Technical Data Sheet

Purified Mouse Anti-MDM2

Product Information

Material Number:	**556353**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	SMP14
Immunogen:	Human MDM2 aa. 154-167
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Reported: Mouse, Rat
Target MW:	95 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

MDM2, originally described as a gene product of mouse double minute chromosomes, is a nuclear oncoprotein that can inhibit the action of certain tumor suppressor proteins. For example, MDM2 binds to the acidic activation domain (residues 2042) of the p53 tumor suppressor protein, and the p53-MDM2 complex down regulates the transcriptional activity of p53. p53 plays a role in the normal cell cycle by activating the transcription of genes that cause arrest in G1. The expression of MDM2 is itself, induced by p53 and may be a way for p53 to self-regulate its activity during the normal cell cycle. However, overexpression of MDM2 results in the loss of p53-regulated growth control and consequently, deregulated cell proliferation. MDM2 also binds to the Retinoblastoma tumor suppressor protein (Rb) and inhibits its growth regulatory function. MDM2 can directly augment proliferation by binding to two transcription factors E2F1 and DP1, and stimulating the activity of the S-phase inducing E2F1/DP1 heterodimer. MDM2 migrates at a reduced molecular weight of ~95 kDa.

The SMP14 clone has been reported to recognize human, mouse and rat MDM2 while exhibiting a slight cross-reactivity with cytokeratins 6, 14 and 16 in some experimental systems. In the immunoprecipitation application, SMP14 has been reported to precipitate MDM2 and p53-MDM2 complexes. MCF7 human breast carcinoma cells (ATCC HTB-22) and NIH/3T3 mouse fibroblasts (ATCC CRL-1658) are suggested as western blot and immunoprecipitation positive controls. SMP14 has been reported to be useful for the immunohistochemical staining of acetone-fixed, frozen sections and of formalin-fixed, paraffin-embedded tissue sections. In addition to a nuclear staining of MDM2, cytoplasmic staining may also be observed which is likely to be atrributable to the slight crossreactivity of the SMP14 clone with cytokeratins.

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

Western blot	Routinely Tested
Immunoprecipitation	Tested During Development
Bioimaging	Tested During Development
Immunohistochemistry-formalin (antigen retrieval required)	Reported
Immunohistochemistry-frozen	Reported

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.

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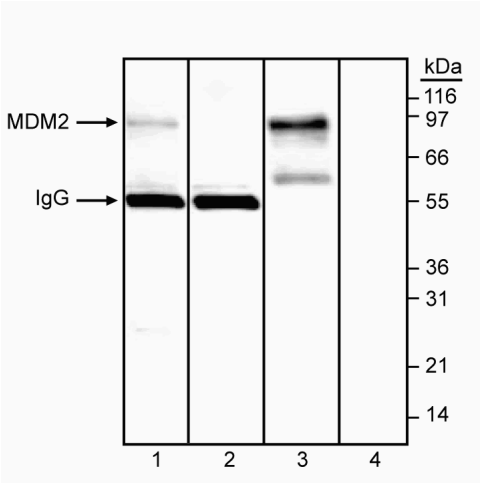
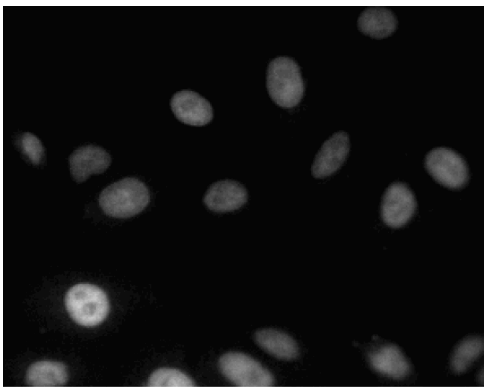
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp
Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml



Immunoprecipitation/western blot analysis of MDM2. *A MCF7 human breast carcinoma cell lysate was used for immunoprecipitation at 2 ug antibody/1 million cells (lanes 1-2) and western blotting at 1 µg/ml (lane 1-4) with the anti-MDM2 antibody (clone SMP14); MCF7 cells (lanes 1 and 3) and isotype control (lanes 2 and 4). MDM2 is identified as a band of ~95 kDa, as well as a putative MDM2 breakdown product at ~62 kDa. The 55 kDa band represents the IgG heavy chain used for immunoprecipitation.*



Immunofluorescent staining of A549 (ATCC CCL-185) cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~ 10,000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the anti-MDM2 antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). The image was taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained HeLa (ATCC CCL-2) and U-2 OS (ATCC HTB-96) cells and can be used with either perm protocol (see Recommended Assay Procedure).*

Suggested Companion Products

Catalog Number	Name	Size	Clone
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611548	MCF7 Cell Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
3. Triton is a trademark of the Dow Chemical Company.
4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Martin K, Trouche D, Hagemeier C, Sorensen TS, La Thangue NB, Kouzarides T. Stimulation of E2F1/DP1 transcriptional activity by MDM2 oncoprotein. *Nature.* 1995; 375(6533):691-694. (Clone-specific: Western blot)

Picksley SM, Vojtesek B, Sparks A, Lane DP. Immunochemical analysis of the interaction of p53 with MDM2;--fine mapping of the MDM2 binding site on p53 using synthetic peptides. *Oncogene.* 1994; 9(9):2523-2529. (Immunogen)