

# Product Datasheet

## ATM Antibody - BSA Free NB100-104

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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**NB100-104**

ATM Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	351 kDa

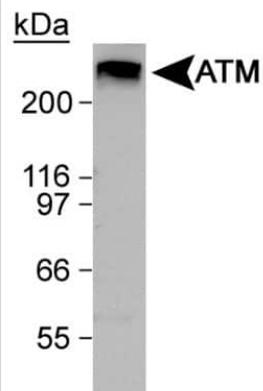
Product Description	
Description	Novus Biologicals Rabbit ATM Antibody - BSA Free (NB100-104) is a polyclonal antibody validated for use in IHC, WB, ELISA, Flow, ICC/IF and IP. Anti-ATM Antibody: Cited in 99 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	472
Gene Symbol	ATM
Species	Human, Mouse, Rat, Canine, Kangaroo
Reactivity Notes	Canine reactivity reported in scientific literature (PMID: 31648115).
Immunogen	ATM Antibody was made to a fragment of the human ATM protein corresponding to the C-terminus (within the last third of the protein sequence). [Uniprot: Q13315]

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, ELISA, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunoprecipitation
Recommended Dilutions	Western Blot 1:500-1:1000, Flow Cytometry, ELISA, Immunohistochemistry 1:100 - 1:200, Immunocytochemistry/ Immunofluorescence 1 - 2 ug/ml, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:100 - 1:200, Immunoblotting reported in scientific literature (PMID 28512243)
Application Notes	In Western blot, it detects a band at ~350 kDa, representing ATM.

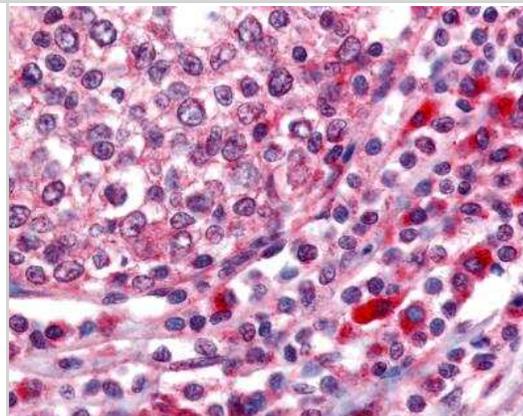


## Images

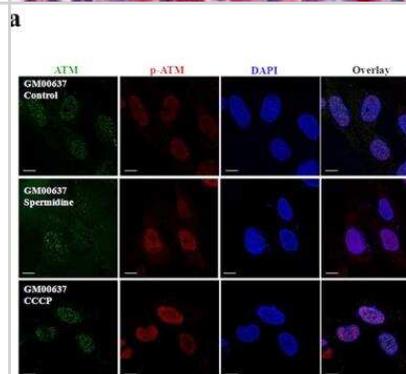
Western Blot: ATM Antibody [NB100-104] - Detection of ATM in HeLa nuclear extract using ATM antibody [NB100-104]. Theoretical molecular weight 351 kDa.



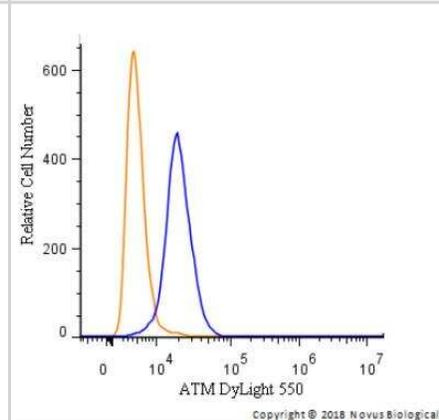
Immunohistochemistry-Paraffin: ATM Antibody [NB100-104] - Staining of human tonsil, germinal center and mantle zone with ATM Antibody [NB100-104].



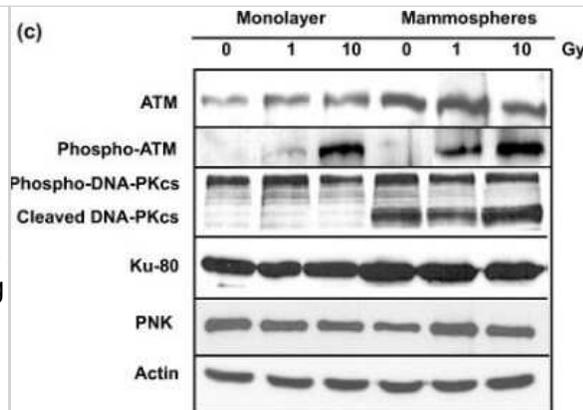
Immunocytochemistry/Immunofluorescence: ATM Antibody [NB100-104] - GM00637 cells with KU55933 pretreatment (a) were exposed to 50  $\mu$ M spermidine or CCCP, followed by immunofluorescence analyses of total and p-ATM on Ser-1981. The scale bar is 10  $\mu$ m. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep24700>) licensed under a CC-BY license.



