Technical Data Sheet

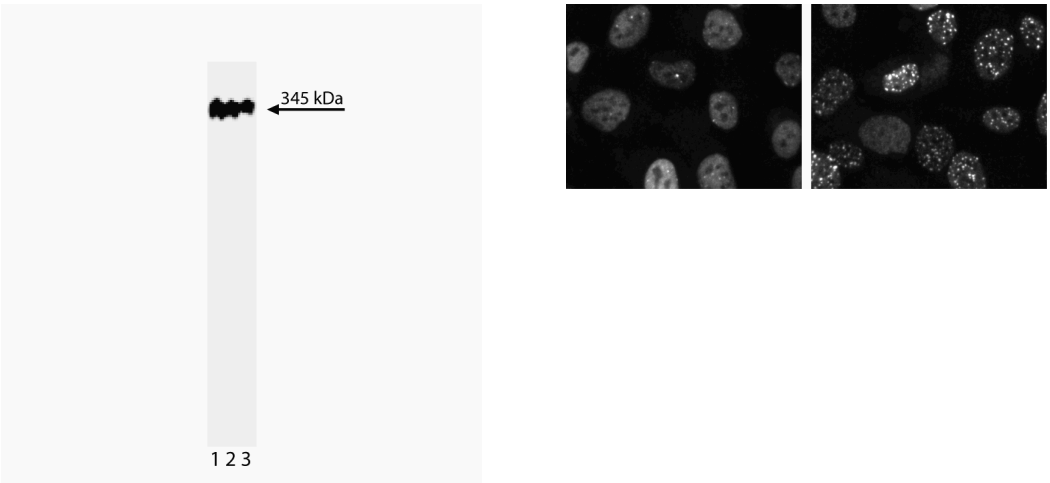
Purified Mouse Anti-Human 53BP1

Product Information

Material Number:	**612523**
Size:	150 µg
Concentration:	250 µg/ml
Clone:	19/53BP1
Immunogen:	Human 53BP1 aa. 149-259
Isotype:	Mouse IgG2b
Reactivity:	QC Testing: Human
Target MW:	345 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

The p53 protein is critical to regulation of normal cell growth and is a suppressor of tumor cell proliferation. Inactivation of p53 by a number of mechanisms, such as missense mutations or interaction with oncogenic viral or cellular proteins, can result in tumor progression. In addition, Bcl2 and p53 are involved in apoptosis in an antagonistic fashion such that overexpressed Bcl2 inhibits p53-induced apoptosis. 53BP1 and 53BP2 were identified in a yeast two-hybrid screen of proteins that bind p53. Both 53BP1 and 53BP2 bind wild type p53, but not mutant p53 found in tumor cells. p53BP1 is localized to the cytoplasm and nucleus, while p53BP2 is found only in the cytoplasm. 53BP1 has BRCT motifs found in proteins involved in cell cycle control and DNA repair. DNA damage leads to 53BP1 hyperphosphorylation, which may be mediated by ATM. 53BP2 has four ankyrin repeats and a SH3 domain that are required for interactions with Bcl2 and p53. Overexpression of 53BP2 in 293 cells inhibits progression of the cell cycle in G2/M phase, while co-transfection of 53BP2 with p53 in H358 cells enhances p53-mediated transcriptional activation. The interaction between 53BP2 and p53 may be regulated by Bcl2, since competition experiments demonstrate that Bcl2 prevents p53 binding to 53BP2. In addition, 53BP2 can also bind the apoptotic-related p65 subunit of NFκB and this subunit can inhibit 53BP2-induced cell death.



Left Figure: Western blot analysis of 53BP1 on a HeLa lysate. *Lane 1: 1:1000, lane 2: 1:2000, lane 3: 1:4000 dilution of the anti-53BP1 antibody.* ***Right Figure: Immunofluorescent staining of HT1080 cells (ATCC CCL-121).*** *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~10,000 cells per well. After overnight incubation, cells were either mock treated (PBS, left) or exposed to hydrogen peroxide (400uM, right) for 30 minutes and allowed to recover in media for 30 minutes. After treatment, cells were stained using the alcohol perm protocol and the anti-53BP1 antibody. The second step reagent was Alexa Fluor® 488 goat anti-mouse IgG (Invitrogen). The image is a confocal collapsed stack, taken on a BD Pathway™ 855 bioimaging system with a 40x objective. This antibody also stains A549 (ATCC CCL-185), HeLa (ATCC CCL-2) and U-2 OS (ATCC HTB-96) cells and can be used with either fix/perm protocol (see Recommended Assay Procedure).*

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Preparation and Storage

Store undiluted at -20°C.

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

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

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.
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/support/resources/protocols/monoclonal_anti.jsp

Suggested Companion Products

Catalog Number	Name	Size	Clone
611449	HeLa 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)
554002	HRP Goat Anti-Mouse Ig	1.0 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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. 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.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Iwabuchi K, Bartel PL, Li B, Marraccino R, Fields S. Two cellular proteins that bind to wild-type but not mutant p53. *Proc Natl Acad Sci U S A.* 1994; 91(13):6098-6102. (Biology)

Iwabuchi K, Li B, Massa HF, Trask BJ, Date T, Fields S. Stimulation of p53-mediated transcriptional activation by the p53-binding proteins, 53BP1 and 53BP2. *J Biol Chem.* 1998; 273(40):26061-26068. (Biology)

Rappold I, Iwabuchi K, Date T, Chen J. Tumor suppressor p53 binding protein 1 (53BP1) is involved in DNA damage-signaling pathways. *J Cell Biol.* 2001; 153(3):613-620. (Biology)