

# Product Datasheet

## RNA Polymerase II/POLR2A Antibody (4H8) - Azide and BSA Free NB200-598SS

Unit Size: 0.025 ml

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

[www.novusbio.com](http://www.novusbio.com)



[technical@novusbio.com](mailto:technical@novusbio.com)

**Reviews: 1 Publications: 13**

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:  
[www.novusbio.com/NB200-598](http://www.novusbio.com/NB200-598)

Updated 10/23/2024 v.20.1

Earn rewards for product reviews and publications.

Submit a publication at [www.novusbio.com/publications](http://www.novusbio.com/publications)

Submit a review at [www.novusbio.com/reviews/destination/NB200-598](http://www.novusbio.com/reviews/destination/NB200-598)



**NB200-598SS**

RNA Polymerase II/POLR2A Antibody (4H8) - Azide and BSA Free

Product Information	
Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	4H8
Preservative	No Preservative
Isotype	IgG1
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	217 kDa
Product Description	
Host	Mouse
Gene ID	5430
Gene Symbol	POLR2A
Species	Human, Mouse, Yeast, Primate (Negative)
Reactivity Notes	Does not cross react with monkey.
Specificity/Sensitivity	Please note that this antibody detects both unphosphorylated and phosphorylated forms of the protein. When using this antibody in western blot and IP, it recognizes both the II0 and IIA form of RPB1 (ie the largest RNAPII subunit). These different forms of RPB1 (as seen in gels) have traditionally been loosely defined as 'hyper-' and 'hypo-phosphorylated', respectively. Here, however, it is important to remember that RPB1 contains no less than 52 repeats of a heptapeptide (TSPTSPS) that can be phosphorylated at different positions (Tyr1, Ser2, Thr4, Ser5 and Ser7; this antibody was raised against a synthetic peptide with 10 of these repeats, mostly but probably not entirely, chemically phosphorylated at Ser5). So, when we say that it recognizes both forms (II0/hyper-phos and IIA/hypo-phos), it might really just mean that the antibody recognizes a few phosphorylated Ser5s among 52 otherwise mostly unphosphorylated repeats. Alternatively, it might conceivably (though less likely) recognize UN-phosphorylated repeats as there might conceivably be a few of those even in the II0/hyper-phos RPB1 form.'
Immunogen	10 repeats of synthetic peptide YSPTSPS using chemically synthesized phospho-Ser 5 YSPTSpPS (Human). [UniProt# P24928]
Notes	
Product Application Details	
Applications	Western Blot, ELISA, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), CyTOF-ready
Recommended Dilutions	Western Blot 1-2 ug/ml, Flow Cytometry 1 ug per million cells, ELISA 1:100-1:2000, Immunocytochemistry/ Immunofluorescence 1:100-1:1000, Immunoprecipitation 1:10-1:500, Flow (Intracellular), Chromatin Immunoprecipitation (ChIP) 1:10-1:500, CyTOF-ready

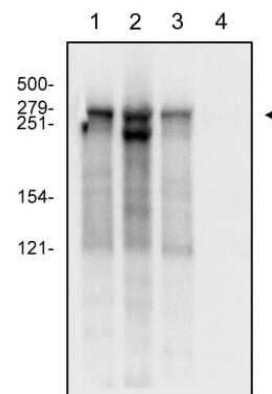


**Application Notes**

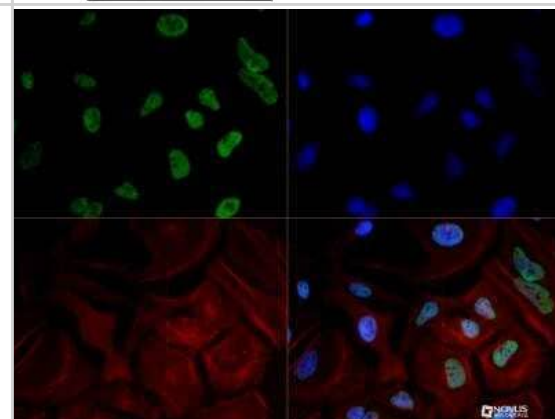
In WB a band can be seen at approximately 217 kDa. Optimal dilutions/concentrations should be determined by the end user.

