abcam

Product datasheet

Anti-ATM antibody [2C1 (1A1)] ab78

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Overview

Product name	Anti-ATM antibody [2C1 (1A1)]
Description	Mouse monoclonal [2C1 (1A1)] to ATM
Host species	Mouse
Specificity	The ATM antibody, clone 2C1, recognizes full-length ATM.
Tested applications	**Suitable for:** Flow Cyt, ICC/IF, IHC-P, WB, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human, Monkey
Immunogen	Fusion protein expressed in E. coli corresponding to amino acids 2577-3056.
Positive control	lymphoblastoid nuclear lysate, human Raji, U87-MG , SK-N-SH (human neuroblastoma), IMR32 , SK-N-AS.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: None Constituents: 10mM PBS, pH 7.4
Purification notes	Purified from ascities fluid by Protein G chromatography to at least 95% homogeneity as determined by SDS-PAGE.
Clonality	Monoclonal
Clone number	2C1 (1A1)
Myeloma	NS1
Isotype	IgG1
Light chain type	kappa

Applications

Our Abpromise guarantee covers the use of **ab78** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt		Use 1-2µg for 10^6 cells. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★	Use at an assay dependent concentration.
IHC-P	★★★★☆	Use a concentration of 1 µg/ml.
WB	★★★★★	1/2000. Detects a band of approximately 350 kDa (predicted molecular weight: 350 kDa).
IP		Use a concentration of 1 - 10 µg/ml.

Target

Function

Serine/threonine protein kinase which activates checkpoint signaling upon double strand breaks (DSBs), apoptosis and genotoxic stresses such as ionizing ultraviolet A light (UVA), thereby acting as a DNA damage sensor. Recognizes the substrate consensus sequence [ST]-Q. Phosphorylates 'Ser-139' of histone variant H2AX/H2AFX at double strand breaks (DSBs), thereby regulating DNA damage response mechanism. Also plays a role in pre-B cell allelic exclusion, a process leading to expression of a single immunoglobulin heavy chain allele to enforce clonality and monospecific recognition by the B-cell antigen receptor (BCR) expressed on individual B lymphocytes. After the introduction of DNA breaks by the RAG complex on one immunoglobulin allele, acts by mediating a repositioning of the second allele to pericentromeric heterochromatin, preventing accessibility to the RAG complex and recombination of the second allele. Also involved in signal transduction and cell cycle control. May function as a tumor suppressor. Necessary for activation of ABL1 and SAPK. Phosphorylates p53/TP53, FANCD2, NFKBIA, BRCA1, CTIP, nibrin (NBN), TERF1, RAD9 and DCLRE1C. May play a role in vesicle and/or protein transport. Could play a role in T-cell development, gonad and neurological function. Plays a role in replication-dependent histone mRNA degradation. Binds DNA ends.

Tissue specificity

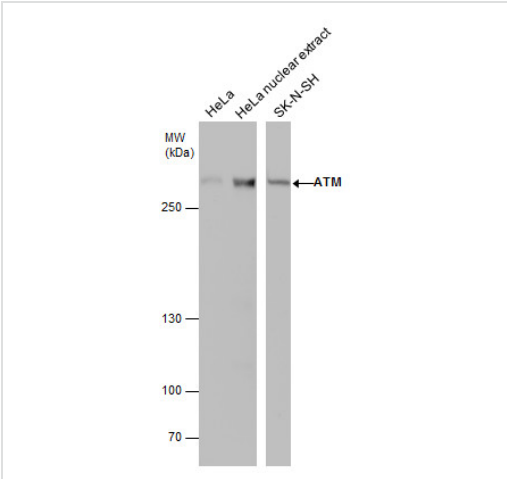
Found in pancreas, kidney, skeletal muscle, liver, lung, placenta, brain, heart, spleen, thymus, testis, ovary, small intestine, colon and leukocytes.

