





Phospho-POLR2A (S2) Recombinant Monoclonal Antibody

Product Code	CSB-RA018327A02phHU
Abbreviation	DNA-directed RNA polymerase II subunit RPB1
Storage	Upon receipt, store at -20°C or -80°C. Avoid repeated freeze.
Uniprot No.	P24928
Immunogen	A synthesized peptide derived from Human Phospho-POLR2A (S2)
Species Reactivity	Human
Tested Applications	ELISA, WB, IHC, IF, IP; Recommended dilution: WB:1:500-1:5000, IHC:1:50-1:200, IF:1:20-1:200, IP:1:200-1:1000
Relevance	DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates. Largest and catalytic component of RNA polymerase II which synthesizes mRNA precursors and many functional non-coding RNAs. Forms the polymerase active center together with the second largest subunit. Pol II is the central component of the basal RNA polymerase II transcription machinery. It is composed of mobile elements that move relative to each other. RPB1 is part of the core element with the central large cleft, the clamp element that moves to open and close the cleft and the jaws that are thought to grab the incoming DNA template. At the start of transcription, a single-stranded DNA template strand of the promoter is positioned within the central active site cleft of Pol II. A bridging helix emanates from RPB1 and crosses the cleft near the catalytic site and is thought to promote translocation of Pol II by acting as a ratchet that moves the RNA-DNA hybrid through the active site by switching from straight to bent conformations at each step of nucleotide addition. During transcription elongation, Pol II moves on the template as the transcript elongates. Elongation is influenced by the phosphorylation status of the C-terminal domain (CTD) of Pol II largest subunit (RPB1), which serves as a platform for assembly of factors that regulate transcription initiation, elongation, termination and mRNA processing. Regulation of gene expression levels depends on the balance between methylation and acetylation levels of tha CTD-lysines (By similarity). Initiation or early elongation steps of transcription of growth-factors-induced immediate early genes are regulated by the acetylation status of the CTD (PubMed:24207025). Methylation and dimethylation have a repressive effect on target genes expression (By similarity).
Form	Liquid
Conjugate	Non-conjugated
Storage Buffer	Rabbit IgG in phosphate buffered saline , pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Purification Method	Affinity-chromatography





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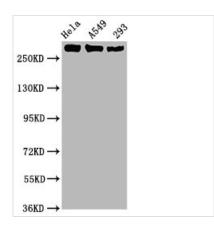
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Isotype	Rabbit IgG
Clonality	Monoclonal
Alias	DNA-directed RNA polymerase II subunit RPB1, RNA polymerase II subunit B1, DNA-directed RNA polymerase II subunit A, DNA-directed RNA polymerase III largest subunit, RNA-directed RNA polymerase II subunit RPB1, POLR2A, POLR2
Immunogen Species	Homo sapiens (Human)
Research Area	Epigenetics and Nuclear Signaling
Gene Names	POLR2A
Clone No.	2G1

Image



Western Blot

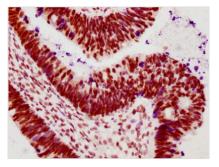
Positive WB detected in Hela whole cell lysate, A549 whole cell lysate, 293 whole cell

All lanes Phospho-POLR2A antibody at 1.02μg/ml

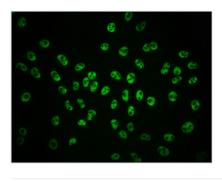
Secondary

Goat polyclonal to rabbit IgG at 1/50000 dilution

Predicted band size: 270 KDa Observed band size: 270 KDa



IHC image of CSB-RA018327A02phHU diluted at 1:100 and staining in paraffin-embedded human ovarian cancer performed on a Leica BondTM system. After dewaxing and hydration, antigen retrieval was mediated by high pressure in a citrate buffer (pH 6.0). Section was blocked with 10% normal goat serum 30min at RT. Then primary antibody (1% BSA) was incubated at 4? overnight. The primary is detected by a biotinylated secondary antibody and visualized using an HRP conjugated SP system.

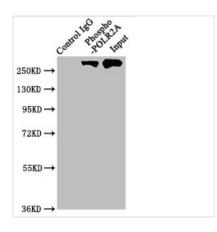


Immunofluorescence staining of Hela cells with CSB-RA018327A02phHU at 1:100,counterstained with DAPI. The cells were fixed in 4% formaldehyde, permeabilized using 0.2% Triton X-100 and blocked in 10% normal Goat Serum. The cells were then incubated with the antibody overnight at 4?. The secondary antibody was Alexa Fluor 488-congugated AffiniPure Goat Anti-Rabbit IgG (H+L).









Immunoprecipitating Phospho-POLR2A in Hela whole cell lysate

Lane 1: Rabbit control IgG(1µg)instead of CSB-RA018327A02phHU in Hela whole cell lysate. For western blotting, a HRP-conjugated Protein G antibody was used as the secondary antibody (1/2000)

Lane 2: CSB-RA018327A02phHU(3µg)+ Hela whole cell lysate(1mg)

Lane 3: Hela whole cell lysate (20µg)

Description

The vectors expressing anti-POLR2A antibody were constructed as follows: immunizing an animal with a synthesized peptide derived from human Phospho-POLR2A (S2), isolating the positive splenocyte and extracting RNA, obtaining DNA by reverse transcription, sequencing and screening POLR2A antibody gene, and amplifying heavy and light chain sequence by PCR and cloning them into plasma vectors. After that, the vector clones were transfected into the mammalian cells for production. The product is the recombinant POLR2A antibody. Recombinant POLR2A antibody in the culture medium was purified using affinity-chromatography. It can react with POLR2A protein from Human and is used in the ELISA, WB, IHC, IF, IP.

POLR2A encodes the largest subunit of RNA polymerase II, the polymerase responsible for the synthesis of eukaryotic messenger RNA. The product of POLR2A contains a carboxy-terminal domain consisting of heptapeptide repeats that is essential for polymerase activity. According to some studies, POLR2A may have the following characteristics.

Potential for pH-responsive nanoparticles and precise targeting of POLR2A in TNBC harboring common TP53 genomic alterations. The clinical consequences of a potentially pathogenic variant in POLR2A depend on its effect on pol-IImediated transcription, as POLR2A variants predicted to cause loss of RPB1 protein are more tolerated than missense variants. BCAR1 promotes proliferation and cell growth, likely through upregulation of POLR2A and subsequent enhancement of catalytic and transferase activity. Humanized monoclonal antibody-induced nuclear localization of CD26 inhibits tumor cell growth by regulating POLR2A transcription.