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Datasheet for 200-301-V01

POL II phospho S5 Antibody

Overview

Description:	Anti-POL II pS5 (MOUSE) Monoclonal Antibody - 200-301-V01
Item No.:	200-301-V01
Size:	50 µg
Applications:	ChIP, ELISA, IF, WB
Reactivity:	Human
Host Species:	Mouse

Product Details

Background:	RNA polymerase II (pol II) is a key enzyme in the regulation and control of gene transcription. It is able to unwind the DNA double helix, synthesize RNA, and proofread the result. Pol II is a complex enzyme, consisting of 12 subunits, of which the B1 subunit is the largest. Together with the second largest subunit, B1 forms the catalytic core of the RNA polymerase II transcription machinery. Anti-Pol II S5p Antibody is ideal for research in Gene Expression, Transcription and Genetics.
Synonyms:	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
Host Species:	Mouse
Clonality:	Monoclonal
Format:	IgG1

Target Details

Gene Name:	POLR2A
Reactivity:	Human
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide

Immunogen:	Anti-Pol II S5p Antibody was produced in mice by repeated immunization with the YSPTSPS repeat in the B1 subunit of RNA polymerase II phosphorylated at Ser5 of the repeated sequence.
Purity/Specificity:	Anti-Pol II pS5 Antibody was purified by Protein A chromatography. This antibody is specific for Pol II protein phosphorylated Ser5. Cross-reactivity with Pol II pS5 from other sources has not been determined.
Relevant Links:	• UniProtKB - P24928 • GeneID - 5430 • NCBI - NP_000928.1

Application Details

Tested Applications:	ChIP, ELISA, IF, WB
Application Note:	Anti-Pol II pS5 Antibody is tested for Chromatin Immunoprecipitation Sequencing, Chromatin Immunoprecipitation, ELISA, Immunofluorescence and Western Blots. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 225 kDa in the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ChIP:	1 µg/ChIP
ELISA:	1:3,000
IF:	1:500
WB:	1:1,000

Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/ml by UV absorbance at 280 nm
Buffer:	0.01 M Sodium Phosphate, 0.25 M Sodium Chloride, pH 7.2
Preservative:	0.05% (w/v) Sodium Azide
Stabilizer:	None

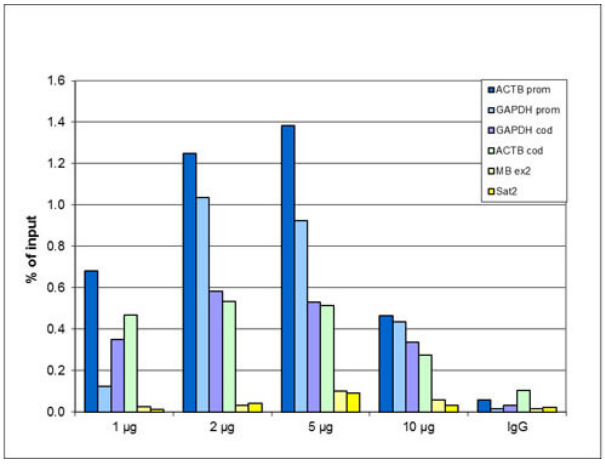
Shipping & Handling

Shipping Condition:	Dry Ice

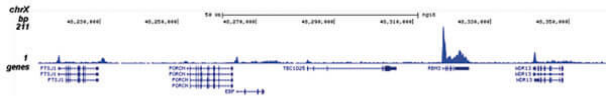
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images

ChIP
Chromatin Immunoprecipitation was performed on sheared chromatin from 1 million HeLaS3 cells using 1 µg of Pol II S5p antibody. The IP'd DNA was subsequently analysed on an Illumina Genome Analyzer. The 36 bp tags were aligned to the human genome using the ELAND algorithm. Fig 4 shows the peak distribution along the complete sequence, a 150 kb region of the X-chromosome (fig 5), and in a two genomic regions surrounding the GAPDH (fig 6) and ACTB positive control genes (fig 7).



ChIP
Chromatin immunoprecipitation assays were performed using sheared chromatin from 1 million HeLa cells for Anti-Pol II S5p Antibody and optimized PCR primer pairs for qPCR. A titration consisting of 1, 2, 5 and 10 µg of antibody per ChIP experiment was analyzed. IgG (2 µg/IP) was used as a negative IP control. Quantitative PCR was performed with primers specific for the promoter and the coding region of the constitutively expressed GAPDH and ACTB genes, used as positive controls, and for exon 2 of the inactive myoglobin (MB) gene and the Sat2 satellite repeat, used as negative controls.



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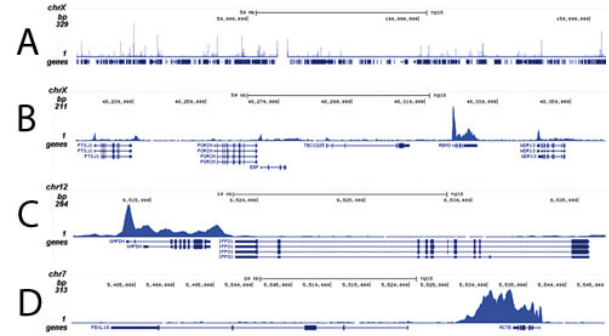

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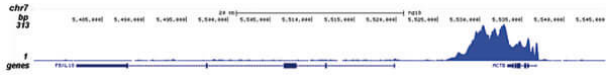

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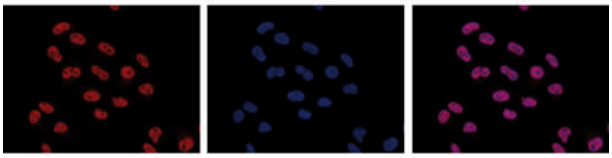
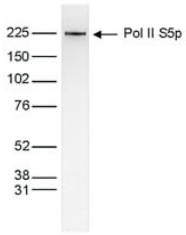


Immunofluorescence Microscopy
Immunofluorescence Microscopy results of Mouse anti-Pol II S5p antibody. Tissue: HeLa cells. Fixation: methanol. Block: PBS/TX-100 containing 5% normal goat serum and 1% BSA. Primary antibody: Pol II S5p antibody at 1:500 for 1 hr at RT (left). Secondary antibody: anti-Mouse Alexa594 secondary antibody at 1:10,000 for 45 min at RT. Staining: Pol II S5p antibody as red fluorescent signal (left), DAPI blue (middle), merge of the two stainings (right).



Western Blot
Western Blot results of Mouse anti-Pol II S5p antibody. Lane 1: 25 µg HeLa nuclear extracts. Primary antibody: Mouse anti-Pol II S5p antibody at 1:1,000. Secondary antibody: Peroxidase anti-mouse secondary antibody at 1:10,000 for 45 min at RT. Block: TBS-Tween containing 5% BLOTTO overnight at 4°C. Predicted/Observed size: ~217 kDa for Mouse Pol II S5p.

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