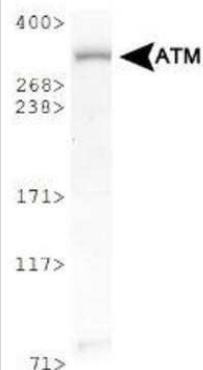
Flow Cytometry: ATM Antibody [NB100-104] - An intracellular stain was performed on HeLa cells with DyLight 550-conjugated ATM Antibody [NB100-104R] (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5  $\mu$ g/mL for 30 minutes at room temperature. Both antibodies were conjugated to DyLight 550.



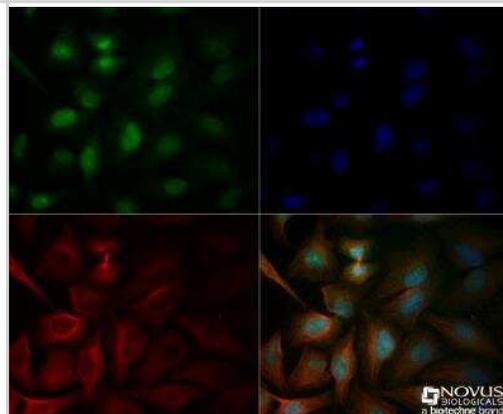
Western Blot: ATM Antibody [NB100-104] - Analysis of double strand break repair in MCF-7 monolayer and mammosphere populations. Expression of proteins involved in the NHEJ pathway of DSB repair in response to increasing doses of ionizing radiation. Lysates were prepared from unirradiated cells and from cells harvested one hour after exposure to 1 or 10-Gy <sup>60</sup>Co I<sup>3</sup>-radiation and analyzed by immunoblotting with antibodies against several DSB repair proteins. Phospho-ATM and phospho-DNA-PKcs refer to phosphorylation of these proteins at Ser1981 and Ser2056, respectively. Actin served as a loading control. Image collected and cropped by Citeab from the following publication (Senescence evasion by MCF-7 human breast tumor-initiating cells. Breast Cancer Res (2010)) licensed under a CC-BY license.



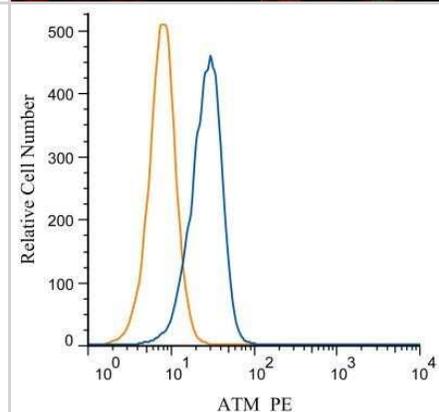
Western Blot: ATM Antibody [NB100-104] - Detection of ATM in Raji whole cell lysate using [NB100-104]. Observed molecular weight ~300 kDa. Theoretical molecular weight 351 kDa.



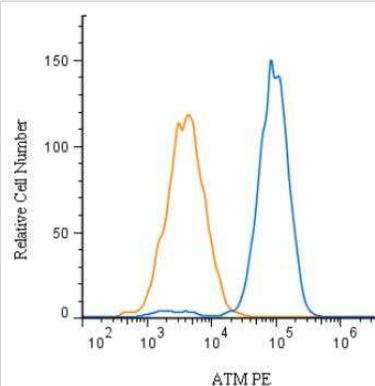
Immunocytochemistry/Immunofluorescence: ATM Antibody [NB100-104] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.05% Triton X-100. Then cells were incubated with [NB100-104] at a 1:100 dilution overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Alpha tubulin was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse DyLight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Flow Cytometry: ATM Antibody [NB100-104] - Analysis of PE conjugate of ATM Antibody [NB100-104PE]. An intracellular stain was performed on HeLa cells with ATM antibody [NB100-104PE] (blue) and a matched isotype control [NBP2-24893PE] (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin.

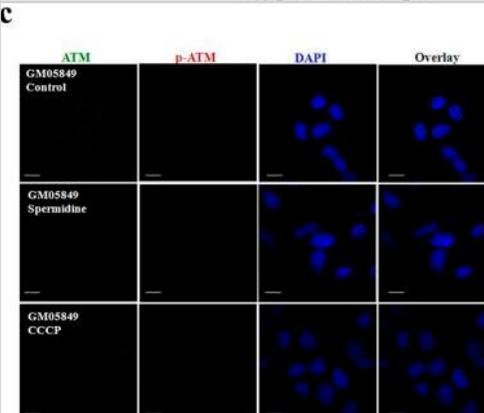


**Flow Cytometry: ATM Antibody [NB100-104]** - An intracellular stain was performed on HepG2 cells with PE-conjugated ATM antibody [NB100-104PE] (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin.

