

Product Datasheet

DDX21 Antibody - BSA Free NB100-1718

Unit Size: 0.1 ml

Store at 4C short term. Store at -20C long term. Avoid freeze-thaw cycles.

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NB100-1718

DDX21 Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	87 kDa

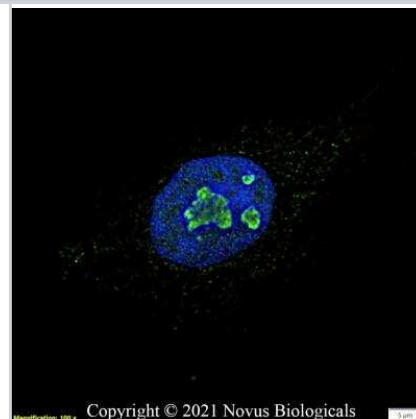
Product Description	
Description	Novus Biologicals Rabbit DDX21 Antibody - BSA Free (NB100-1718) is a polyclonal antibody validated for use in IHC, WB, Flow, ICC/IF, IP and ChIP. Anti-DDX21 Antibody: Cited in 11 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	9188
Gene Symbol	DDX21
Species	Human, Mouse, Zebrafish
Reactivity Notes	Based on 100% sequence identity, this antibody is predicted to react with Panda, Orangutan, Gorilla, Chimpanzee, African elephant, Northern white-cheeked gibbon, and Thirteen-lined ground squirrel. Use in Zebrafish reported in scientific literature (PMID:32231306).
Immunogen	The immunogen recognized by this antibody maps to a region between residue 725 and the C-terminus (residue 783) of human DEAD/H (Asp-Glu-Ala-Asp/His) Box Polypeptide 21 using the numbering given in entry NP_004719.2 (GeneID 9188).

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Chromatin Immunoprecipitation, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Knockdown Validated
Recommended Dilutions	Western Blot 1:2000-1:10000, Chromatin Immunoprecipitation reported in scientific literature (PMID 32231306), Flow Cytometry 1:1000, Immunohistochemistry 1:10-1:500, Immunocytochemistry/ Immunofluorescence 1:50 - 1:500, Immunoprecipitation 2 - 10 ug/mg of lysate, Immunohistochemistry-Paraffin 1:10-1:500, Chromatin Immunoprecipitation (ChIP), Knockdown Validated reported in scientific literature (PMID 35440492)

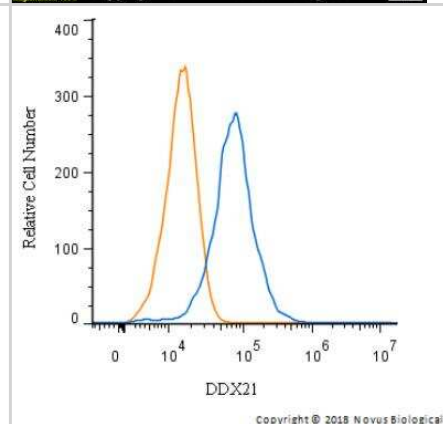


Images

Immunocytochemistry/Immunofluorescence: DDX21 Antibody [NB100-1718] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-DDX21 Antibody NB100-1718 at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



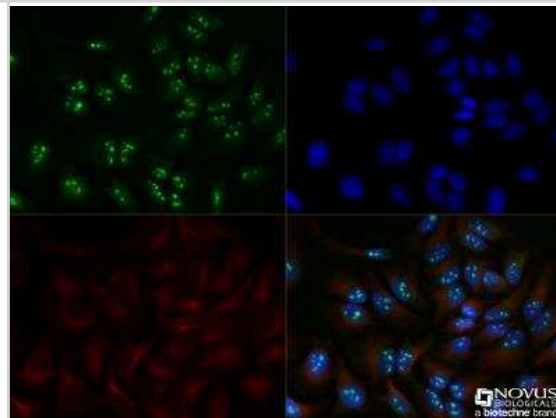
Flow Cytometry: DDX21 Antibody [NB100-1718] - An intracellular stain was performed on Jurkat Cells with DDX21 Antibody NB100-1718 and a matched isotype control. Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG APC-conjugated Secondary Antibody (R&D Systems, F0111).



