



p53 polyclonal antibody

Other names: TP53, P53, TRP53, LSF1

Cat. No. C15410083

Type: Polyclonal

ChIP-grade / ChIP-seq grade

Source: Rabbit

Lot #: A61-0012

Size: 50 µg/ 19 µl

Concentration: 2.73 µg/µl

Specificity: Human: positive

Other species: not tested

Purity: Affinity purified polyclonal antibody in PBS containing 0.05% azide and 0.05% ProClin 300.

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: Polyclonal antibody raised in rabbit against human p53 (tumor protein p53), using a KLH-conjugated synthetic peptide containing a sequence from the C-terminal part of the protein.

Applications

	Suggested dilution	Results
ChIP*	1 µg/ChIP	Fig 1, 2
ELISA	1:4,000	Fig 3
Western blotting	1:2,000	Fig 4

*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

The transcription factor p53 (UniProt/Swiss-Prot entry P04637) is a tumour suppressor that regulates the cellular response to diverse cellular stresses. Upon activation, p53 induces several target genes which leads to cell cycle arrest and DNA repair, or alternatively, to apoptosis. In unstressed cells, p53 is kept inactive by the ubiquitin ligase MDM2 which inhibits the activity and promotes the degradation. Mutations in p53 are involved in a vast majority of human cancers.

Results

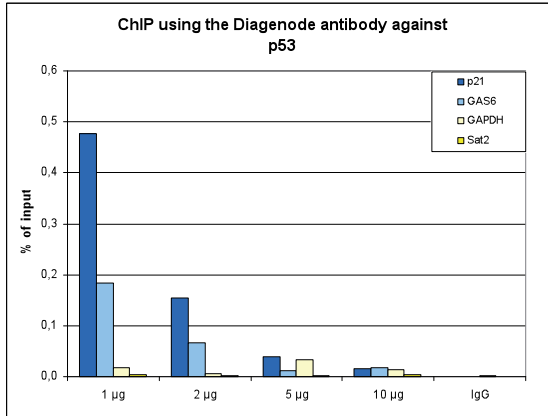


Figure 1. ChIP results obtained with the Diagenode antibody directed against p53”

ChIP assays were performed using human U2OS cells, treated with camptothecin, the Diagenode antibody against p53 (Cat. No. C15410083) and optimized PCR primer sets for qPCR. ChIP was performed on sheared chromatin from 4 million cells. A titration of the antibody consisting of 1, 2, 5, and 10 µg per ChIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control. qPCR was performed with primers for the p21 and GAS6 genes used as positive controls, and for GAPDH 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).

