TECHNICAL DATASHEET

LOT: 001

HOLOGIC® Diagenode

Pol II antibody

Cat. No. C15200253

Lot:	001
Size:	50 µg
Type:	Monoclonal, **ChIP-seq grade**
Isotype:	IgG2a
Source:	Mouse
Concentration:	2.2 µg/µl

Specificity:	Human, Mouse, Xenopus: positive. Other species: not tested.
Purity:	Protein A purified monoclonal antibody.

Storage buffer: PBS containing 0.05% Na-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 B1 subunit of RNA polymerase II (polymerase (RNA) II (DNA directed) polypeptide A) of wheat germ. Interacts with the highly conserved C-terminal domain of the protein containing the YSPTSPS repeat

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq*	0.5 – 1 µg per ChIP	Fig 1, 2
Western blotting	1:1,000	Fig 3

*Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 0.5–5 µg per IP.

Target description

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.

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Last update: August, 2024

Results

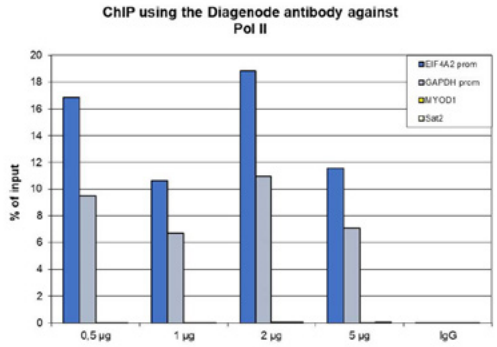

Figure 1: ChIP results obtained with the Hologic Diagenode monoclonal antibody directed against Pol II

ChIP assays were performed using human HeLa cells, the Hologic Diagenode monoclonal antibody against Pol II (cat. No. C15200253) 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 0.5, 1, 2 and 5 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 of the GAPDH and EIF4A2 genes, used as positive controls, and for the MYOD1 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).

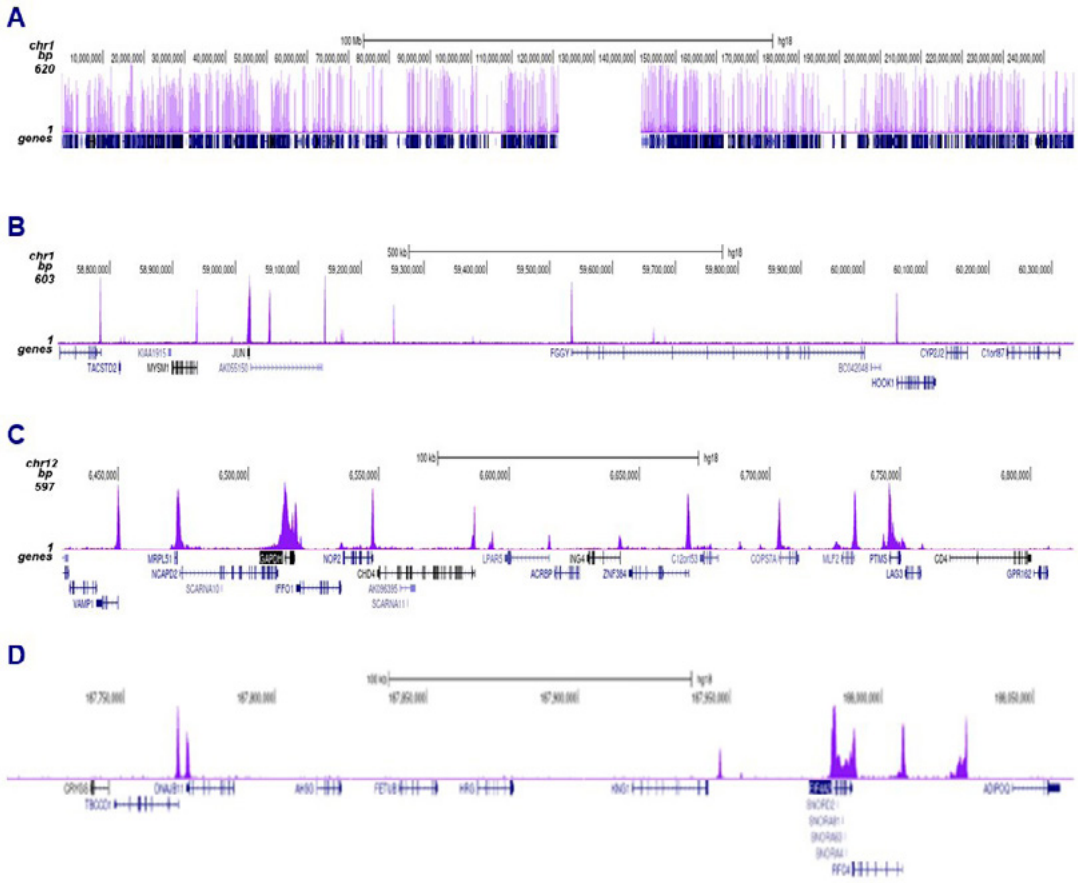

Figure 2: ChIP-seq results obtained with the Hologic Diagenode monoclonal antibody directed against Pol II

ChIP was performed on sheared chromatin from 1 million HeLa cells using 0.5 µg of the Hologic Diagenode antibody against Pol II (cat. No. C15200253) as described above. The IP'd DNA was subsequently analysed on an Illumina NovaSeq. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 50 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the enrichment along the complete sequence and a 1.5 Mb region of chromosome 1 (fig 2A and B) and in genomic regions of chromosome 12 and 3, surrounding the GAPDH and EIF4A2 positive control genes (fig 2C and D).

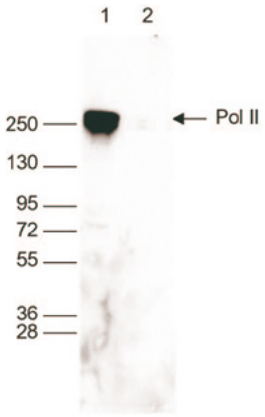

Figure 3: Western blot analysis using the Hologic Diagenode monoclonal antibody directed against Pol II

Whole cell extracts (40 µg) from HeLa cells transfected with Pol II siRNA (lane 2) and from an untransfected control (lane 1) were analysed by Western blot using the Hologic Diagenode antibody against Pol II (cat. No. C15200253) 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.