

## Pol II antibody

### Cat. No. C15200004

Lot:	001–15
Size:	10 µg / 50 µg
Type:	Monoclonal, <b>ChIP-grade, ChIP-seq grade, CUT&amp;Tag-grade</b>
Isotype:	IgG1
Source:	Mouse
Concentration:	1 µg/µl

Specificity:	Human, Xenopus, yeast: positive Other species: not tested
Purity:	Protein A purified monoclonal antibody
Storage buffer:	PBS containing 0.05% azide.

**Storage:** Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.

**Precautions:** This product is for research use only. Not for use in diagnostic or therapeutic procedures.

**Description:** Monoclonal antibody raised in mouse against the YSPTSPS repeat in the B1 subunit of RNA polymerase II.

## Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq*	1 µg per ChIP	Fig 1, 2
CUT&Tag	1 µg	Fig 3
ELISA	1:3,000	Fig 4
Western blotting	1:1,000	Fig 5, 6
Immunofluorescence	1:500	Fig 7

\*Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 µg per IP.

## Target description

RNA polymerase II (Pol II) is a key enzyme in the regulation and control of gene transcription. It can unwind the DNA double helix, synthesize RNA, and proofread the result. Pol II is a complex enzyme composed of 12 subunits, among 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.

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## Results

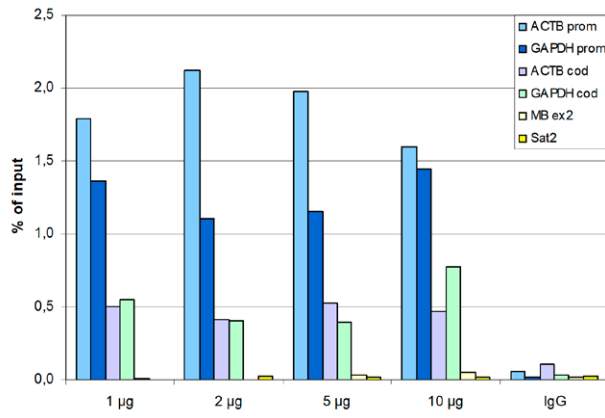


Figure 1: ChIP results obtained with the monoclonal antibody directed against Pol II

ChIP assays were performed using human HeLa cells, the monoclonal antibody against Pol II (cat. No. C15200004), and optimized PCR primer pairs for qPCR. ChIP was performed with the iDeal ChIP-seq kit (cat. No. C01010051), using sheared chromatin from 1 million cells. 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. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).



Figure 2: ChIP-seq results obtained with the monoclonal antibody directed against Pol II

ChIP was performed on sheared chromatin from 1 million HeLaS3 cells using 1 µg of the antibody against Pol II (cat. No. C15200004) as described above. The IP'd DNA was subsequently analyzed on an Illumina Genome Analyzer. Library preparation, cluster generation, and sequencing were performed according to the manufacturer's instructions. The 36 bp tags were aligned to the human genome using the ELAND algorithm. Figure 2 shows the peak distribution across the complete sequence and a 400 kb region of the X-chromosome (Figure 2A and 2B, respectively), and in two genomic regions surrounding the GAPDH and ACTB positive control genes (Figure 2C and 2D).

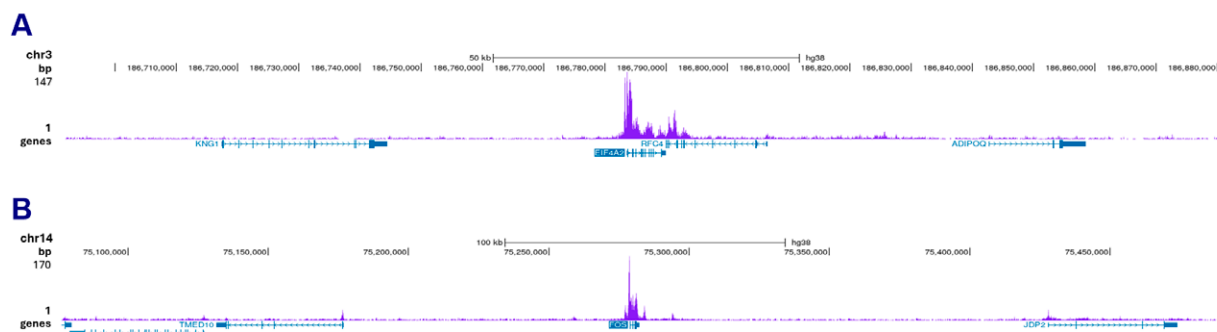


Figure 3: Cut&Tag results obtained with the monoclonal antibody directed against Pol II

