abcam

Product datasheet

Anti-ATM antibody [EPR20100] - BSA and Azide free ab223533

KO VALIDATED Recombinant RabMAb

10 Images

Overview

Product name	Anti-ATM antibody [EPR20100] - BSA and Azide free
Description	Rabbit monoclonal [EPR20100] to ATM - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ChIP, IP, ICC/IF, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human testis lysate; HeLa, PC-12, RAW 264.7, 293 and SH-SY5Y whole cell lysates. ICC/IF: SH-SY5Y and HeLa cells. Flow Cyt (intra): HEK-293 cells. IP: HEK-293 whole cell lysate. ChIP: Chromatin prepared from HCT 116 cells treated with 1mM Hydroxyurea for 16h.
General notes	ab223533 is the carrier-free version of **ab201022**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid

Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20100
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab223533 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 (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ChIP		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 351 kDa (predicted molecular weight: 351 kDa).

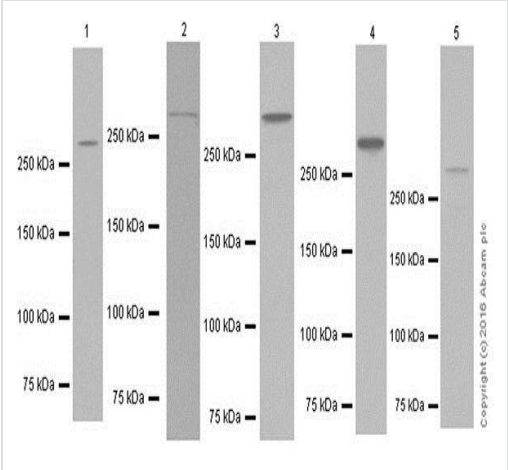
Target

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 [EPR20100] - BSA and Azide free (ab223533)

All lanes : Anti-ATM antibody [EPR20100] - ChIP Grade (**ab201022**) at 1/1000 dilution

Lane 1 : Human testis lysate

Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate

Lane 3 : RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate

Lane 4 : 293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lane 5 : SH-SY5Y (Human neuroblastoma cell line from bone marrow) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

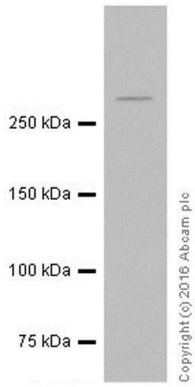
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 351 kDa
Observed band size: 351 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab201022**).

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1 and 2: 3 minutes; Lane 3: 30 seconds; Lane 4: 5 seconds; Lane 5: 1 second



Western blot - Anti-ATM antibody [EPR20100] - BSA and Azide free (ab223533)

Anti-ATM antibody [EPR20100] - ChIP Grade (**ab201022**) at 1/5000 dilution + HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 10 µg

Secondary

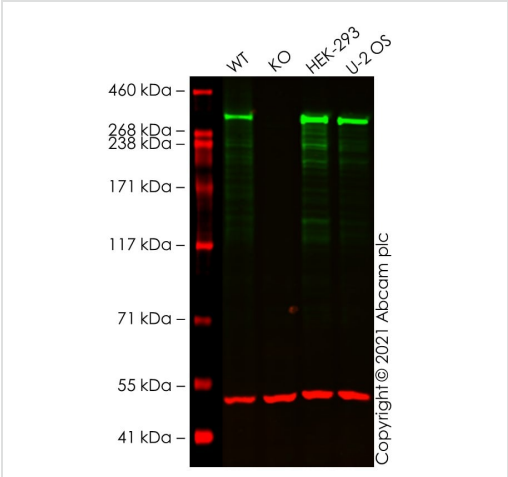
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 351 kDa
Observed band size: 351 kDa

Exposure time: 10 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab201022**).

