PS:** paraffin sections

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Antibodies for p53 Research *(continued...)*

Name	Cat. No.	Comments	Size	Price
PhosphoDetect™ Anti-p53 (pSer^{20}) Rabbit pAb	DR1023	Liquid, purified. Immunogen used was a a synthetic phosphopeptide corresponding to amino acids surrounding the Ser^{20} phosphorylation site of human p53. Recognizes the ~53 kDa p53 protein phosphorylated at Ser^{20}. Does not recognize unphosphorylated p53. Reacts with human and mouse. **IB, IC, IF, PS**	50 µl	
PhosphoDetect™ Anti-p53 (pSer^{392}) (Ab-4) Rabbit pAb	PC387	Liquid, purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acids surrounding the Ser^{392} phosphorylation site of human p53. Recognizes the ~53 kDa p53 protein phosphorylated at Ser^{392}. Does not recognize unphosphorylated p53. Reacts with human and mouse. **IB**	50 µl	
PhosphoDetect™ Anti-p53 (pSer^{392}) Mouse mAb (9F4)	506133	Immunogen used was a synthetic phosphopeptide corresponding to amino acids surrounding the Ser^{392} phosphorylation site of human p53, conjugated to KLH. Recognizes the ~53 kDa p53 protein phosphorylated at Ser^{392}. Does not recognize unphosphorylated p53. Reacts with human and mouse. **ELISA, IB**	100 µg	
PhosphoDetect™ Anti-p53 (pSer^{46}) Rabbit pAb	DR1024	Liquid, purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acids surrounding the Ser^{46} phosphorylation site of human p53. Recognizes the ~53 kDa p53 kDa protein phosphorylated at Ser^{46} in MCF7 cells treated with etoposide. Does not recognize unphosphorylated p53. Reacts with human. **IB, IC, IP**	50 µl	
Anti-p53 Binding Protein 1 (Ab-1) Rabbit pAb	PC712	Liquid, purified. Immunogen used was a recombinant protein consisting of human 53BP1 fused to a His•Tag® sequence. Recognizes the ~230 kDa 53BP1 protein in HeLa cell nuclear extract (Cat. No. WB64). Reacts with human and mouse. **IB, IF, IP**	100 µl	
Anti-p53 Binding Protein 1 Mouse mAb (BP13)	DR1003	Undiluted ascites. Immunogen used was N-terminal human p53BP1/GST-fusion protein. Recognizes the ~230 kDa 53BP1 protein in HeLa nuclear extract (Cat. No. WB64) and HEK293 whole cell lysate. **IB, IC, IP**	50 µl	

AC: affinity chromatography; **ELISA:** enzyme linked immunosorbent assay; **FC:** flow cytometry; **FFS:** free floating sections; **FS:** frozen sections; **GS:** gel shift; **IB:** immunoblotting; **IC:** immunocytochemistry; **IF:** immunofluorescence; **IP:** immunoprecipitation; **PS:** paraffin sections

p53 Antibody Sampler Kit

Contains 20 µg of each of the following antibodies.

- Anti-p53 (Ab-1) (Pantropic) Mouse mAb (PAb421) Cat. No. OP03
- Anti-p53 (Ab-2) (Pantropic) Mouse mAb (PAb1801) Cat. No. OP09
- Anti-p53 (Ab-3) (Mutant) Mouse mAb (PAb240) Cat. No. OP29
- Anti-p53 (Ab-4) (Wild type) Mouse mAb (PAb246) Cat. No. OP32
- Anti-p53 (Ab-5) (Wild type) Mouse mAb (PAb1620) Cat. No. OP33
- Anti-p53 (Ab-6) (Pantropic) Mouse mAb (DO-1) Cat. No. OP43

Cat. No. ASK07 **1 kit**

p53 ELISA^{PLUS} (Autoantibody) Kit

Provided in a 96-well format. Plate is pre-coated with recombinant human wild-type p53. The detection antibody used is purified goat anti-human polyclonal antibody conjugated with horseradish peroxidase. Designed to measure circulating antibodies to p53 in human serum samples.

