

Product datasheet for **TA347269**

POLR2A Mouse Monoclonal Antibody

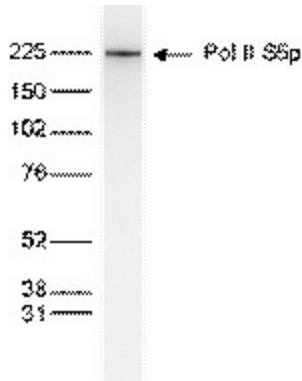
Product data:

Product Type:	Primary Antibodies
Applications:	ELISA, IF, WB
Recommended Dilution:	ChIP (1-2ug/ChIP); ELISA (1,3000); Western blotting (1:1,000); Immunofluorescence (1:500)
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	The immunogen for anti-Pol II S5p antibody: the YSPTSPS repeat in the B1 subunit of RNA polymerase II, phosphorylated at Ser2 of the repeat sequence
Concentration:	lot specific
Purification:	Protein A purified monoclonal antibody in PBS containing 0.05% azide.
Conjugation:	Unconjugated
Storage:	Store at -20°C as received.
Stability:	Stable for 12 months from date of receipt.
Gene Name:	polymerase (RNA) II subunit A
Database Link:	NP_000928 Entrez Gene 5430 Human P24928
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 (UniProt/Swiss-Prot entry P24928) is the largest. Together with the second largest subunit, B1 forms the catalytic core of the RNA polymerase II transcription machinery.
Synonyms:	hRPB220; hsRPB1; POLR2; POLRA; RPB1; RPBh1; RpiILS; RPO2; RPOL2
Protein Pathways:	Huntington's disease, Metabolic pathways, Purine metabolism, Pyrimidine metabolism, RNA polymerase

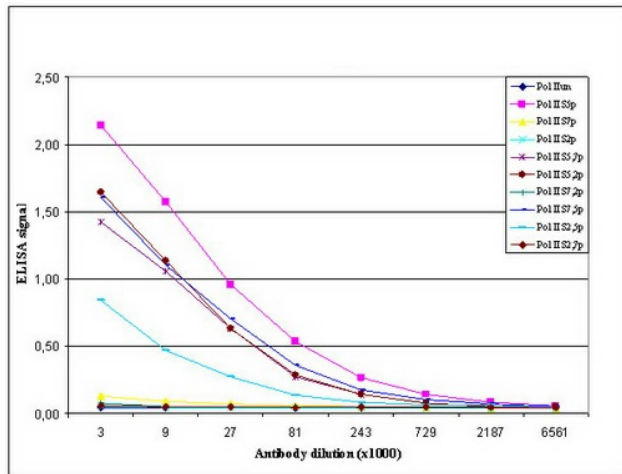


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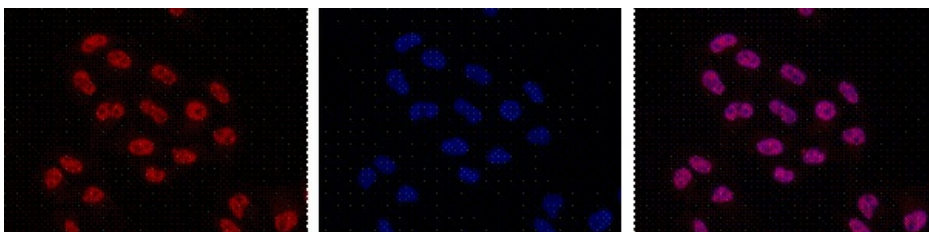
Product images:



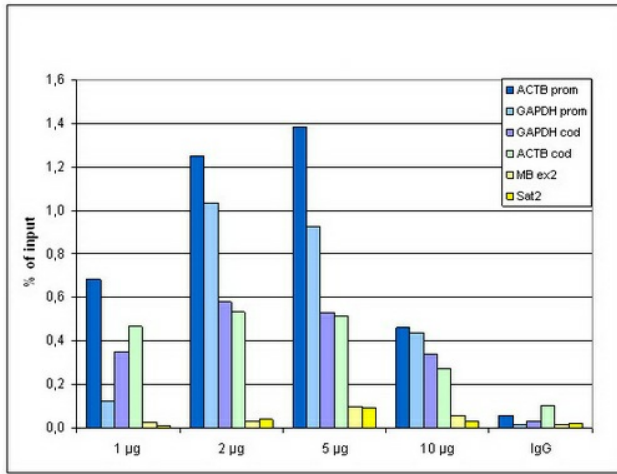
WB using the antibody against Pol II S5p diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.



Cross reactivity of the antibody against Pol IIS5p
To test the specificity an ELISA was performed using a serial dilution of the antibody against Pol IIS5p. The wells were coated with peptides containing the unmodified C-terminal repeat sequence as well as different phosphorylated peptides. Image shows the specificity of the antibody for the S5 phosphorylation.

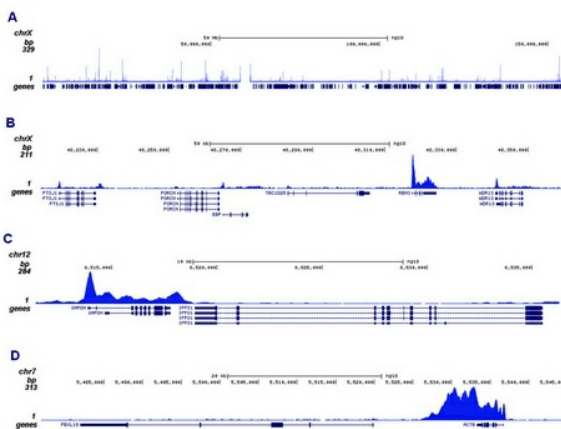


HeLa cells were stained with the antibody against Pol II S5p and with DAPI. Cells were fixed with methanol and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the Pol II S5p antibody (left) diluted 1:500 in blocking solution followed by an anti-mouse antibody conjugated to Alexa594. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.



ChIP assays using HeLa cells: ChIP-seq™ kit, using sheared chromatin from 1 million cells. A titration of 1, 2, 5 and 10 µg ab was used. IgG (2µg/IP) was negative control. qPCR primers were specific for the promoter and the coding region of the constitutively expressed GAPDH and ACTB genes as positive controls, and for exon 2 of the inactive myoglobin (MB) gene and the Sat2 satellite repeats as negative controls. Image shows the recovery, expressed as a % of input (the relative amount of IP'd DNA compared to input DNA after qPCR).

Figure 2



ChIP was performed on sheared chromatin from 1 million HeLaS3 cells using 1 µg of the ab against Pol II S5p. 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. Image shows the peak distribution along the complete sequence and a 150 kb region of the X-chromosome (figure 2A and B, respectively), and in a two genomic regions surrounding the GAPDH and ACTB positive control genes (figure 2C and D).