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-ATM antibody [EPR20100] - BSA and Azide free (ab223533)

All lanes : Anti-ATM antibody [EPR20100] - ChIP Grade (**ab201022**) at 1/1000 dilution

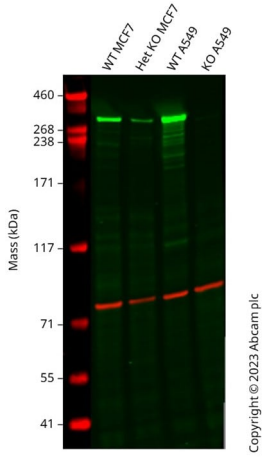
Lane 1 : Wild-type A549 cell lysate
Lane 2 : ATM knockout A549 cell lysate
Lane 3 : HEK-293 cell lysate
Lane 4 : U-2 OS cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 351 kDa

False colour image of Western blot: Anti-ATM antibody [EPR20100] - ChIP Grade staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab201022** was shown to bind specifically to ATM. A band was observed at 350 kDa in wild-type A549 cell lysates with no signal observed at this size in ATM knockout cell line **ab276095** (knockout cell lysate **ab283834**). To generate this image, wild-type and ATM knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-ATM antibody [EPR20100] - BSA and Azide free (ab223533)

All lanes : Anti-ATM antibody [EPR20100] - ChIP Grade (**ab201022**) at 1/1000 dilution

Lane 1 : Wild-type MCF7 cell lysate
Lane 2 : ATM knockout MCF7 cell lysate
Lane 3 : Wild-type A549 cell lysate
Lane 4 : ATM knockout A549 **ab283811** cell lysate

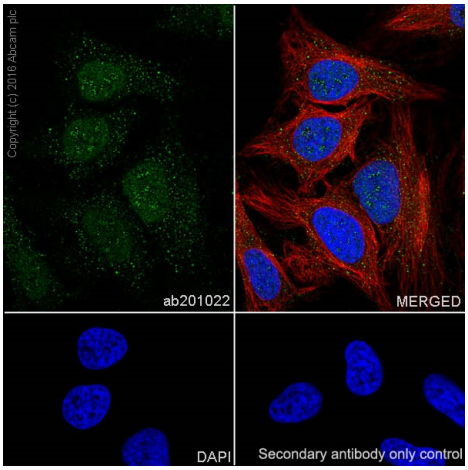
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 351 kDa
Observed band size: 350 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab201022**).

Anti-ATM antibody [EPR20100] (**ab201022**) staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (**ab238078**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab201022** was shown to bind specifically to ATM. A band was observed at 350 kDa in wild-type MCF7 cell lysates with a reduction in signal observed at this size in ATM heterozygous knockout cell line **ab282630**. To generate this image, wild-type and ATM heterozygous knockout MCF7 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween$®$ 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution



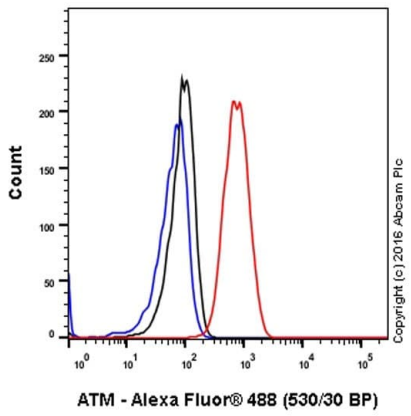
Immunocytochemistry/ Immunofluorescence - Anti-ATM antibody [EPR20100] - BSA and Azide free (ab223533)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling ATM with **ab201022** at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear and weak cytoplasmic staining on HeLa cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.

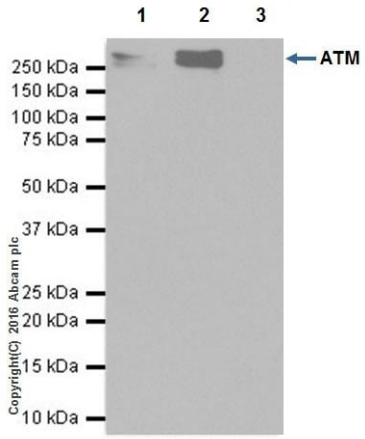
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab201022**).