Assay range: 0.16-1 antibody titer units

Cat. No. QIA53 **1 kit**

p53, MDM-2 Binding Domain, N-Terminal 17-26, Cell-Permeable

(ETFSDLWKLL-KKWKMRRNQFWVKVQRG)

Amino acids residues 17-26, derived from the N-terminal MDM-2 binding domain of p53, and linked to the *Antennapedia* leader sequence at the C-terminus. Directly binds to MDM-2 and induces non-apoptotic cell-death (IC_{50} = 40 µg/ml in TUC-3 cells) that is independent of p53 activation. Does not affect the growth of normal cells. *Purity: ≥95% by HPLC.* M.W. 3509.2

Cat. No. 506136 **500 µg**

Ref.: Kanovsky, M., et al. 2001. *Proc. Natl. Acad. Sci. USA* **98,** 12438

p53 (1-342, C-Terminal Deletion), His•Tag®, Human, Recombinant, *S. frugiperda*

(p53 del 1-342)

A human p53 mutant protein (amino acids 1-342) with deletion of the C-terminal 51 amino acids, including the entire basic domain and part of the tetramerization domain. This mutant protein can be used as a unique tool to study specific function of p53 related to the C-terminus. *Activity: 1 unit/ng protein. Purity: ≥95% by SDS-PAGE.* M.W. 40,000

Cat. No. 506146 **5000 units**

Ref.: Waterman, M.J., et al. 1996. *Cancer Res.* **56,** 158; Ishioka, C., et al. 1995; *Oncogene* **10,** 1485; Pellegata, N.S., et al. 1995. *Oncogene* **11,** 337; el-Deiry, W.S., et al. 1992. *Nat. Genet.* **1,** 45; Hupp, T.R., et al. 1992. *Cell* **71,** 875.

p53, Wild-Type, His•Tag®, Human, Recombinant, *S. frugiperda*

Human p53 expressed in a baculovirus expression system. *Activity: 1 unit/ng protein. Purity: ≥95% by SDS-PAGE.* M.W. 53,000

Cat. No. 506147 **5000 units**

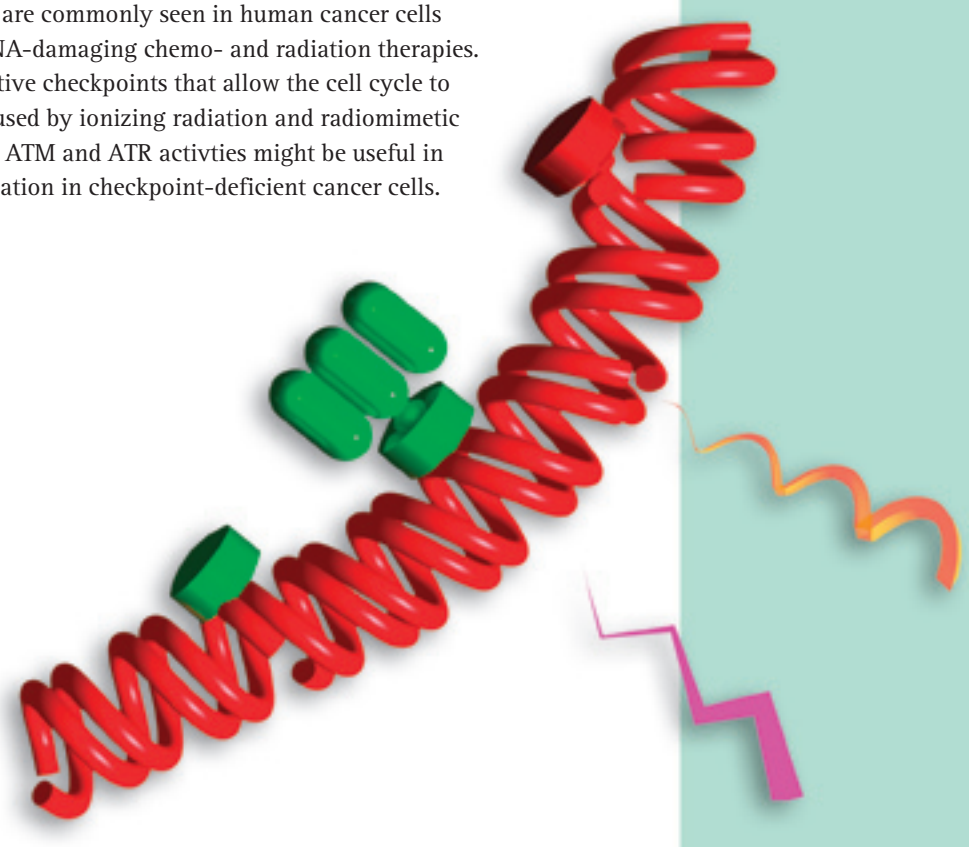
Ref.: Liu, G., et al. 2003. *J. Biol. Chem.* **278,** 17557; Zhang, L., et al. 2000. *Cancer Res.* **60,** 3655; Bennett, W.P., et al. 1992. *Chest* **101,** 19S; Hollstein, M., et al. 1991. *Science* **253,** 49; Fields, S. and Jang, S.K. 1990. *Science* **249,** 1046

