abcam

Product datasheet

Anti-ATM antibody [EPR20100] - ChIP Grade ab201022

KO 評価済 リコンビナント RabMAb

4 References 画像数 10

製品の概要

製品名	Anti-ATM antibody [EPR20100] - ChIP Grade
製品の詳細	Rabbit monoclonal [EPR20100] to ATM - ChIP Grade
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), ICC/IF, IP, ChIP, WB
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	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.
特記事項	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR20100

アイソタイプ IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab201022の使用に適用されます**

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/800. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/500.
IP		1/40.
ChIP		Use 2 µg for 25 µg of chromatin.
WB		1/1000. Detects a band of approximately 351 kDa (predicted molecular weight: 351 kDa).

ターゲット情報

機能 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.

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

関連疾患 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.

配列類似性

Belongs to the PI3/PI4-kinase family. ATM subfamily.
Contains 1 FAT domain.
Contains 1 FATC domain.
Contains 1 PI3K/PI4K domain.

ドメイン

The FATC domain is required for interaction with KAT5.

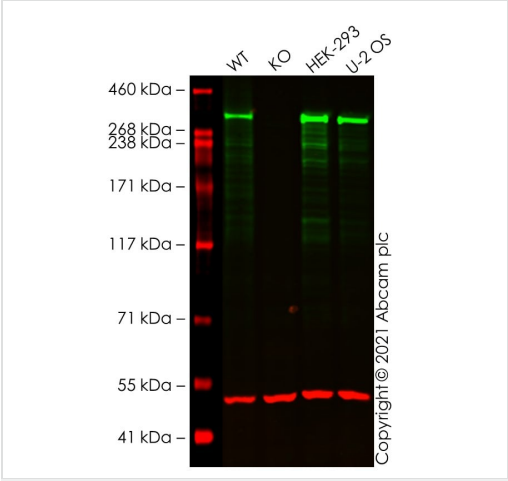
翻訳後修飾

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.

細胞内局在

Nucleus. Cytoplasmic vesicle. Primarily nuclear. Found also in endocytic vesicles in association with beta-adaptin.

画像



Western blot - Anti-ATM antibody [EPR20100] - ChIP Grade (ab201022)

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

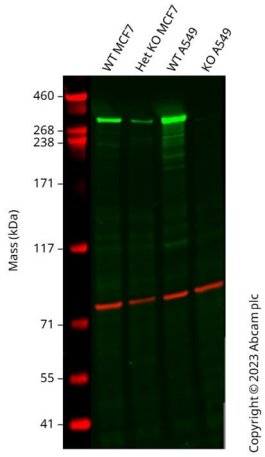
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 351 kDa
Observed band size: 350 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] - ChIP Grade (ab201022)

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

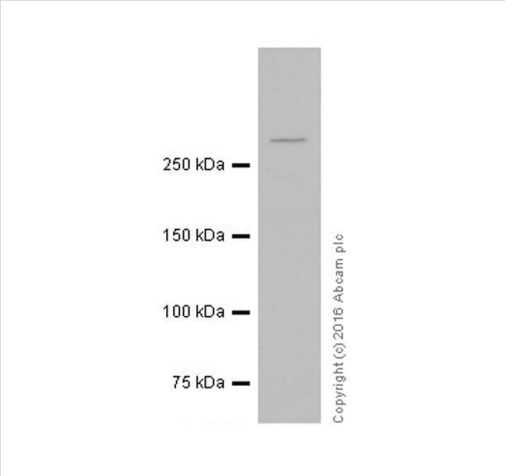
Lane 1 : Wild-type MCF7 cell lysate
Lane 2 : ATM knockout MCF7 cell lysate
Lane 3 : Wild-type A549 cell lysate
Lane 4 : ATM knockout A549 **ab283811** cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

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



Western blot - Anti-ATM antibody [EPR20100] - ChIP Grade (ab201022)

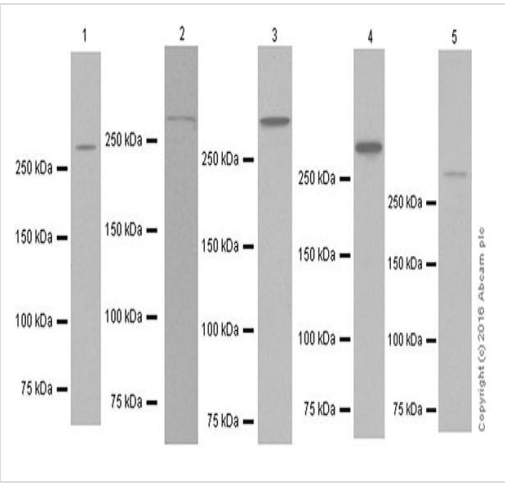
Anti-ATM antibody [EPR20100] - ChIP Grade (ab201022) at 1/5000 dilution + HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 10 µg

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

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-ATM antibody [EPR20100] - ChIP Grade (ab201022)

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.