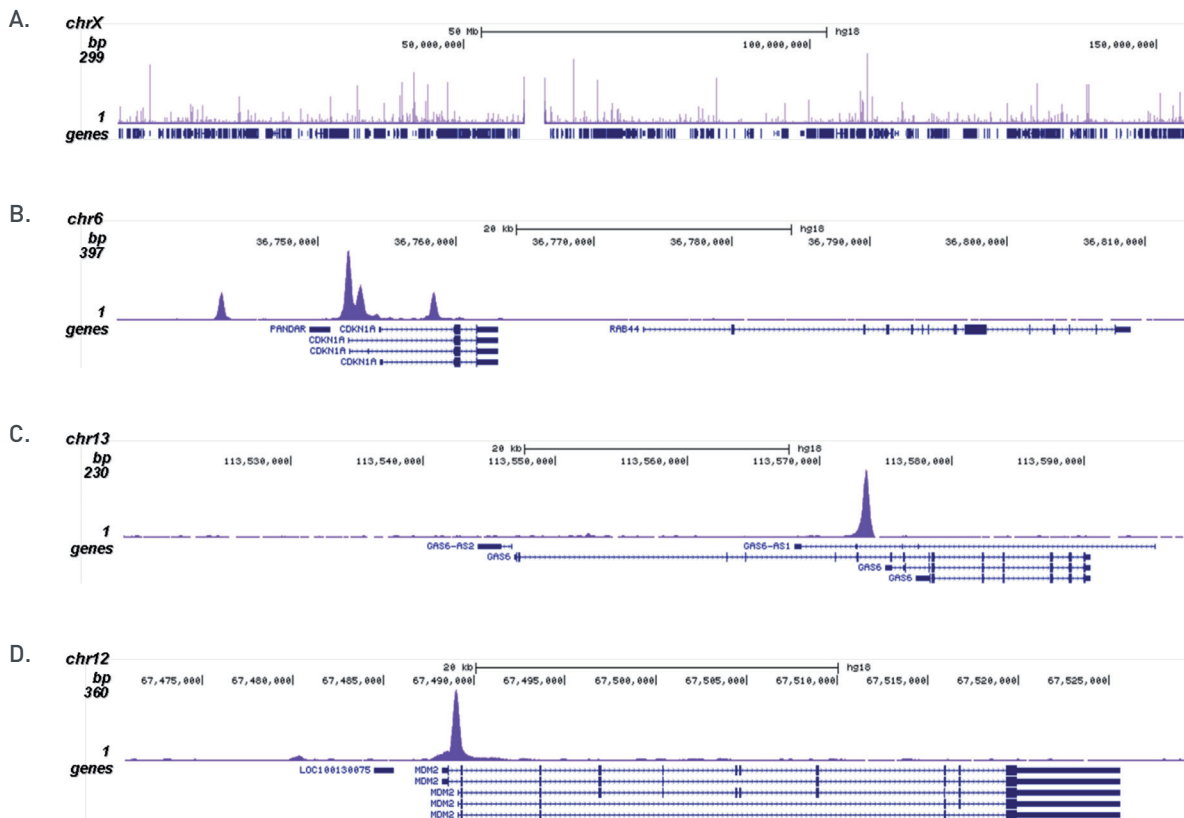


Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against p53

ChIP was performed on sheared chromatin from 4 million U2OS cells using 1 µg of the Diagenode antibody against p53 (Cat. No. C15410083) as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 51 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the peak distribution along the X-chromosome (fig 2A) and in 3 genomic regions of chromosome 6, 13 and 12, surrounding p21 (CDKN1A), GAS6 and MDM2, 3 known targets genes of p53 (fig 2B, C and D, respectively).

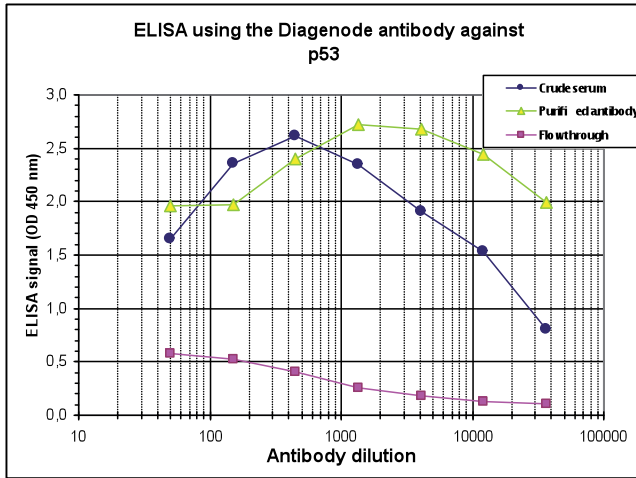


Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of Diagenode antibody directed against human p53 (Cat. No. C15410083), crude serum and flow through in antigen coated wells. By plotting the absorbance against the antibody dilution (Figure 3), the titer of the antibody was estimated to be 1:308,000.

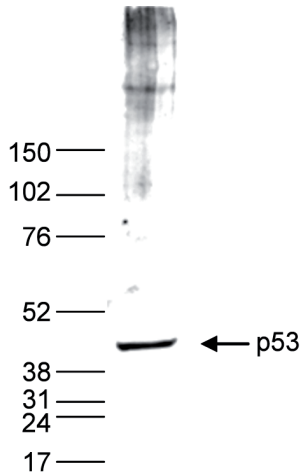


Figure 4. Western blot analysis using the Diagenode antibody directed against p53

Nuclear extracts of HeLa cells (40 µg) were analysed by Western blot using the Diagenode antibody against p53 (Cat. No. C15410083) diluted 1:2,000 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.