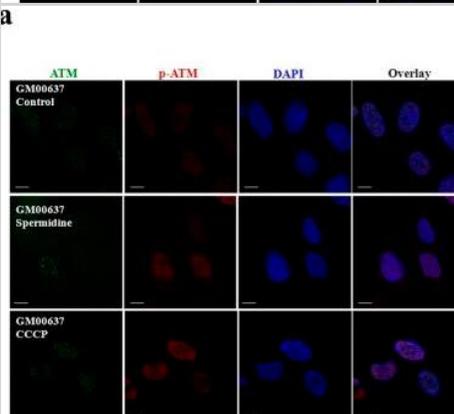


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**Immunocytochemistry/ Immunofluorescence: ATM Antibody [NB100-104]**  
- ATM is activated by spermidine in GM00637 cells. GM00637 cells with or without KU55933 pretreatment (a,b), & GM05849 cells (c) were exposed to 50  $\mu$ M spermidine or CCCP, followed by immunofluorescence analyses of total & p-ATM on Ser-1981. The scale bar is 10  $\mu$ m. Ratios of cells expressing p-ATM Ser-1981 to cells expressing total ATM were presented (d). 20–45 cells/condition from three experiments were collected. Values are mean  $\pm$  SD, \* $p$  < 0.05 vs. control. Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep24700>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

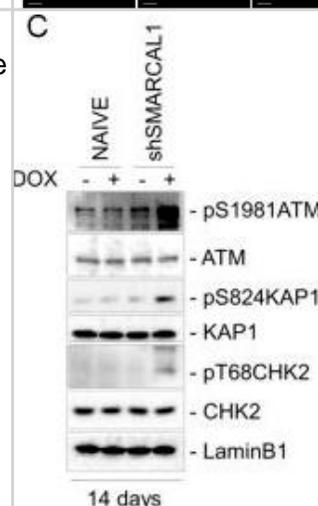


**Immunocytochemistry/ Immunofluorescence: ATM Antibody [NB100-104]**  
- ATM is activated by spermidine in GM00637 cells. GM00637 cells with or without KU55933 pretreatment (a,b), & GM05849 cells (c) were exposed to 50  $\mu$ M spermidine or CCCP, followed by immunofluorescence analyses of total & p-ATM on Ser-1981. The scale bar is 10  $\mu$ m. Ratios of cells expressing p-ATM Ser-1981 to cells expressing total ATM were presented (d). 20–45 cells/condition from three experiments were collected. Values are mean  $\pm$  SD, \* $p$  < 0.05 vs. control. Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep24700>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

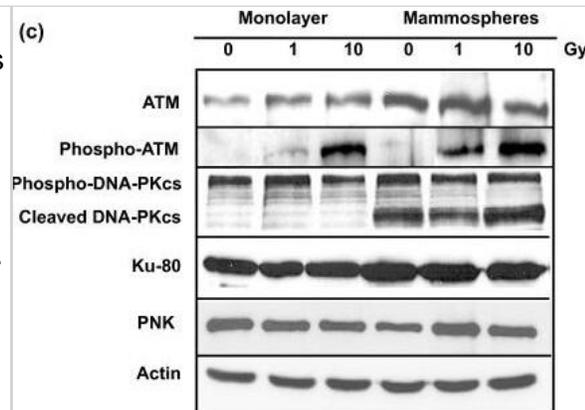


**Western Blot: ATM Antibody [NB100-104]** - Depletion of SMARCAL1 induces DNA damage & checkpoint activation in iSML1 iPSCs. (A,B) The iSML1 iPSCs were cultured for 7 & 14 days in the presence of doxycycline (DOX) to induce SMARCAL1 downregulation & then immunostained. The graphs (top) show quantification of the number of  $\gamma$ -H2AX-positive cells (A) or ATM-pSer1981-positive cells (B).

