

Product Datasheet

CIP2A Antibody (2G10) - BSA Free NB110-59722

Unit Size: 0.1 ml

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

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NB110-59722

CIP2A Antibody (2G10) - 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	Monoclonal
Clone	2G10
Preservative	0.02% Sodium Azide
Isotype	IgG2b Kappa
Purity	Protein G purified
Buffer	PBS

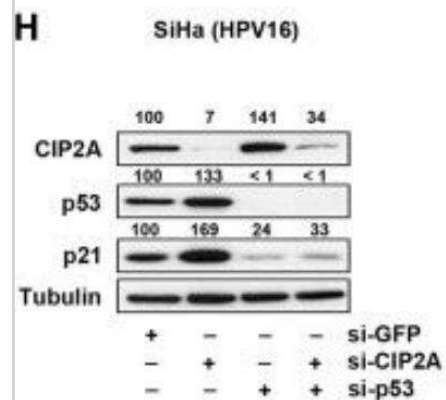
Product Description	
Description	Novus Biologicals Mouse CIP2A Antibody (2G10) - BSA Free (NB110-59722) is a monoclonal antibody validated for use in IHC, WB, Flow and ICC/IF. Anti-CIP2A Antibody: Cited in 18 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	57650
Gene Symbol	CIP2A
Species	Human, Mouse, Rat, Primate
Reactivity Notes	Other species have not been tested.
Immunogen	A synthetic peptide made to a C-terminal portion of the human CIP2A protein. [UniProt# Q8TCG1]

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Knockdown Validated
Recommended Dilutions	Western Blot 1:1000-1:2000, Flow Cytometry 1 ug per million cells, Immunohistochemistry 1:100 - 1:200, Immunocytochemistry/ Immunofluorescence 1:40, Immunohistochemistry-Paraffin reported in scientific literature (PMID 25965834), Knockdown Validated
Application Notes	In ICC/IF, strong cytoplasmic staining is observed in U20s cells. In Western Blot analysis, a band is seen at ~90 kDa representing CIP2A.

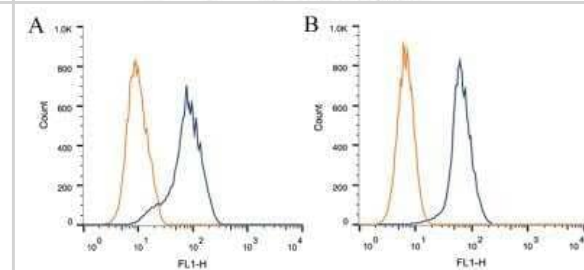


Images

Western Blot: CIP2A Antibody (2G10) [NB110-59722] - miR-375 increases p21, p53, and RB in HPV16- and 18-positive cancer. Protein levels of CIP2A, p53, and p21 in SiHa cells transfected with si-CIP2A and/or si-p53 were measured by Western blot analysis. Protein levels of CIP2A, p53, and p21 in SiHa cells transfected with si-p53 and/or miR-375-mimic were measured by Western blot analysis. Image collected and cropped by CiteAb from the following publication (<https://molecular-cancer.biomedcentral.com/articles/10.1186/1476-4598-13-80>) licensed under a CC-BY license.



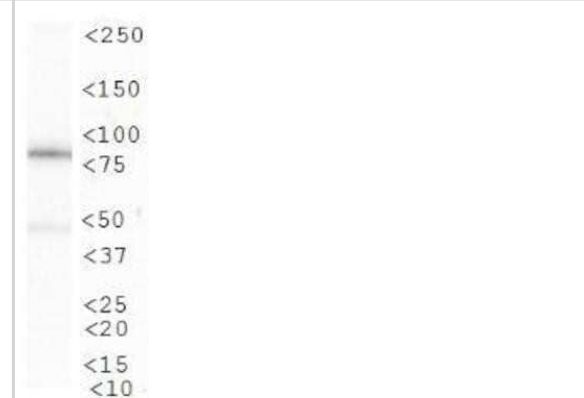
Flow Cytometry: CIP2A Antibody (2G10) [NB110-59722] - Intracellular flow cytometric staining of 1×10^6 CHO (A) and HEK-293 (B) cells using CIP2A antibody (dark blue). Isotype control shown in orange. An antibody concentration of $1 \mu\text{g}/1 \times 10^6$ cells was used.