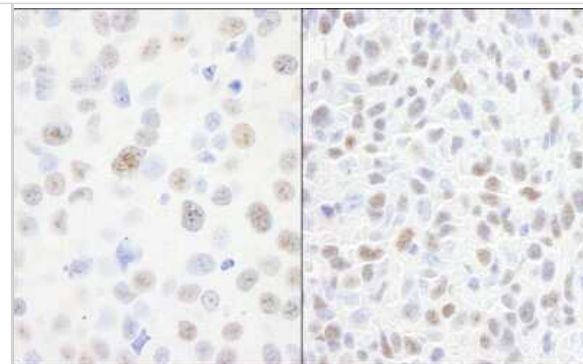
Immunocytochemistry/Immunofluorescence: DDX21 Antibody [NB100-1718] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with DDX21 Antibody conjugated to Alexa Fluor 488 (NB100-1718AF488) at 5 ug/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



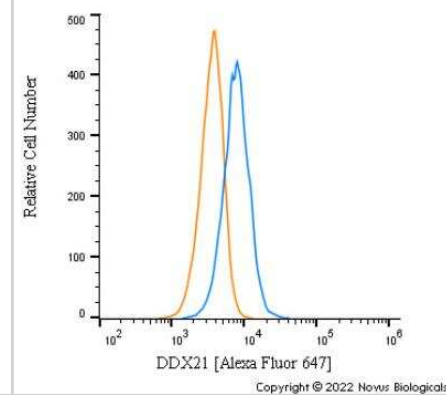
Immunocytochemistry/Immunofluorescence: DDX21 Antibody [NB100-1718] - ICC/IF detection of DDX21 in HeLa cells which were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-DDX21 [NB100-1718] at a 1:200 dilution overnight at 4C and detected with an anti-rabbit DylightTM 488 (green) at a 1:500 dilution. Alpha tubulin (DM1A) [NB100-690] was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse DylightTM 550 (red) at a 1:500 dilution. Nuclei were counterstained with DAPI (blue) [NBP2-31156].



Immunohistochemistry: DDX21 Antibody [NB100-1718] - Sample: FFPE section of human breast carcinoma (left) and mouse squamous cell carcinoma (right). Antibody: Affinity purified rabbit anti-DDX21 used at a dilution of 1:200 (1ug/ml) . Detection: DAB.



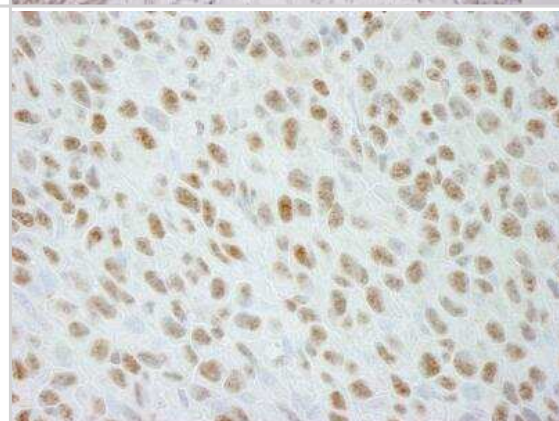
Flow Cytometry: DDX21 Antibody - BSA Free [NB100-1718] - An intracellular stain was performed on A431 cells with DDX21 NB100-1718AF647 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.



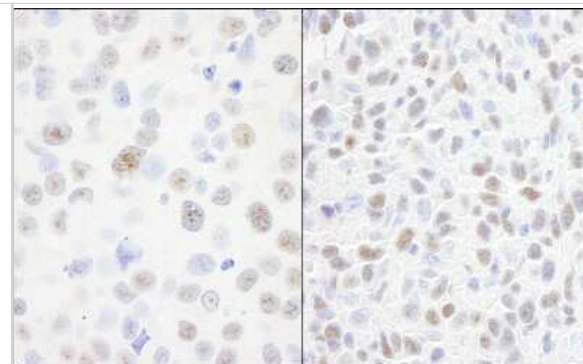
Immunohistochemistry-Paraffin: DDX21 Antibody [NB100-1718] - FFPE section of human larynx squamous cell carcinoma. Antibody used at a dilution of 1:250. Detection: DAB staining using Immunohistochemistry Accessory Kit.