Cut&Tag was performed on 50,000 K562 cells using 1 µg of the monoclonal antibody against Pol II (cat. No. C15200004), the pA-Tn5 transposase (C01070001), and the Universal Cut&Tag kit (cat. No. C01070024). The libraries were subsequently analyzed on an Illumina NovaSeq sequencer (2x50 paired-end reads) according to the manufacturer's instructions. The tags were aligned to the human genome (hg38) using the BWA algorithm. Figure 3 shows the peak distribution in two genomic regions on chromosomes 3 and 14 (Figure 3A and 3B, respectively).

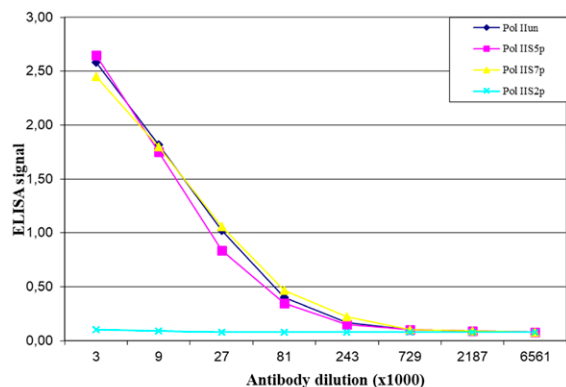


Figure 4: Cross-reactivity of the monoclonal antibody directed against Pol II

To test the specificity, an ELISA was performed using a serial dilution of the monoclonal antibody against Pol II (cat. No. C15200004). The wells were coated with peptides containing the unmodified C-terminal repeat sequence as well as different phosphorylated peptides. Figure 4 shows that the antibody recognizes the unphosphorylated Pol II as well as most phosphorylated forms.

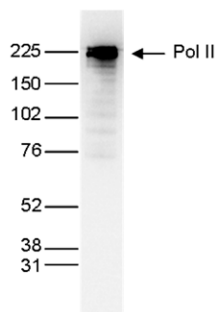


Figure 5: Western blot analysis using the monoclonal antibody directed against Pol II

Nuclear extracts (25 µg) from HeLa cells were analyzed by Western blot, using the monoclonal antibody against Pol II (cat. No. C15200004) 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.

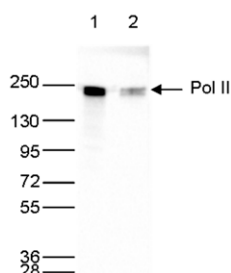


Figure 6: Western blot analysis using the monoclonal antibody directed against Pol II

Whole cell extracts (40 µg) from HeLa cells transfected with Pol II siRNA (lane 2) and from an untransfected control (lane 1) were analyzed by Western blot using the antibody against Pol II (cat. No. C15200004) 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.

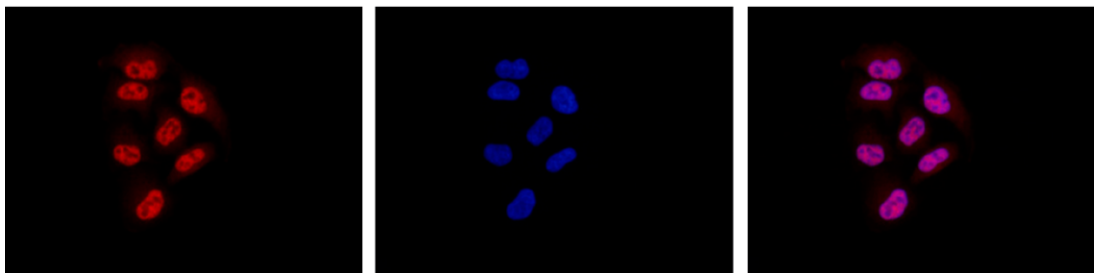


Figure 7: Immunofluorescence using the monoclonal antibody directed against Pol II

HeLa cells were stained with the antibody against Pol II (cat. No. C15200004) 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 labeled with the Pol II 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 stains is shown on the right.