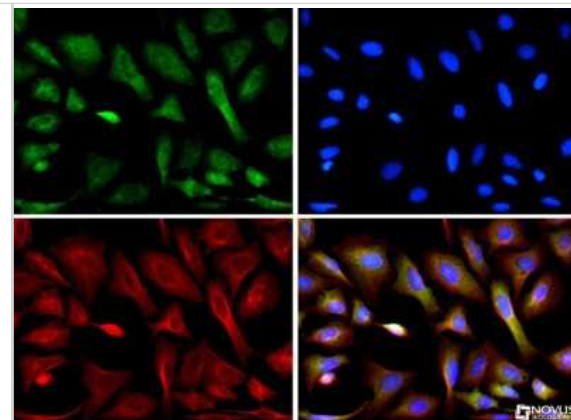
Western Blot: CIP2A Antibody (2G10) [NB110-59722] - Detection of CIP2A in HeLa whole cell lysate.



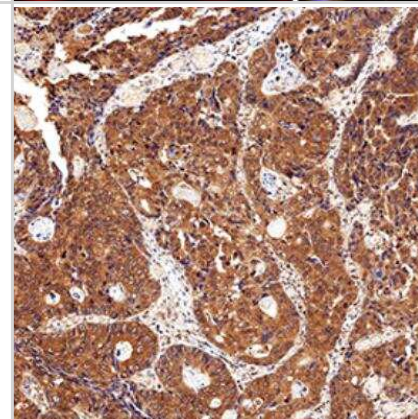
Western Blot: CIP2A Antibody (2G10) [NB110-59722] - Analysis of CIP2A in NIH/3T3 cell lysate.



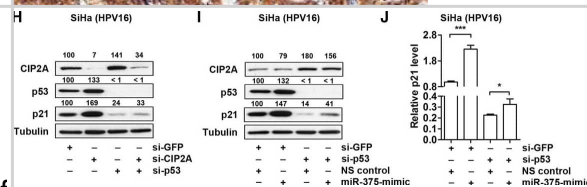
Immunocytochemistry/Immunofluorescence: CIP2A Antibody (2G10) [NB110-59722] - CIP2A antibody was tested in U2OS cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



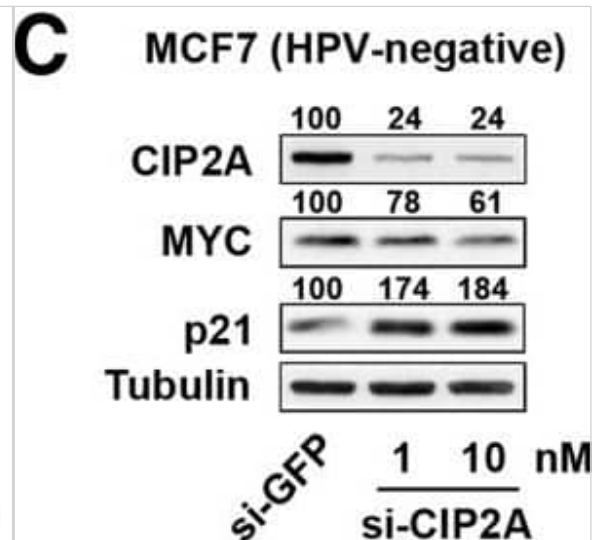
Immunohistochemistry-Paraffin: CIP2A Antibody (2G10) [NB110-59722] - IHC analysis of formalin fixed paraffin-embedded (FFPE) human colon cancer using CIP2A antibody at 1:200 on a Bond Rx autostainer (Leica Biosystems). The assay involved 20 minutes of heat induced antigen retrieval (HIER) using 10mM sodium citrate buffer (pH 6.0) and endogenous peroxidase quenching with peroxide block. The sections were incubated with primary antibody for 30 minutes and Bond Polymer Refine Detection (Leica Biosystems) with DAB was used for signal development followed by counterstaining with hematoxylin. Whole slide scanning and capturing of representative images was performed using Aperio AT2 (Leica Biosystems). Staining was performed by Histowiz.