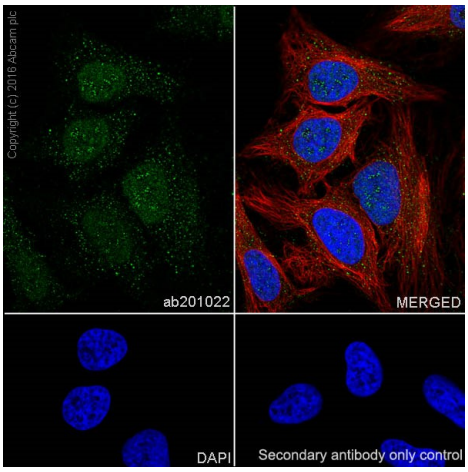
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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

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

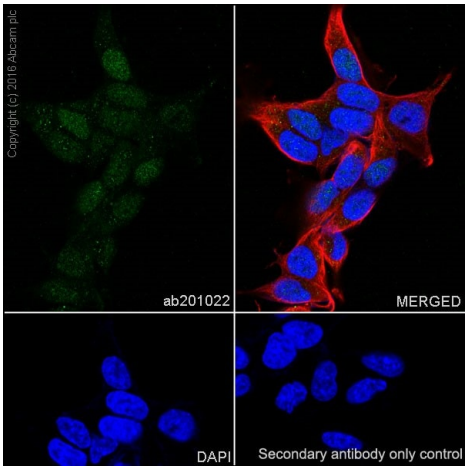


Immunocytochemistry/ Immunofluorescence - Anti-ATM antibody [EPR20100] - ChIP Grade (ab201022)

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.

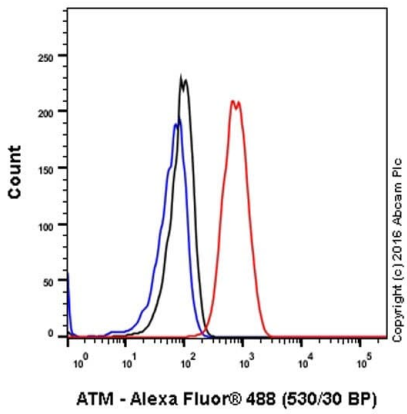


Immunocytochemistry/ Immunofluorescence - Anti-ATM antibody [EPR20100] - ChIP Grade (ab201022)

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.

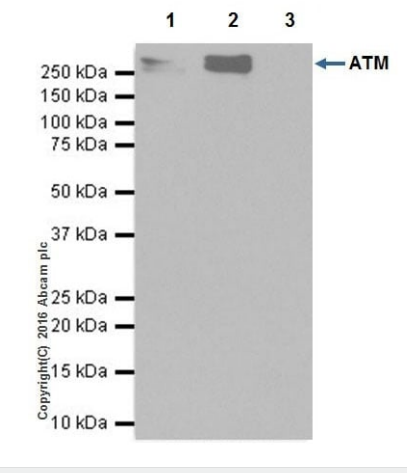
The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

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.



Flow Cytometry (Intracellular) - Anti-ATM antibody [EPR20100] - ChIP Grade (ab201022)

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 Fluorr® 488) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-ATM antibody [EPR20100] - ChIP Grade (ab201022)

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 Reaction (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

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

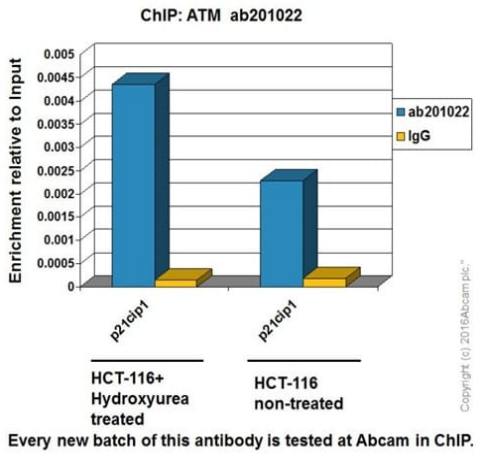
Lane 2: ab201022 IP in HEK-293 whole cell lysate.

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

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

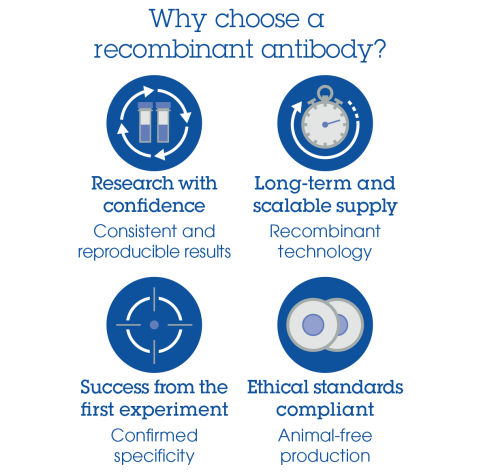
Exposure time: 3 minutes.

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



ChIP - Anti-ATM antibody [EPR20100] - ChIP Grade (ab201022)

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).



Anti-ATM antibody [EPR20100] - ChIP Grade (ab201022)

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