Representative images from triplicate experiments are shown (bottom). (C) Immunoblot detection of the indicated DDR proteins in iSML1 iPSCs after 14 days of continuous treatment with DOX. Lamin B1 was used as the loading control. Data are mean  $\pm$  s.e.m. from three independent experiments. \* $P$   $\leq$  0.05, \*\* $P$   $\leq$  0.01, \*\*\* $P$   $\leq$  0.001 (two-way ANOVA test). ns, not significant. Scale bars: 10  $\mu$ m. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31515241>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: ATM Antibody [NB100-104] - Analysis of double strand break repair in MCF-7 monolayer & mammosphere populations. (a) Cells were exposed to 4 Gy 60Co  $\gamma$ -radiation & the relative degree of double-strand breakage (DSB) was determined by the comet assay under neutral conditions immediately after exposure & at the times indicated after exposure. (b) The 'comets' (n of about 100) were categorized according to the NIH LISTSERV (Comet Assay Interest Group web site) in which type 1 comets display the least DNA damage & type 5 the most. The error bars represent the mean  $\pm$  standard error of the mean in both panels. The comets of the unirradiated cells are labeled Cont. (c) Expression of proteins involved in the NHEJ pathway of DSB repair in response to increasing doses of ionizing radiation. Lysates were prepared from unirradiated cells & from cells harvested one hour after exposure to 1 or 10-Gy 60Co  $\gamma$ -radiation & analyzed by immunoblotting with antibodies against several DSB repair proteins. Phospho-ATM & phospho-DNA-PKcs refer to phosphorylation of these proteins at Ser1981 & Ser2056, respectively. Actin served as a loading control. Image collected & cropped by CiteAb from the following publication (<http://breast-cancer-research.biomedcentral.com/articles/10.1186/bcr2583>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Urushihara Y, Hashimoto T, Fujishima Y, Hosoi Y. AMPK/FOXO3a Pathway Increases Activity and/or Expression of ATM, DNA-PKcs, Src, EGFR, PDK1, and SOD2 and Induces Radioresistance under Nutrient Starvation International Journal of Molecular Sciences 2023-08-15 [PMID: 37629008] (Immunoprecipitation, Western Blot, Human)

Roulston A, Zimmermann M, Papp R et al. RP-3500: A Novel, Potent, and Selective ATR Inhibitor that is Effective in Preclinical Models as a Monotherapy and in Combination with PARP Inhibitors Molecular Cancer Therapeutics 2022-02-01 [PMID: 34911817] (Immunoprecipitation, Western Blot, Human)

Celeste E Suart, Alma M Perez, Ismael Al-Ramahi, Tamara Maiuri, Juan Botas, Ray Truant Spinocerebellar Ataxia Type 1 protein Ataxin-1 is signaled to DNA damage by ataxia-telangiectasia mutated kinase. Human molecular genetics 2022-03-28 [PMID: 33772540]

Nagelli S CIP2A IS A CRITICAL DNA DAMAGE RESPONSE PROTEIN THAT DRIVES BASAL-LIKE BREAST CANCER Thesis 2023-01-01 (WB)

Habib R, Kim R, Neitzel H et al. Telomere attrition and dysfunction: a potential trigger of the progeroid phenotype in nijmegen breakage syndrome Aging (Albany NY) 2020-06-22 [PMID: 32564008]

Hashimoto T, Urushihara Y, Murata Y et al. AMPK increases expression of ATM through transcriptional factor Sp1 and induces radioresistance under severe hypoxia in glioblastoma cell lines Biochemical and biophysical research communications 2021-12-23 [PMID: 34973534] (WB, Human)

Sato H, Singer RH Cellular variability of nonsense-mediated mRNA decay Nature communications 2021-12-10 [PMID: 34893608] (ICC/IF, Human)

Nishiyama Y, Morita A, Tatsuta S Et al. Isorhamnetin Promotes 53BP1 Recruitment through the Enhancement of ATM Phosphorylation and Protects Mice from Radiation Gastrointestinal Syndrome Genes 2021-09-26 [PMID: 34680909] (WB, Human)

Chakraborty P, Hiom K DHX9-dependent recruitment of BRCA1 to RNA promotes DNA end resection in homologous recombination Nature communications 2021-07-05 [PMID: 34226554] (WB)

Gupta M, Liu X, Teraoka SN et al. Genes affecting ionizing radiation survival identified through combined exome sequencing and functional screening Human mutation 2021-06-21 [PMID: 34153142]

Zhang JQJ, Saravanabavan S, Chandra AN et al. Up-regulation of DNA Damage Response Signaling in Autosomal Dominant Polycystic Kidney Disease The American journal of pathology 2021-02-04 [PMID: 33549515]

Xu L, Ma E et al. ATM deficiency promotes progression of CRPC by enhancing Warburg effect. Endocr Relat Cancer 2019-01-01 [PMID: 30400006] (WB, Human)

More publications at <http://www.novusbio.com/NB100-104>

## Procedures

### Western Blot Protocol for ATM Antibody (NB100-104)

#### Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturer's instructions.

### Immunocytochemistry/ Immunofluorescence Protocol for ATM Antibody (NB100-104)

#### Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 4% paraformaldehyde to the dish and fix at room temperature for 10 minutes.
2. Remove the paraformaldehyde and wash the cells in PBS.
3. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 2 min.
4. Remove the permeabilization buffer and wash three times for 5 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 5 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 5 minutes each.
10. Counter stain DNA with DAPI if required.



**Immunohistochemistry-Paraffin Protocol for ATM Antibody (NB100-104)**

## Immunohistochemistry-Paraffin Embedded Sections

## Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

## Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.





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General: novus@novusbio.com

### **Products Related to NB100-104**

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NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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