Involvement in disease

Defects in ATM are the cause of ataxia telangiectasia (AT) [MIM:208900]; also known as Louis-Bar syndrome, which includes four complementation groups: A, C, D and E. This rare recessive disorder is characterized by progressive cerebellar ataxia, dilation of the blood vessels in the conjunctiva and eyeballs, immunodeficiency, growth retardation and sexual immaturity. AT patients have a strong predisposition to cancer; about 30% of patients develop tumors, particularly lymphomas and leukemias. Cells from affected individuals are highly sensitive to damage by ionizing radiation and resistant to inhibition of DNA synthesis following irradiation.
Note=Defects in ATM contribute to T-cell acute lymphoblastic leukemia (TALL) and T-prolymphocytic leukemia (TPLL). TPLL is characterized by a high white blood cell count, with a predominance of prolymphocytes, marked splenomegaly, lymphadenopathy, skin lesions and serous effusion. The clinical course is highly aggressive, with poor response to chemotherapy and short survival time. TPLL occurs both in adults as a sporadic disease and in younger AT patients.
Note=Defects in ATM contribute to B-cell non-Hodgkin lymphomas (BNHL), including mantle cell lymphoma (MCL).
Note=Defects in ATM contribute to B-cell chronic lymphocytic leukemia (BCLL). BCLL is the commonest form of leukemia in the elderly. It is characterized by the accumulation of mature

	CD5+ B lymphocytes, lymphadenopathy, immunodeficiency and bone marrow failure.
Sequence similarities	Belongs to the PI3/PI4-kinase family. ATM subfamily. Contains 1 FAT domain. Contains 1 FATC domain. Contains 1 PI3K/PI4K domain.
Domain	The FATC domain is required for interaction with KAT5.
Post-translational modifications	Phosphorylated by NUAK1/ARK5. Autophosphorylation on Ser-367, Ser-1893, Ser-1981 correlates with DNA damage-mediated activation of the kinase. Acetylation, on DNA damage, is required for activation of the kinase activity, dimer-monomer transition, and subsequent autophosphorylation on Ser-1981. Acetylated in vitro by KAT5/TIP60.
Cellular localization	Nucleus. Cytoplasmic vesicle. Primarily nuclear. Found also in endocytic vesicles in association with beta-adaptin.

Images

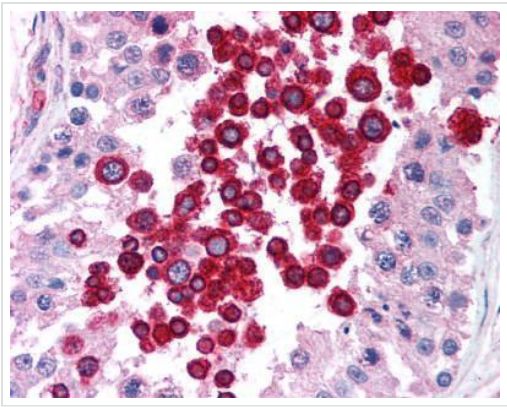


Western blot - Anti-ATM antibody [2C1 (1A1)] (ab78)

All lanes : Anti-ATM antibody [2C1 (1A1)] (ab78) at 1/500 dilution

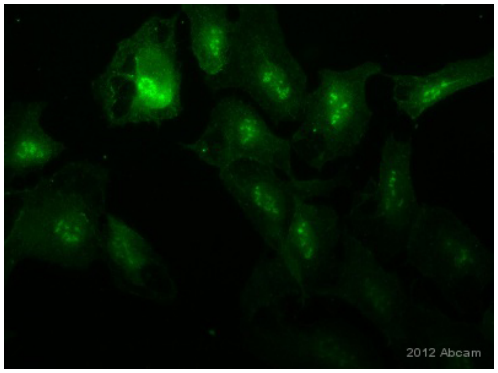
Lane 1 : 30ug HeLa
Lane 2 : 30ug HeLa nuclear extract
Lane 3 : 30ug SK-N-SH

Predicted band size: 350 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATM antibody [2C1 (1A1)] (ab78)

ab78 staining ATM in Human Testis sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Samples were incubated with primary antibody (5ug/ml) and a Biotin-conjugated rabbit anti-mouse IgG was used as the secondary antibody.

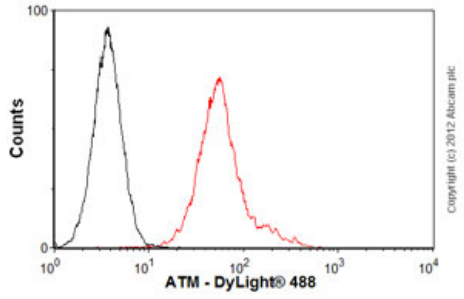


Immunocytochemistry/ Immunofluorescence - Anti-ATM antibody [2C1 (1A1)] (ab78)

This image is courtesy of an anonymous Abreview

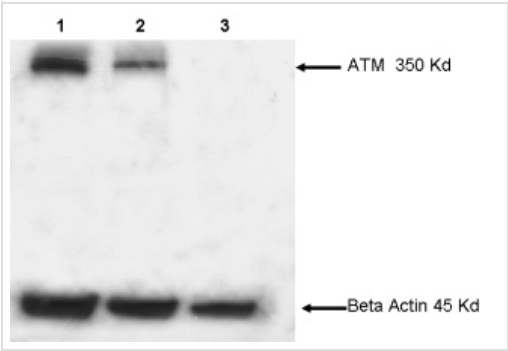
ab78 staining ATM in Human U2OS cells by Immunocytochemistry/ Immunofluorescence.