ATM/ATR:
Sensors of DNA Damage and Response

ATM (Ataxia-telangiectasia mutated) and ATR (ATM- and Rad3-related), members of phosphatidyl inositol 3-kinase-like kinase (PIKK) family, are activated in response to DNA damage. ATM is primarily activated in response to double-strand breaks (DSBs) induced by ionizing radiation and radiomimetic drugs, while ATR acts in response to UV damage. ATR is reported to bind to UV-damaged DNA with greater affinity than to undamaged DNA. Also, damaged DNA stimulates the ATR kinase activity to a significantly higher level than does undamaged DNA. ATM and ATR are usually found localized near the damaged regions within several minutes indicating that these two kinases may also have a damage-sensing role.

After their recruitment to sites of DNA damage, ATM and ATR phosphorylate several intracellular substrates, including Chk1 and Chk2 that in turn target other proteins to induce cell-cycle arrest and allow DNA repair to proceed. In normal cells ATM is present as inert dimers or multimers, which dissociate into highly active ATM monomers following any DNA damage. During this process, ATM undergoes autophosphorylation on Ser^{1981} and the activated ATM undergoes additional phosphorylations and acetylation reactions. It is believed that after rapid activation of ATM and subsequent phosphorylation of its substrates, ATR is independently activated and maintains phosphorylation of these substrates.

Defects in ATM/ATR signaling pathways are commonly seen in human cancer cells and affect the sensitivity of tumors to DNA-damaging chemo- and radiation therapies. In addition, most cancer cells have defective checkpoints that allow the cell cycle to proceed even in the presence of DSBs caused by ionizing radiation and radiomimetic drugs. Hence, designing drugs that block ATM and ATR activties might be useful in promoting chemo- and radiation sensitization in checkpoint-deficient cancer cells.



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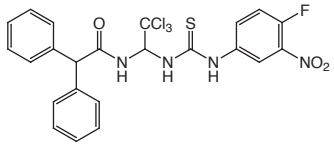
ATM/ATR Kinase Inhibitor

A cell-permeable thiourea compound that selectively inhibits the kinase activity of ATM and ATR (IC_{50} ~ 200 nM) without significantly affecting other PIKK family members (PI3K, mTOR, and DNA-PK) or kinases that are known to phosphorylate p53. *Purity: ≥95% by HPLC.* M.W. 555.8



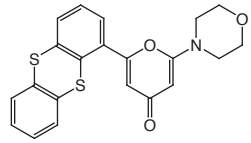
Cat. No. 118501 **5 mg**

Ref.: Won, J., et al. 2006. *Nat. Chem. Biol.* **2,** 369.

