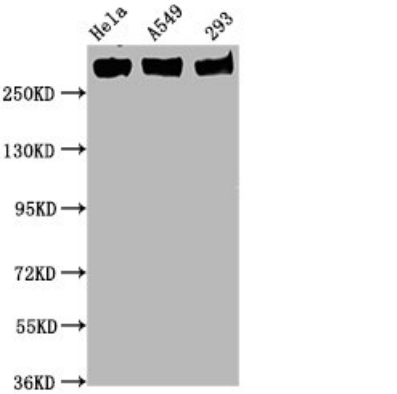
Phospho-POLR2A (S5) Recombinant Monoclonal Antibody

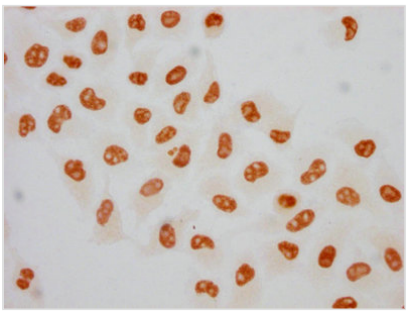
Product Code	CSB-RA018327A05phHU
Abbreviation	DNA-directed RNA polymerase II subunit RPB1
Storage	Upon receipt, store at -20°C or -80°C. Avoid repeated freeze.
Uniprot No.	P24928
Immunogen	A synthesized peptide derived from Human Phospho-POLR2A (S5)
Species Reactivity	Human
Tested Applications	ELISA, WB, IHC, IF; Recommended dilution: WB:1:500-1:5000, IHC:1:50-1:200, IF:1:20-1:200
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

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.	2H4

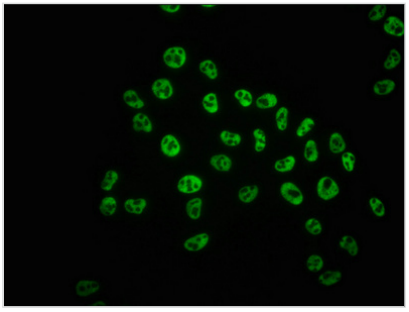
Image



Western Blot
Positive WB detected in Hela whole cell lysate,A549 whole cell lysate,293 whole cell lysate
All lanes Phospho-POLR2A antibody at 0.75µg/ml
Secondary
Goat polyclonal to rabbit IgG at 1/50000 dilution
Predicted band size: 270 KDa
Observed band size: 270 KDa



Immunocytochemistry analysis of CSB-RA018327A05phHU diluted at 1:75 and staining in Hela cells(treated with 100ng/ml EGF for 4h) performed on a Leica BondTM system. The cells were fixed in 4% formaldehyde, permeabilized using 0.2% Triton X-100 and 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-RA018327A05phHU at 1:100,counter-stained 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).

Description

To produce the phospho-POLR2A (S5) recombinant monoclonal antibody, the initial step entails isolating the genes encoding the POLR2A antibody from rabbits that have been previously immunized with a synthesized peptide derived

from the human POLR2A protein phosphorylated at S5. Subsequently, these antibody genes are cloned into specialized expression vectors. Following this genetic modification, the vectors are introduced into host suspension cells. Following successful transfection, positive cells are cultured to promote the expression and secretion of antibodies. The phospho-POLR2A (S5) recombinant monoclonal antibody is then meticulously purified from the cell culture supernatant using affinity chromatography. Finally, the antibody's functionality is rigorously tested through a battery of assays, including ELISA, WB, IHC, and IF, confirming its ability to effectively react with human POLR2A protein phosphorylated at S5.

Phosphorylation of POLR2A at S5 is a fundamental regulatory step in eukaryotic gene expression. It helps orchestrate the complex transcriptional processes that lead to the synthesis of functional mRNA molecules, ensuring proper gene expression and cellular function. Dysregulation of this phosphorylation event can have significant implications for gene expression and is associated with various diseases and developmental disorders.