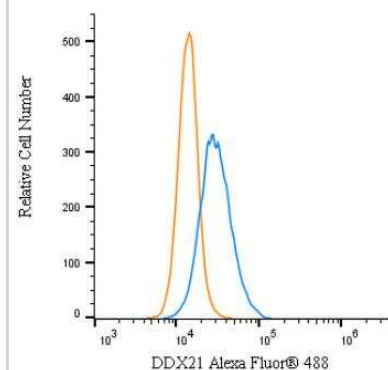
Immunohistochemistry-Paraffin: DDX21 Antibody [NB100-1718] - FFPE section of mouse squamous cell carcinoma. Antibody used at a dilution of 1:250. Detection: DAB staining using Immunohistochemistry Accessory Kit.



Immunohistochemistry: DDX21 Antibody [NB100-1718] - Sample: FFPE section of human breast carcinoma (left) and mouse squamous cell carcinoma (right). Antibody: Affinity purified rabbit anti-DDX21 used at a dilution of 1:200 (1ug/ml). Detection: DAB.

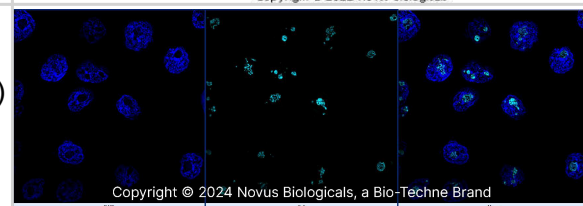


Flow Cytometry: DDX21 Antibody [NB100-1718] - An intracellular stain was performed on HeLa cells with DDX21 Antibody NB100-1718AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.



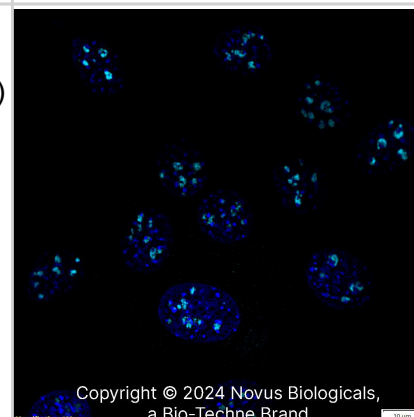
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DDX21 was detected in immersion fixed A431 human skin carcinoma cell line using Rabbit anti-DDX21 Antigen Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NB100-1718AF647) (light blue) at 5 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



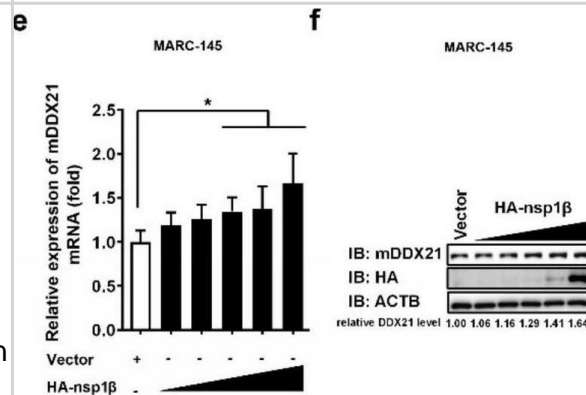
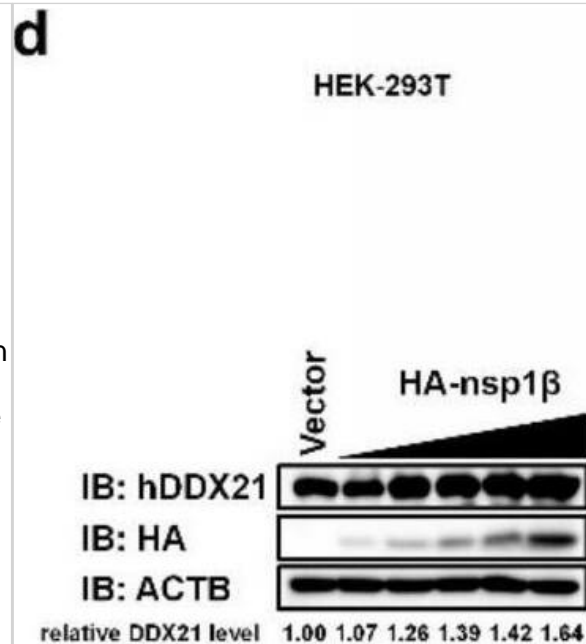
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DDX21 was detected in immersion fixed NIH3T3 Mouse fibroblast cell line using Rabbit anti-DDX21 Antigen Affinity-purified Polyclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NB100-1718AF647) (light blue) at 5 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.