Cells were fixed with paraformaldehyde, permeabilized with 0.5% NP40/PBS and blocked with 3% BSA for 1 hour at 25°C. Samples were incubated with primary antibody (1/100 in 3% BSA/PBS) for 12 hours at 4°C. An AlexaFluor®488-conjugated goat anti-rabbit polyclonal IgG (1/200) (ab150077) was used as the secondary antibody.



Flow Cytometry - Anti-ATM antibody [2C1 (1A1)] (ab78)

Overlay histogram showing HeLa cells stained with ab78 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab78, 1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Western blot - Anti-ATM antibody [2C1 (1A1)] (ab78)

This image is courtesy of Simona Cavalieri, University of Turin, Italy

All lanes : Anti-ATM antibody [2C1 (1A1)] (ab78) at 1/2000 dilution

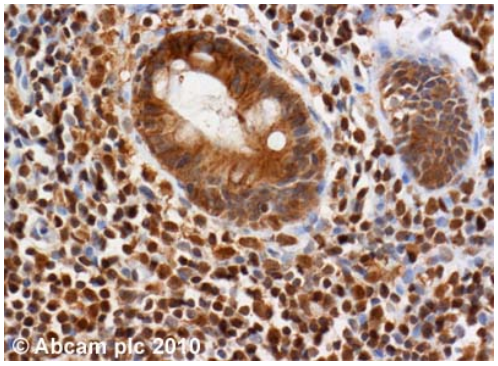
Lane 1 : 50ug lymphoblastoid nuclear lysate, anti-beta actin antibody.
Lane 2 : 25ug lymphoblastoid nuclear lysate, anti-beta actin antibody.
Lane 3 : No ATM negative control, anti-beta actin antibody.

Secondary
All lanes : Anti-mouse secondary antibody at 1/2000 dilution

Performed under reducing conditions.

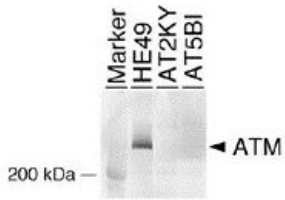
Predicted band size: 350 kDa

Arrows denote the location of the 45kDa beta actin protein, and the 350kDa ATM protein.



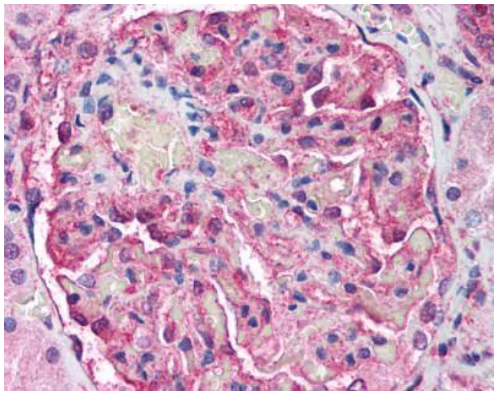
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATM antibody [2C1 (1A1)] (ab78)

ab78 (2µg/ml) staining ATM in human colonic mucosa, using an automated system (DAKO Autostainer Plus). Using this protocol there is strong nuclear staining of mucosal epithelium and lymphocytes.
Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer EDTA pH 9.0 in a DAKO PT link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako envision flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.



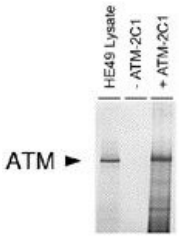
Western blot - Anti-ATM antibody [2C1 (1A1)] (ab78)

Detection of human ATM protein using anti-ATM 2C1 monoclonal antibody (ab78) by western blot.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATM antibody [2C1 (1A1)] (ab78)

ab78 staining ATM in Human Kidney sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Samples were incubated with primary antibody (5ug/ml) and a Biotin-conjugated rabbit anti-mouse IgG was used as the secondary antibody.



Immunoprecipitation - Anti-ATM antibody [2C1 (1A1)] (ab78)

Detection of human ATM protein using anti-ATM 2C1 monoclonal antibody (ab78) by immunoprecipitation.

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