ATM Kinase Inhibitor

(2-Morpholin-4-yl-6-thianthren-1-yl-pyran-4-one)

A cell-permeable, potent, and ATP-competitive inhibitor of ATM kinase (IC_{50} = 13 nM; Ki = 2.2 nM). Displays excellent selectivity over other PIKK family kinases (IC_{50} = 2.5, 9.3, 16.6 µM for DNA-PK, mTOR, PI 3-K, respectively; IC_{50} > 100 µM for PI 4-K and ATR). Inhibits ATM-dependent cellular protein phosphorylation following ionizing radiation treatment and sensitizes cells with wild-type ATM, but not mutant ATM, to the cytotoxic effects of DNA-damaging agents. *Purity: ≥98% by HPLC.* M.W. 395.5



Cat. No. 118500 **2 mg**

Ref.: Pereg, Y., et al. 2005. *Proc. Natl. Acad. Sci. USA* **102,** 5056; Lau, A., et al. 2005. *Nat. Cell Biol.* **7,** 493; Hickson, I., et al. 2004. *Cancer Res.* **64,** 9152.

Antibodies for ATM and ATR Research

Name	Cat. No.	Comments	Size	Price
Anti-ATM (Ab-1) (13-24) Rabbit pAb	PC85	Liquid, purified. Immunogen used was a synthetic peptide corresponding to amino acids 13-24 of human ATM. Recognizes the ~350 kDa ATM protein in Daudi, HeLa, and AT169a cells. **IB**	100 µg	
Anti-ATM (Ab-2) Mouse mAb (6B7)	OP90	Liquid, purified. Immunogen used was a synthetic peptide corresponding to amino acids 368-380 of human ATM. Recognizes the ~350 kDa ATM protein in Daudi and HeLa cells. **IP**	200 µg	
Anti-ATM (Ab-3) (819-844) Rabbit pAb	PC116	Liquid, purified. Immunogen used was a synthetic peptide corresponding to amino acids 819-844 of human ATM. Recognizes the ~350 kDa ATM protein in Daudi and HeLa cells. **IB, IP**	100 µg	
Anti-ATR (Ab-2) (2122-2644) Rabbit pAb	PC538	Undiluted serum. Immunogen used was a synthetic peptide corresponding to amino acids 2122-2644 of human ATR. Recognizes the ~300 kDa ATR protein in human and mouse testis tissue extracts. **IB**	50 µl	

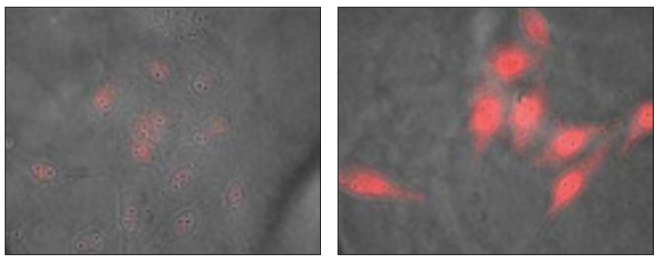
IB: immunoblotting; **IP:** immunoprecipitation

PhosphoDetect™ Anti-ATM (pSer^{1981}) Mouse mAb (10H11.E12)

Liquid, Protein G purified. Immunogen used was a synthetic phosphopeptide corresponding to amino acids 1974-1988 surrounding the Ser^{1981} phosphorylation site of human ATM. Recognizes the ~370 kDa ATM protein phosphorylated on Ser^{1981} in camptothecin-treated HeLa cells. Suitable for immunoblotting and immunocytochemistry.

Sold under license of U.S. Patent 7,108,992.