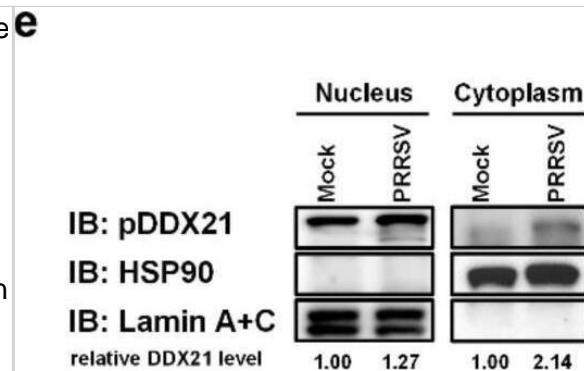
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PRRSV nsp1 β upregulates DDX21 transcription and expression. (a,b) HEK-293T cells were transfected with 3 μ g of plasmids expressing HA-tagged nsp1 α , nsp1 β , nsp4, nsp12, or N protein. At 30 h post-transfection, the cells were collected to detect the mRNA expression of DDX21 by qRT-PCR (a) or DDX21 protein expression by Western blotting (b). (c–h) HEK-293T cells (c,d) and MARC-145 cells (e,f) were transfected with increasing amounts (0.1875 μ g, 0.375 μ g, 0.75 μ g, 1.5 μ g, 3 μ g) of plasmid encoding HA-tagged nsp1 β or empty vector (3 μ g). iPAM cells (g,h) were transfected with increasing amounts (0.375 μ g, 0.75 μ g, 1.5 μ g, 3 μ g) of plasmid encoding HA-tagged nsp1 β or empty vector (3 μ g). At 30 h post-transfection, the cells were collected for use in qRT-PCR (c,e,g) or Western blotting (d,f,h). All experiments were performed in triplicate. The data are shown as the means \pm SD of three independent experiments (n.s, no significant differences; * $p < 0.05$; ** $p < 0.01$). The relative levels of DDX21 in experimentally transfected cells as compared with in empty vector-transfected cells were analyzed by ImageJ software, and the resulting ratio is displayed below the images as the fold change. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/35336874>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

