

Technical Data Sheet

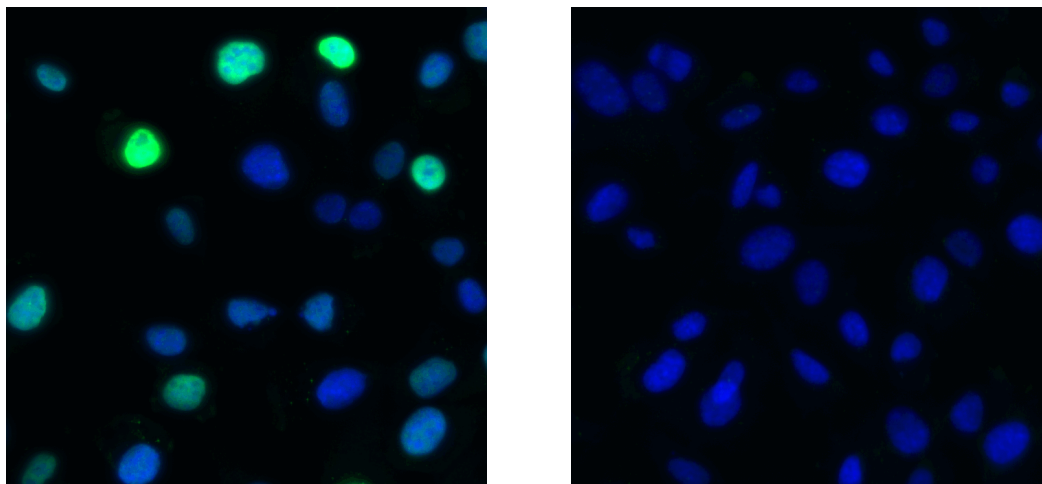
Purified Mouse anti-ATM (pS1981)**Product Information**

Material Number:	560007
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	K88-534
Immunogen:	Phosphorylated Mouse ATM Peptide
Isotype:	Mouse (BALB/c) IgG2b, κ
Reactivity:	Confirmed by Bioimaging: Human Confirmed by western blot: Mouse
Target MW:	349 kDa
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

Description

ATM is the gene that is mutated in ataxia telangiectasia, an autosomal recessive hereditary disease that is characterized by delayed motor and sexual development, weakened immunity, multiple skin changes, increased sensitivity to ionizing radiation, and increased risk of developing certain cancers. The protein encoded by ATM is a phosphoinositide-3-kinase-related protein kinase that is found in the nucleus and is responsible for early responses to the changes in chromatin structure caused by DNA double-strand breaks. Inactive wild type ATM protein forms homodimers or higher order multimers in which the C-terminal kinase domains are inhibited. It has been proposed that multimeric ATM is recruited to DNA double-strand breaks through interactions between its N-terminal HEAT domains and specific chromatin protein complexes. The inhibition of the kinase domains is lifted, resulting in auto-phosphorylation of the ATM at a single serine site and disruption of the multimers. The phosphorylated state of ATM is a rapid and highly sensitive indicator that cells have been exposed to agents that cause DNA double-strand breaks. The kinase domains of the resulting ATM monomers are accessible to a wide variety of substrates that are involved in DNA repair, cell-cycle regulation, and apoptosis. Thus ATM is a central regulator of cellular responses to ionizing radiation, and cells that lack ATM undergo radioresistant DNA synthesis and are resistant to γ radiation-induced apoptosis.

The K88-534 monoclonal antibody recognizes human ATM phosphorylated at serine 1981 (S1981) and mouse ATM phosphorylated at its orthologous site, S1987.



Immunofluorescent detection of ATM phosphorylation in human fibrosarcoma cells. HT-1080 cells (ATCC CCL-121) were seeded in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) at ~10,000 cells per well. After overnight culture, DNA damage was induced by exposing the cells to bleomycin sulfate (250 units/ml) in serum-free medium for 2 hours at 37°C (left panel) or the cells were untreated (right panel). To allow time for DNA repair processes to occur, the cells were washed, replenished with fresh complete medium, and returned to the incubator for 30 minutes. After treatment, the cells were fixed, permeabilized with cold 90% methanol, stained with Purified Mouse anti-ATM (pS1981) (pseudo-colored green), and counter-stained with Hoechst 33342 (pseudo-colored blue) according to the Recommended Assay Procedure. The second-step reagent was Alexa Fluor® 488 goat anti-mouse Ig (Invitrogen). Confocal images were captured on a BD Pathway™ 855 Bioimager System using a 20x (0.75 NA) objective. Five sections, separated by 1 μ m each, were captured and collapsed for viewing purposes using BD Attovision™ software.

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Preparation and Storage

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

Store undiluted at 4° C.

Application Notes

Application

Bioimaging	Routinely Tested
Western blot	Reported

Recommended Assay Procedure:

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 fresh 3.7% Formaldehyde in PBS or BD Cytotfix™ fixation buffer (Cat. No. 554655) to each well and incubating for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells by adding 100 µl of -20°C 90% methanol or -20°C BD™ Phosflow Perm Buffer III (Cat. No. 558050) to each well and incubating for 5 minutes at RT.
4. Remove the permeabilizer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of blocking buffer (1% BSA in 1× PBS) to each well and incubating for 30 minutes at RT.
6. Remove the blocking buffer, dilute the antibody in blocking buffer, and stain the cells by adding 50 µl of the diluted antibody to each well and incubating for 1 hour at RT.
7. Remove the diluted antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, dilute the second-step reagent in blocking buffer, and stain the cells by adding 50 µl of the diluted second-step reagent to each well and incubating for 1 hour at RT.
9. Remove the diluted 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 100 µl of a 2 µg/ml solution of Hoechst 33342 (eg, 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.

Suggested Companion Products

Catalog Number	Name	Size	Clone
353219	BD Falcon™ 96-well Imaging Plate	1 box	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.
3. 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.

References

Bakkenis CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature*. 2003; 421:499-506.(Biology)
Pellegrini M, Celeste A, Difilippantonio S, et al. Autophosphorylation at serine 1987 is dispensable for murine Atm activation in vivo. *Nature*. 2006; 443:222-225.
(Clone-specific: Western blot)
You Z, Chahwan C, Bailis J, Hunter T, Russell P. ATM activation and its recruitment to damaged DNA require binding to the C terminus of Nbs1. *Mol Cell Biol*. 2005; 25(13):5363-5379.(Biology)