Cat. No. DR1002 **50 µg**



HeLa cells | HeLa cells + camptothecin

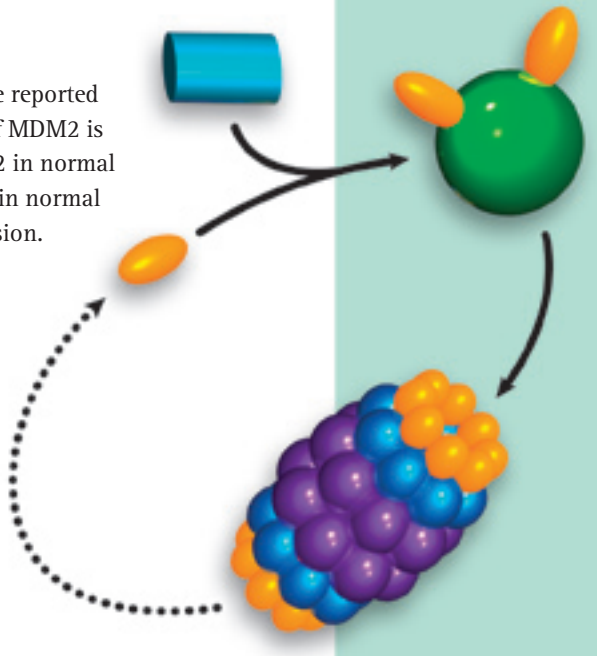
Detection of human ATM phosphorylated at (Ser^{1981}) by immunofluorescence. Samples: Untreated (left panel) and camptothecin-treated (10 µM, 2 h) HeLa cells, fixed with 100% methanol (right panel). Primary antibody: PhosphoDetect™ Anti-ATM (pSer^{1981}) Mouse mAb (10H11.E12) (Cat. No. DR1002) (1.5 µg/ml).

MDM2:
The Regulator of p53 Degradation

MDM2, a p53-inducible phosphoprotein, binds to the N-terminus of the p53 and negatively regulates its activity. *Mdm2* is a p53 responsive gene and its transcription can be activated by p53. Hence, in the presence of high levels of p53, MDM2 levels are also elevated. p53 interacts with MDM2 at Phe^{19}, Trp^{23}, and Leu^{26} to fill up a complementary hydrophobic pocket of MDM2. The three amino acids are also essential for transactivation of p53. Binding of MDM2 to p53 antagonizes the transcriptional activity of p53 and blocks its acetylation and transactivation by interfering with p300/CBP.

MDM2 is believed to function as an E3 ligase to ubiquitinate p53 and force its export from the nucleus to the cytoplasm where it is degraded in the proteasome. Some researchers believe that E3 ubiquitin ligase activity of MDM2 alone is not sufficient to trigger p53 degradation. This is due to the fact that MDM2 mono-ubiquitinates p53 at multiple sites, but does not catalyze the addition of polyubiquitin chains, which are essential for recognition by the proteasome. One possibility exists that mono-ubiquitination of p53 exposes a nuclear export signal; polyubiquitination and degradation can then proceed in the cytoplasm. Although p53-mediated transactivation is a nuclear event, p53 degradation occurs in the cytoplasm. Hence, the ubiquitin ligase function of MDM2 could serve as a cellular mechanism for turnover of p53-MDM2 complexes after their function is completed. It is generally agreed that the nuclear export signal of MDM2 is required for p53 degradation. Studies have shown that Leptomycin B that blocks nuclear export complex formation also prevents nuclear-cytoplasmic shuttling of MDM2 and p53. p53, if sequestered in the cytoplasm, is resistant to degradation by MDM2. Import of p53 from the cytoplasm to the nucleus and export back to cytoplasm seems to be essential for its degradation, and the shuttling of MDM2 and p53 may be a mechanism to prevent their premature degradation.

Malignant tumors, particularly breast tumors and soft tissue sarcomas, are reported to frequently overexpress MDM2. In breast cancer cells, overexpression of MDM2 is correlated with lack of p21 expression. However, overexpression of MDM2 in normal cells is known to cause G_1 arrest. Hence, MDM2 induced by DNA damage in normal cells may have a protective role in preventing untimely cell cycle progression.



References:

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Lee, M.H. and Lozano G. 2006. *Semin. Cancer Biol.* **16,** 225.
Deb, S.P. 2003. *Mol. Cancer Res,* **1,** 1009.
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Dang, J., et al. 2002. *Cancer Res.* **62,** 1222.
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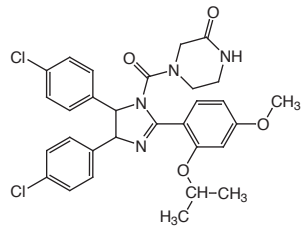
Freedman, D.A. and Levine, A.J. 1998. *Mol. Cell Biol.* **18,** 7288.
Sigalas, I., et al. 1996. *Nat. Med.* **2,** 912.