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PRRSV infection promotes pDDX21 translocation from the nucleus to the cytoplasm. (a,b) iPAM cells were infected with PRRSV (MOI = 1). At 12, 24, and 36 hpi, these cells were collected for use in qRT-PCR to determine the number of mRNA copies of pDDX21 (a) or in Western blotting with antibodies against DDX21, PRRSV N protein, and β -actin (b). (c,d) iPAM cells were infected with increasing doses of PRRSV (0.01 MOI, 0.1 MOI, or 1.0 MOI). At 24 hpi, these cells were collected for use in qRT-PCR (c) and Western blotting (d) as described in (a,b), respectively. (e) iPAM cells were grown until they formed a monolayer on 100-mm plates and then infected with PRRSV (MOI = 0.1). These cells were collected at 24 hpi, and a nuclear cytosol fractionation assay was performed to detect the pDDX21 expression in the nucleus or cytoplasm. Data are presented as the means \pm SD of three independent experiments (* $p < 0.05$; *** $p < 0.001$). The relative levels of pDDX21 in PRRSV-infected cells as compared with in mock-infected cells were analyzed by ImageJ software, and the ratio is displayed below the images as the fold change. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/35336874>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Survival motor neuron (SMN)-deficient motor neurons exhibit reduced DDX21 levels in a cell autonomous manner. (A) p75 enriched motor neurons derived from SMN Δ 7 and wild type E13 embryos were labelled with DDX21 antibody, at DIV7. Scale bars represent 5 μ m. (B, C, D) Nuclear, (C) nucleoplasmic and (D) foci fluorescence intensity of DDX21 staining normalised to control. Bar graphs of mean \pm s.d. * $P < 0.05$; paired two-tailed t test ($P = 0.0417$). (E) iPSC-derived motor neurons isolated from healthy individuals (csi4i and miff) and spinal muscular atrophy (SMA) type I patients (SMA86 and SMA32) were labelled with DDX21 antibody. Scale bars represent 5 μ m. (F) Nuclear fluorescence intensity of DDX21 staining normalised to the average of control samples. (G) Nuclear fluorescence intensity where control samples (csi4i and miff) and SMA samples (SMA68 and SMA32) have been pulled together. (H) Nucleoplasmic fluorescence intensity of DDX21 staining normalised to the average of control samples. (I) Nucleoplasmic fluorescence intensity where control samples (csi4i and miff) and SMA samples (SMA68 and SMA32) have been pulled together. (J) Foci fluorescence intensity of DDX21 staining normalised to the average of control samples. (K) Foci fluorescence intensity where control samples (csi4i, miff) and SMA samples (SMA68, SMA32) have been pulled together. Bar graphs of mean \pm s.d. * $P < 0.05$; paired two-tailed t test ($P = 0.0344$). Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/35440492>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

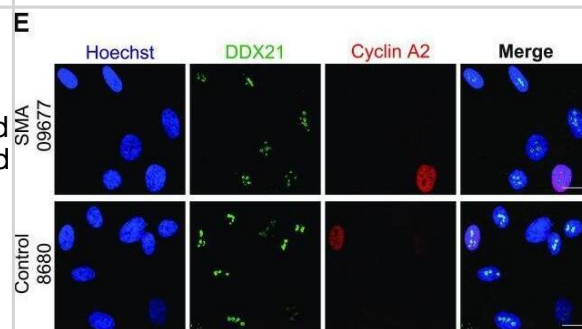


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DDX21/Hoechst

DDX21 levels in spinal muscular atrophy (SMA) embryonic cortical neurons and fibroblasts derived from SMA patients. (A) SMA and control embryonic cortical neurons were labelled with DDX21 antibody, at DIV7. Scale bars represent 5 μ m. (B, C, D) Nuclear (B), nucleoplasmic (C), and foci (D) fluorescence intensity of DDX21 staining is presented normalised to control. Bar graphs of mean \pm s.d (N = 3). ns, not significant (P > 0.05). (E) Fibroblasts derived from SMA type I patient (SMA) and a healthy individual (control) were double-stained with DDX21 and cyclin A1. Scale bars represent 10 μ m. Cyclin A1-positive cells were excluded from the analysis. (F, G, H) Nuclear (F), nucleoplasmic (G), and foci (H) fluorescence intensity of DDX21 staining is presented normalised to control. Bar graphs of mean \pm s.d (N = 3). ns, not significant (P > 0.05). Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/35440492>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Freibaum BD, Messing J, Yang P et al. High-fidelity reconstitution of stress granules and nucleoli in mammalian cellular lysate *Journal of Cell Biology* 2021-03-01 [PMID: 33502444] (Western Blot, Human)

Wang X, Wu Z, Qin W et al. Long non-coding RNA ZFAS1 promotes colorectal cancer tumorigenesis and development through DDX21-POLR1B regulatory axis *Aging (Albany NY)* 2020-11-16 [PMID: 33202381] (Western Blot, Human)

