Technical Data Sheet

Purified Mouse anti-ATM (pS1981)

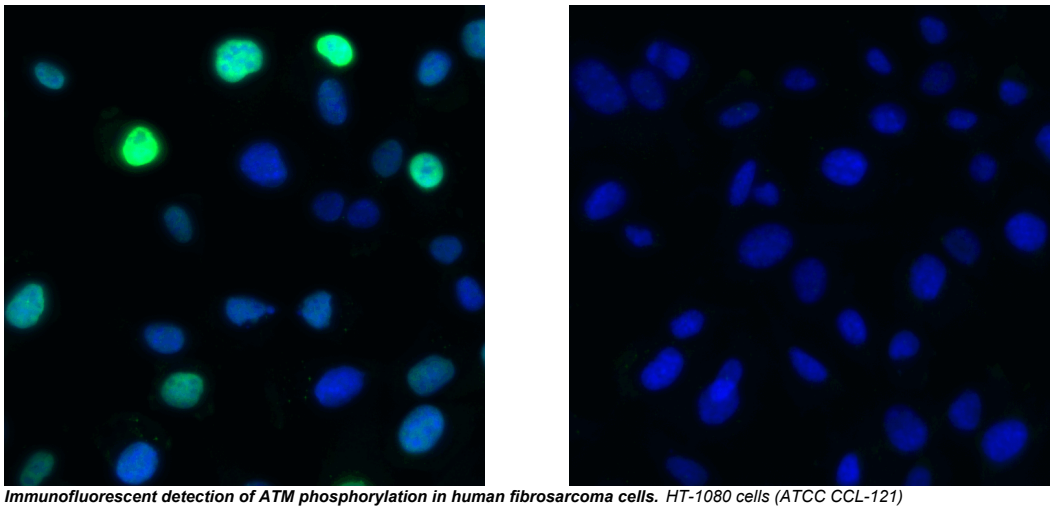
Product Information

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 ≤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 phophoinositide-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, cells were stained using the alcohol perm protocol and the 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 Bioimaging System using a 20x (0.75 NA) objective. Five sections, separated by 1 µm each, were captured and collapsed for viewing purposes using BD Attovision™ software.*

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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

Bioimaging	Routinely Tested
Western blot	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.
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

Suggested Companion Products

Catalog Number	Name	Size	Clone
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

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 dipensable 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)