MDM2 Antagonist, Nutlin-3, Racemic

A cell-permeable *cis*-imidazoline compound that acts as a potent and selective MDM2 antagonist (IC_{50} = 90 nM for Nutlin-3a and 13.6 µM for Nutlin-3b). Activates the p53 pathway by binding MDM2 in the p53-binding pocket and inhibits MDM2-p53 interaction. *Purity: ≥98% by HPLC.* M.W. 581.5



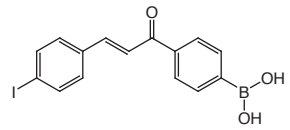
Cat. No. 444143 **1 mg**

Ref.: Thompson, T., et al. 2004. *J. Biol. Chem.* **279,** 53015; Vassilev, L.T., et al. 2004. *Science* **303,** 844.

MDM2 Inhibitor

(*trans*-4-Iodo, 4'-boranyl-chalcone)

A cell-permeable boranyl-chalcone that binds strongly to MDM2 and irreversibly disrupts the MDM2/p53 protein complex. Exhibits selective toxicity towards MDM2 overexpressing human breast cancer cell lines (IC_{50} = 10, 8.8, and 7 µM for MDA-MB-435, MDA-MB-231, and Wt-MCF7, respectively) compared to normal breast cell lines (IC_{50} = 75 and 63 µM for MCF-10A and MCF-12A, respectively). *Purity: ≥95% by HPLC.* M.W. 378.0



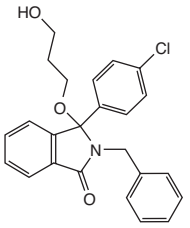
Cat. No. 444145 **10 mg**

Ref.: Kumar, S.K., et al. 2003. *J. Med. Chem.* **46,** 2813.

MDM2 Antagonist III

(2-Benzyl-3-(4-chlorophenyl)-3-(3-hydroxypropoxy)-2,3-dihydroisoindol-1-one)

A cell-permeable isoindolinone compound that binds to MDM2 and disrupts MDM2-p53 interaction (IC_{50} = 15.9 µM). Shown to upregulate p53-dependent luciferase activity and cellular levels of MDM2 and p21 in SJSA cells. *Purity: ≥95% by HPLC.* M.W. 407.9



Cat. No. 444149 **10 mg**

Ref.: Hardcastle, I.R., et al. 2006. *J. Med. Chem.* **49,** 6209; Hardcastle, I.R., et al. 2006. *Bioorg. Med. Chem. Lett.* **15,** 1515.

MDM2 Human, Recombinant, *E. coli*

Recombinant, human MDM2 expressed in *E. coli* and purified by affinity chromatography using FPLC. Useful for *in vitro* transcription assays and DNA-protein and protein-protein interaction assays. *Biological activity: 20-200 ng is sufficient for an in vitro protease assay and 100 ng is sufficient for a protein-protein interaction assay. One unit is defined as 1 ng purified protein. Purity: ≥95% by SDS-PAGE.*

Cat. No. 444146 **10,000 U**

Ref.: Thompson, T., et al. 2004. *J. Biol. Chem.* **279,** 53015; Vassilev, L.T., et al. 2004. *Science* **303,** 844.

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Antibodies for MDM2 Research

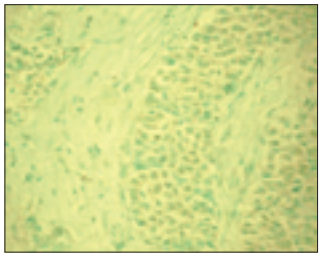
Name	Cat. No.	Comments	Size	Price
Anti-MDM2 (Ab-1) Mouse mAb (IF2)	OP46	Liquid, purified. Immunogen used was human MDM2. Recognizes the ~90 kDa (apparent MW) MDM2 protein. Also recognizes isoforms of ~57 kDa and ~74/76 kDa by immunoblotting. **FS, IB, IF, IP, PS**	20 µg 100 µg	
Anti-MDM2 (Ab-2) Mouse mAb (2A10)	OP115	Liquid, purified. Immunogen used was human MDM2. Recognizes the ~90 kDa (apparent MW) MDM2 protein. **IB, IF, IP, PS**	100 µg	
Anti-MDM2 (Ab-3) Mouse mAb (4B11)	OP143	Liquid, purified. Immunogen used was a full length recombinant human MDM2. Recognizes the ~90 kDa (apparent MW) MDM2 protein. May also recognize the ~60 kDa and ~90 kDa isoforms of MDM2. **IB, IF, IP, PS**	10 µg 100 µg	