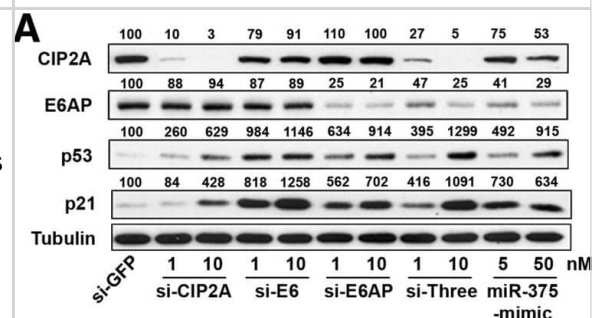
Western Blot: CIP2A Antibody (2G10) [NB110-59722] - miR-375 increases p21, p53, & RB in HPV16- & 18-positive cancer. (A) Protein levels of p21, p53, & RB in SiHa cells transfected w/ miR-375 inhibitor, -mimic, or NS control measured by Western blot analysis. 25%, 50%, 100% amounts of untreated cell lysates included to calibrate the semiquantitative measurement. (B) Relative endogenous mRNA levels of p21, p53, & RB measured in SiHa cells transfected w/ miR-375 inhibitor, -mimic, or NS control using qRT-PCR. (C) One hundred thousand SiHa cells seeded on 24-well plate & the number of cells counted by trypan blue exclusion staining assay 48 h post-transfection. (D) Protein levels of p21, p53, & RB in HeLa cells transfected w/ miR-375 inhibitor, -mimic, or NS control measured by Western blot analysis. (E) Relative p21 mRNA levels measured in HeLa cells transfected w/ miR-375 inhibitor, -mimic, or NS control. (F) Trypan blue exclusion staining assay used to analyze the proliferation rate of HeLa cells 48 h post-transfection. (G) Flow cytometry analysis demonstrates G1 arrest of SiHa cells 48 h after transfection w/ miR-375-mimic compared to miR-375 inhibitor or NS control. (H) Protein levels of CIP2A, p53, & p21 in SiHa cells transfected w/ si-CIP2A and/or si-p53 measured by Western blot analysis. (I) Protein levels of CIP2A, p53, & p21 in SiHa cells transfected w/ si-p53 and/or miR-375-mimic measured by Western blot analysis. (J) mRNA levels of p21 in SiHa cells transfected w/ si-p53 and/or miR-375-mimic measured by qRT-PCR. The concentrations of siRNA or miRNA used in panels H, I, & J 10 nM & 25 nM, respectively. Results are expressed as mean \pm SD from three independent experiments. * $p < 0.05$, *** $p < 0.001$, & **** $p < 0.0001$. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/24708873>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