**Images**

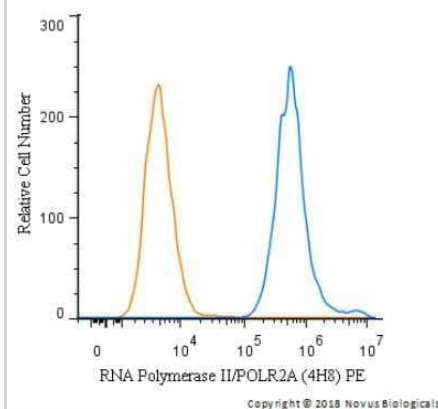
**Western Blot: RNA Polymerase II/POLR2A Antibody (4H8) [NB200-598]**  
- Whole cell protein from HeLa (lane 1), MCF7 (lane 2), NIH-3T3 (lane 3) and Cos7 (lane 4) were separated by SDS-PAGE on a 6% gel, transferred to PVDF and probed with 2 ug/mL NB200-598. RNA Pol II reactivity (arrowhead) is observed in HeLa, MCF and 3T3 cells.



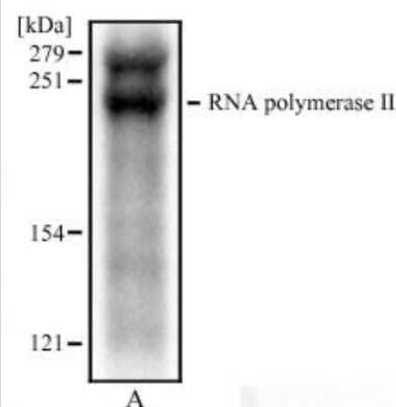
**Immunocytochemistry/Immunofluorescence: RNA Polymerase II/POLR2A Antibody (4H8) [NB200-598]** - RNA Polymerase II/POLR2A antibody was tested in HeLa cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).



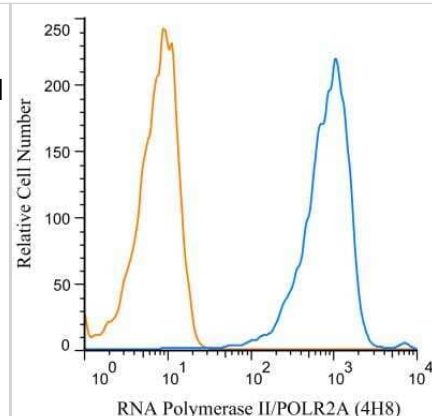
**Flow Cytometry: RNA Polymerase II/POLR2A Antibody (4H8) [NB200-598]** - An intracellular stain was performed on HeLa cells with RNA Polymerase II/POLR2A (4H8) antibody NB200-598PE (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes. Both antibodies were conjugated to phycoerythrin.



**Western Blot: RNA Polymerase II/POLR2A Antibody (4H8) [NB200-598]**  
- Analysis of MCF7 cell lysata (A) using RNA polymerase II antibody at 2 ug/mL.



**Flow (Intracellular): RNA Polymerase II/POLR2A Antibody (4H8) [NB200-598]** - An intracellular stain was performed on Daudi cells with RNA Polymerase II/POLR2A (4H8) antibody NB200-598 (blue) and a matched isotype control NBP2-27287 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1  $\mu$ g/mL for 30 minutes, followed by mouse F(ab)<sub>2</sub> IgG (H+L) APC-conjugated secondary antibody (F0101B, R&D Systems).