FS: frozen sections; **IB:** immunoblotting; **IF:** immunofluorescence; **IP:** immunoprecipitation; **PS:** paraffin sections

Anti-MDM2 (Ab-4) Mouse mAb (2A9C1.18)

Liquid, purified. Immunogen used was human recombinant MDM2. Recognizes the ~90 kDa (apparent MW) MDM2 protein. Suitable for immunoblotting, immunofluorescence, immunoprecipitation, and immunohistochemistry (paraffin sections).

Cat. No. OP144 20 µg, 100 µg

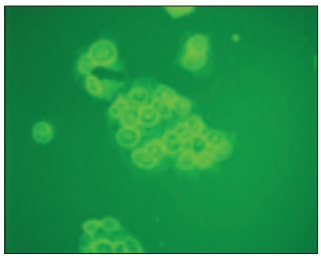


Detection of human MDM2 by staining paraffin sections. Sample: Human breast carcinoma, paraffin-embedded. Primary antibody: Anti-MDM2 (Ab-4) Mouse mAb (2A9C1.18) (Cat. No. OP144) (2.5 µg/ml). Detection: DAB.

Anti-MDM2 (Ab-5) Mouse mAb (4B2C1.11)

Liquid, purified. Immunogen used was human recombinant MDM2. Recognizes the ~90 kDa (apparent MW) MDM2 protein. Suitable for immunoblotting, immunofluorescence, immunoprecipitation, and immunohistochemistry (paraffin sections).

Cat. No. OP145 100 µg

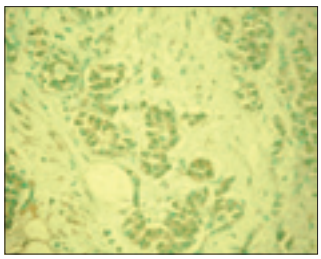


Detection of human MDM2 by immunocytochemistry. Sample: MCF7 cells. Primary antibody: Anti-MDM2 (Ab-5) Mouse mAb (4B2C1.11) (Cat. No. OP145) (2.5 µg/ml). Detection: fluorescence.

Anti-MDM2 (Ab-6) Mouse mAb (5B10C)

Liquid, purified. Immunogen used was human recombinant MDM2. Recognizes the ~90 kDa (apparent MW) MDM2 protein. Suitable for immunoblotting, immunofluorescence, immunoprecipitation, and immunohistochemistry (paraffin sections).

Cat. No. OP146 100 µg



Detection of human MDM2 by staining paraffin sections. Sample: Breast carcinoma tissue. Primary antibody: Anti-MDM2 (Ab-6) Mouse mAb (5B10C) (Cat. No. OP146) (2.5 µg/ml) Detection: DAB

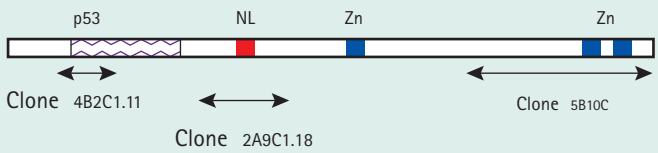
MDM2 Antibody Sampler Kit-Human

Contains 20 µg of each of the following anti-MDM2 antibodies:

- MDM2 (Ab-4) Monoclonal (2A9C1.18) (Cat. No. OP144)
- MDM2 (Ab-5) Monoclonal (4B2C1.11) (Cat. No. OP145)
- MDM2 (Ab-6) Monoclonal (5B10C) (Cat. No. OP146)

Each antibody is suitable for immunoblotting, immunoprecipitation, immunofluorescence, and immunohistochemistry (paraffin sections).

Cat. No. ASK26 1 kit



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