Daigh LH, Saha D, Rosenthal DL et al. Uncoupling of mTORC1 from E2F activity maintains DNA damage and senescence *Nature Communications* 2024-10-24 [PMID: 39448567]

Aryan F, Detrés D, Luo CC et al. Nucleolus activity-dependent recruitment and biomolecular condensation by pH sensing *Molecular cell* 2023-11-14 [PMID: 37979585]

Yu Z, Wang Q, Zhu G et al. Decoding the genomic landscape of chromatin-associated biomolecular condensates *bioRxiv* 2023-08-25 (WB)

Gritti I, Basso V, Rinchai D et al. Loss of ribonuclease DIS3 hampers genome integrity in myeloma by disrupting DNA:RNA hybrid metabolism *The EMBO journal* 2022-10-10 [PMID: 36215697] (ICC/IF)

Karyka E, Berrueta Ramirez N, Webster CP et al. SMN-deficient cells exhibit increased ribosomal DNA damage *Life science alliance* 2022-08-01 [PMID: 35440492] (ICC/IF, KD, Human)

Li J, Wang D, Fang P et al. DEAD-Box RNA Helicase 21 (DDX21) Positively Regulates the Replication of Porcine Reproductive and Respiratory Syndrome Virus via Multiple Mechanisms *Viruses* 2022-02-24 [PMID: 35336874] (WB, Porcine)

Calo E, Gu B, et al. Tissue-selective effects of nucleolar stress and rDNA damage in developmental disorders. *Nature* 2018-02-01 [PMID: 29364875] (IF/IHC, Mouse)

Santoriello C, Sporrij A, Yang S et al. RNA helicase DDX21 mediates nucleotide stress responses in neural crest and melanoma cells *Nat. Cell Biol.* 2020-04-01 [PMID: 32231306] (Chemotaxis, WB, Zebrafish)

Shao Z, Flynn RA, Crowe JL et al. DNA-PKcs has KU-dependent function in rRNA processing and haematopoiesis *Nature* 2020-02-26 [PMID: 32103174] (IF/IHC, Mouse)

Song C, Hotz-Wagenblatt A, Voit R, Grummt I. SIRT7 and the DEAD-box helicase DDX21 cooperate to resolve genomic R loops and safeguard genome stability. *Genes Dev.* 2017-08-08 [PMID: 28790157] (WB, Human)

Procedures

Western Blot Protocol for DDX21 Antibody (NB100-1718)

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.

Immunohistochemistry-Paraffin Protocol for DDX21 Antibody (NB100-1718)

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.

Immunocytochemistry/ Immunofluorescence Protocol for DDX21 Antibody (NB100-1718)**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 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 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 10 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 10 minutes each.
10. Counter stain DNA with DAPI if required.



Flow (Intracellular) Protocol for DDX21 Antibody (NB100-1718)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2×10^5 and 1×10^6 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 μ L for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.
 - a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Re-suspend cells to a concentration of 1×10^6 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 1 mL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

Optional: Perform cell surface staining as described in the previous section.

1. Fix the cells by adding 100 μ L fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 μ L of a permeabilization buffer to every 1×10^6 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.
 - a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.
 - b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).
3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 5 minutes at 400 RCF.
5. Discard supernatant and re-suspend in 1 mL of staining buffer + 0.1% permeabilizer.
6. Stain each sample at 1 μ L/ 1×10^6 cells of primary antibody or 1-3 μ L/ 1×10^6 cells for directly conjugated antibodies. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
7. Following the primary/conjugate incubation, add 2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 5 minutes at 400 RCF.
8. Remove supernatant and re-suspend each sample in 2 mL staining buffer + 0.1% permeabilizer, repeat wash for 5 minutes at 400 RCF.
9. If using a directly conjugated antibody, after the second wash, re-suspend cell pellet to a final volume of 500 μ L per sample and proceed with flow analysis.



Novus Biologicals USA

10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

Bio-Techne Canada

21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd

19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info.EMEA@bio-techne.com

General Contact Information

www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

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