## Publications

Hensel A, Stahl P, Moews L et al. The Taspase1/Myosin1f-axis regulates filopodia dynamics iScience 2022-06-17 [PMID: 35601920]

Kim YC, Qi M, Dong X et al. Transgenic mice lacking FGF15/19-SHP phosphorylation display altered bile acids and gut bacteria, promoting nonalcoholic fatty liver disease The Journal of biological chemistry 2023-06-20 [PMID: 37348559] (ChIP, Mouse)

Li F, Negi V, Yang P et al. TEAD1 regulates cell proliferation through a pocket-independent transcription repression mechanism Nucleic acids research 2022-12-09 [PMID: 36484096] (WB, ChIP, Human)

Zhang L, Qu J, Qi Y et al. EZH2 engages TGF beta signaling to promote breast cancer bone metastasis via integrin  $\beta$ 1-FAK activation Nature communications 2022-05-10 [PMID: 35538070] (Chemotaxis, Human)

Appanah R, Lones EC, Aiello U et al. Sen1 Is Recruited to Replication Forks via Ctf4 and Mrc1 and Promotes Genome Stability Cell Rep 2020-02-18 [PMID: 32075754] (WB, Yeast)

Eng J, Thibault G, Luoh SW et al. Cyclic Multiplexed-Immunofluorescence (cmIF), a Highly Multiplexed Method for Single-Cell Analysis Methods Mol. Biol. 2019-09-10 [PMID: 31502168]

Lin J. R, Izar B, et al. Highly multiplexed immunofluorescence imaging of human tissues and tumors using t-CyCIF and conventional optical microscopes. Elife 2018-07-11 [PMID: 29993362] (ICC/IF, Human)

### Details:

Citation using the Alexa Fluor 488 format of this antibody.

Luo W, Chen I, Chen Y et al. PRDX2 and PRDX4 are negative regulators of hypoxia-inducible factors under conditions of prolonged hypoxia Oncotarget. 2016-02-09 [PMID: 26837221] (WB)

### Details:

The mechanism for feedback inhibition of hypoxia-inducible factors during prolonged hypoxia is clarified through studying the interaction of PRDX2 and PRDX4 with HIF-1 alpha and HIF-2 alpha.

Chan EA, Teng G, Corbett E et al. Peripheral subnuclear positioning suppresses Tcrb recombination and segregates Tcrb alleles from RAG2. Proc Natl Acad Sci U S A. 2013-11-26 [PMID: 24218622] (ICC/IF, Mouse)

Kato H, Okazaki K, Iida T et al. Spt6 prevents transcription-coupled loss of posttranslationally modified histone H3. Sci Rep 2013-07-15 [PMID: 23851719] (Chemotaxis, Yeast)

Cheng J, Torkamani A, Peng Y et al. Plasma membrane associated transcription of cytoplasmic DNA Proc Natl Acad Sci U S A 2012-06-18 [PMID: 22711823] (WB, ICC/IF, Human)

Walsh HE Shupnik MA. Proteasome regulation of dynamic transcription factor occupancy on the GnRH-stimulated luteinizing hormone beta-subunit promoter. Mol Endocrinol;23(2):237-50. 2009-02-01 [PMID: 19095772] (Chemotaxis, Human)

More publications at <http://www.novusbio.com/NB200-598>

## Procedures

### Western Blot protocol for RNA Polymerase II/POLR2A Antibody (NB200-598)

#### 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 RNA Polymerase II/POLR2A Antibody (NB200-598)

#### 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. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization 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.





### **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](http://www.novusbio.com)  
Technical Support: nb-technical@bio-techne.com  
Orders: nb-customerservice@bio-techne.com  
General: novus@novusbio.com

### **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.

For more information on our 100% guarantee, please visit [www.novusbio.com/guarantee](http://www.novusbio.com/guarantee)

Earn gift cards/discounts by submitting a review: [www.novusbio.com/reviews/submit/NB200-598](http://www.novusbio.com/reviews/submit/NB200-598)

Earn gift cards/discounts by submitting a publication using this product:  
[www.novusbio.com/publications](http://www.novusbio.com/publications)