Flow Cytometry (Intracellular) - Anti-ATM antibody [EPR20100] - BSA and Azide free (ab223533)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HEK-293 (Human epithelial cell line from embryonic kidney) cells labeling ATM with **ab201022** at 1/800 dilution (red) compared with a rabbit monoclonal IgG isotype control (**ab172730**; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab201022**).



Immunoprecipitation - Anti-ATM antibody [EPR20100] - BSA and Azide free (ab223533)

ATM was immunoprecipitated from 0.35 mg of HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate with **ab201022** at 1/40 dilution. Western blot was performed from the immunoprecipitate using **ab201022** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

Lane 1: HEK-293 whole cell lysate, 10 µg (Input).

Lane 2: **ab201022** IP in HEK-293 whole cell lysate.

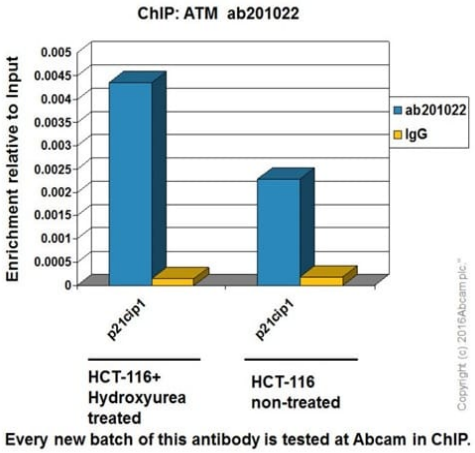
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab201022** in HEK-293 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes.

ATM cleavage has been documented previously and the fragment pattern is consistent with what has been described in the literature PMID:16849690

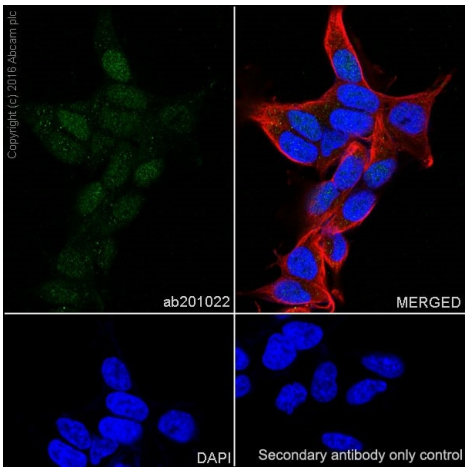
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab201022**).



ChIP - Anti-ATM antibody [EPR20100] - BSA and Azide free (ab223533)

Chromatin was prepared from HCT 116 (Human colorectal carcinoma cell line) cells treated with 1mM Hydroxyurea for 16h and non-treated according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 2µg of **ab201022** (blue), and 20µl of Anti rabbit IgG sepharose beads. 2µg of rabbit normal IgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab201022**).



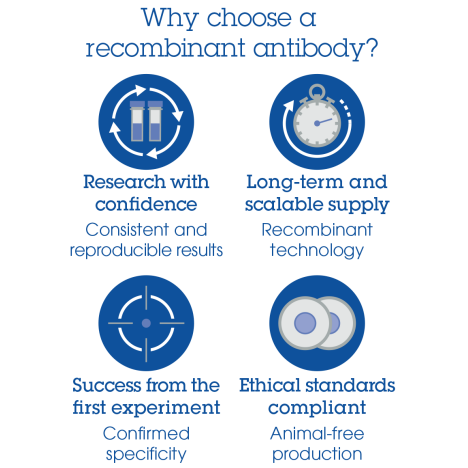
Immunocytochemistry/ Immunofluorescence - Anti-ATM antibody [EPR20100] - BSA and Azide free (ab223533)

This ICC data was generated using the same anti-ATM antibody clone [EPR20100] in a different buffer formulation (cat# **ab201022**).

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized SH-SY5Y (Human neuroblastoma cell line from bone marrow) cells labeling ATM with **ab201022** at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear and weak cytoplasmic staining on SH-SY5Y cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.



Anti-ATM antibody [EPR20100] - BSA and Azide free (ab223533)

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