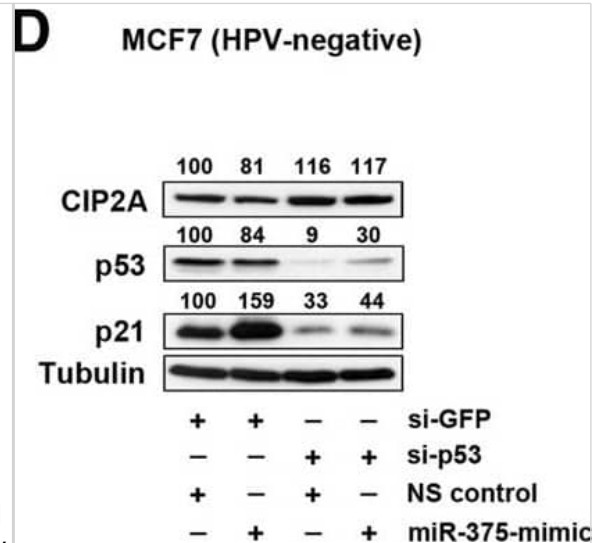
Western Blot: CIP2A Antibody (2G10) [NB110-59722] - miR-375 control on CIP2A-MYC pathway also contributes to p21 elevation. (A) p21, p53, RB, CIP2A, & MYC protein levels in MCF7 cells transfected with miR-375 inhibitor, -mimic, or NS control were measured by Western blot analysis. Tubulin expression was used as internal control. 25%, 50%, 100% amounts of untreated cell lysates were included to calibrate the semiquantitative measurement. (B) Transfection with miR-375-mimic significantly upregulated p21 mRNA in MCF7. Relative endogenous p21 mRNA levels were measured in MCF7 cells transfected with miR-375 inhibitor, -mimic, or NS control for 48 h using qRT-PCR. (C) CIP2A & MYC protein levels were effectively silenced by si-CIP2A transfection with 1 & 10 nM concentrations for 48 h. Increased p21 protein levels were detected in si-CIP2A dose-dependent manner. 10 nM of si-GFP was used as a control. (D) Protein levels of CIP2A, p53, & p21 in MCF7 cells transfected with si-p53 and/or miR-375-mimic were measured by Western blot analysis. (E) mRNA levels of p21 in MCF7 cells transfected with si-p53 and/or miR-375-mimic were measured by qRT-PCR. (F) Flow cytometry analysis demonstrates G1 arrest of MCF7 cells 48 h after transfection with miR-375-mimic compared to miR-375 inhibitor or NS control. The concentrations of siRNA or miRNA used in panels D, E, & F were 10 nM & 25 nM, respectively. (G) Schematic depiction of miR-375-mediated repression of CIP2A, E6, E6AP, & E7 in HPV16-positive cells that simultaneously increases tumor suppressor p53, p21, & RB, & causes cell cycle arrest. Results are expressed as mean \pm SD from three independent experiments. * $p < 0.05$, ** $p < 0.01$, & *** $p < 0.001$. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/24708873>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: CIP2A Antibody (2G10) [NB110-59722] - miR-375-mediated repression of HPV16, E6AP, & CIP2A activates the p53-p21 network & suppresses telomerase activity. (A) miR-375 demonstrates upregulation of p53 & p21 comparable to that of the single or combined CIP2A, E6, & E6AP knockdown. CIP2A, E6AP, p53, & p21 protein levels in SiHa cells transfected with siRNA targeting CIP2A, HPV16-E6, & E6AP (si-CIP2A, si-E6, & si-E6AP, respectively) were analyzed by Western blot. 1 nM & 10 nM siRNA concentrations & 5 nM & 50 nM for miR-375-mimic were used for transfection. si-Three is a combination of the three siRNAs indicated above. 10 nM of si-GFP was used as a control. Tubulin expression was used as internal control. (B) The increase in p21 protein levels correlate to its mRNA levels. Relative endogenous p21 mRNA levels transfected with siRNAs or miR-375 were measured using qRT-PCR. (C) miR-375 exerted a similar or stronger reduction in TERT mRNA levels when compared to E6 & E6AP knockdown in SiHa cells. (D) SiHa cells transfected with miR-375-mimic significantly reduced telomerase activity. Relative telomerase activities in SiHa cells transfected with NS control, miR-375-mimic, & miR-375 inhibitor were measured by SYBR real-time PCR TRAP assay. Heat-inactivated telomerase extracts were used to normalize this data. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, & **** $p < 0.0001$. ns, not significant. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/24708873>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: CIP2A Antibody (2G10) [NB110-59722] - miR-375 control on CIP2A-MYC pathway also contributes to p21 elevation. (A) p21, p53, RB, CIP2A, & MYC protein levels in MCF7 cells transfected with miR-375 inhibitor, -mimic, or NS control were measured by Western blot analysis. Tubulin expression was used as internal control. 25%, 50%, 100% amounts of untreated cell lysates were included to calibrate the semiquantitative measurement. (B) Transfection with miR-375-mimic significantly upregulated p21 mRNA in MCF7. Relative endogenous p21 mRNA levels were measured in MCF7 cells transfected with miR-375 inhibitor, -mimic, or NS control for 48 h using qRT-PCR. (C) CIP2A & MYC protein levels were effectively silenced by si-CIP2A transfection with 1 & 10 nM concentrations for 48 h. Increased p21 protein levels were detected in si-CIP2A dose-dependent manner. 10 nM of si-GFP was used as a control. (D) Protein levels of CIP2A, p53, & p21 in MCF7 cells transfected with si-p53 and/or miR-375-mimic were measured by Western blot analysis. (E) mRNA levels of p21 in MCF7 cells transfected with si-p53 and/or miR-375-mimic were measured by qRT-PCR. (F) Flow cytometry analysis demonstrates G1 arrest of MCF7 cells 48 h after transfection with miR-375-mimic compared to miR-375 inhibitor or NS control. The concentrations of siRNA or miRNA used in panels D, E, & F were 10 nM & 25 nM, respectively. (G) Schematic depiction of miR-375-mediated repression of CIP2A, E6, E6AP, & E7 in HPV16-positive cells that simultaneously increases tumor suppressor p53, p21, & RB, & causes cell cycle arrest. Results are expressed as mean \pm SD from three independent experiments. * $p < 0.05$, ** $p < 0.01$, & *** $p < 0.001$. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/24708873>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Chuang H, Pan J, Cai Y et al. Reciprocal regulation of CIP2A and AR expression in prostate cancer cells *Research Square* 2022-07-25 [PMID: 36098827]

Sikorski K, Mehta A et al. A high-throughput pipeline for validation of antibodies. *Nat Methods* 2018-01-11 [PMID: 30377371] (Human)

Details:

Antibody validation based on denaturing gel electrophoresis of biotinylated cell lysates (PAGE) followed by mass spectrometry (MS) and antibody array analysis (MAP).

Miller RA Long-lived mice with reduced growth hormone signaling have a constitutive upregulation of hepatic chaperone-mediated autophagy *Autophagy* 2020-02-12 [PMID: 32013718] (WB, Mouse)

Details:

Snell dwarf mice

Khanna A, Thoms JAI, Stringer BW et al. Constitutive CHK1 expression drives a pSTAT3-CIP2A circuit that promotes glioblastoma cell survival and growth *Mol. Cancer Res.* 2020-02-20 [PMID: 32079743] (IF/IHC, Human, Mouse)

Li W, Zhang H, Yang L, Wang Y. Cancerous inhibitor of protein phosphatase 2A regulates cisplatin resistance in ovarian cancer. *J. Cell. Physiol.* 2018-11-11 [PMID: 30655886] (WB, IF/IHC, Human)

Liu CY, Hsu CC, Huang TT et al. ER stress-related ATF6 upregulates CIP2A and contributes to poor prognosis of colon cancer *Mol Oncol* 2018-10-01 [PMID: 30063110] (IF/IHC, Human)

Chao Ting-Ting, Wang Cheng-Yi, Chen Yen-Lin et al. Afatinib induces apoptosis in NSCLC without EGFR mutation through Elk-1-mediated suppression of CIP2A. *Oncotarget* 2015-01-01 [PMID: 25537503] (IF/IHC, Human)

Zhao S, Gao X, Zang S et al. MicroRNA-383-5p acts as a prognostic marker and inhibitor of cell proliferation in lung adenocarcinoma by cancerous inhibitor of protein phosphatase 2A *Oncology Letters* 2017-07-18 [PMID: 28927114] (WB, Human)

Wang X, Gao P, Wang M et al. Feedback between E2F1 and CIP2A regulated by human papillomavirus E7 in cervical cancer: implications for prognosis.. *Am J Transl Res* 2017-05-31 [PMID: 28559983] (WB, ICC/IF, IF/IHC, Human)

Khanna A, Rane JK, Kivinummi KK et al. CIP2A is a candidate therapeutic target in clinically challenging prostate cancer cell populations. *Oncotarget.* 2015-04-19 [PMID: 25965834] (IHC-P, Human)

Jung HM, Phillips BL, Chan EK. miR-375 activates p21 and suppresses telomerase activity by coordinately regulating HPV E6/E7, E6AP, CIP2A, and 14-3-3zeta. *Mol. Cancer* 2014-04-17 [PMID: 24708873] (WB, Human)

Zhai M, Cong L, Han Y et al. CIP2A is overexpressed in osteosarcoma and regulates cell proliferation and invasion. *Tumour Biol.* 2013-09-08 [PMID: 24014087]

More publications at <http://www.novusbio.com/NB110-59722>

Procedures

Western Blot Protocol specific for p90 Autoantigen Antibody (NB110-59722)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
 2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
 3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
 4. Rinse the blot.
 5. Block the membrane using standard blocking buffer for at least 1 hour.
 6. Wash the membrane in wash buffer three times for 10 minutes each.
 7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
 8. Wash the membrane in wash buffer three times for 10 minutes each.
 9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
 10. Wash the blot in wash buffer 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 manufacturers instructions.
- Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunocytochemistry/Immunofluorescence protocol for CIP2A Antibody (NB110-59722)

Immunocytochemistry Protocol

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

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.





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Products Related to NB110-59722

NB800-PC1	HeLa Whole Cell Lysate
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
NBP1-43317-0.5mg	Mouse IgG2b Kappa Light Chain Isotype Control (MG2b)

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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