diagenode
A Hologic Company

TECHNICAL DATASHEET

TARDBP polyclonal antibody - Classic

Other name: TDP43, TDP-43, ALS1

Cat. No. C15410266

Type: Polyclonal **ChIP-grade, ChIP-seq grade**

Source: Rabbit

Lot #: 40135

Size: 25 µl/100 µl

Concentration: 1.01 µg/µl

Specificity: Human, mouse, rat

Purity: Affinity purified polyclonal antibody in 0.1 M Tris-HCl containing 0.1 M Glycine, 20% glycerol and 0.01% thimerosal.

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 TARDBP (TAR DNA Binding Protein), using a recombinant protein.

Applications

Applications	Suggested dilution/amount	Results
ChIP*	2 µg per ChIP	Fig 1, 2
Western blotting	1:1,000 - 1:3,000	Fig 3, 4, 5
Immunoprecipitation	2 µg per IP	Fig 6
Immunofluorescence	1:100 - 1:1,000	Fig 7
Immunohistochemistry	1:100 - 1:1,000	Fig 8

* 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

TARDBP (UniProt/Swiss-Prot entry Q13148) is a DNA and RNA-binding protein which regulates transcription and splicing. It binds to the chromosomally integrated TAR DNA from HIV-1 thereby repressing HIV-1 transcription. TARDBP is also involved in the regulation of CFTR splicing where it promotes CFTR exon 9 skipping. The resulting aberrant splicing is associated with pathological features typical of cystic fibrosis.

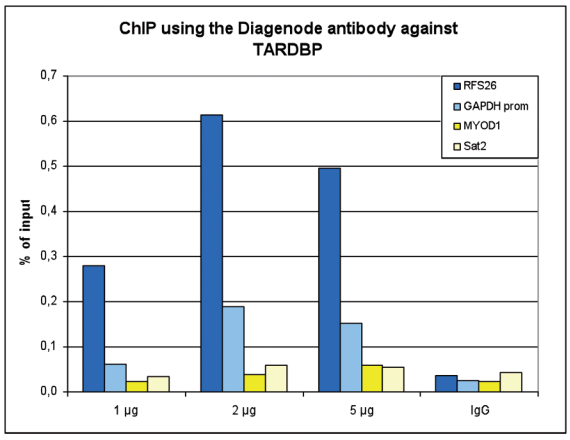

Figure 1. ChIP results obtained with the Diagenode antibody directed against TARDBP

ChIP assays were performed using K562 cells, the Diagenode antibody against TARDBP (Cat. No. C15410266) and optimized primer sets for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (Cat. No. C01010055), using sheared chromatin from 4 million cells. A titration of the antibody consisting of 1, 2 and 5 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control. QPCR was performed with primers for the GAPDH and RFS26 promoters, 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).

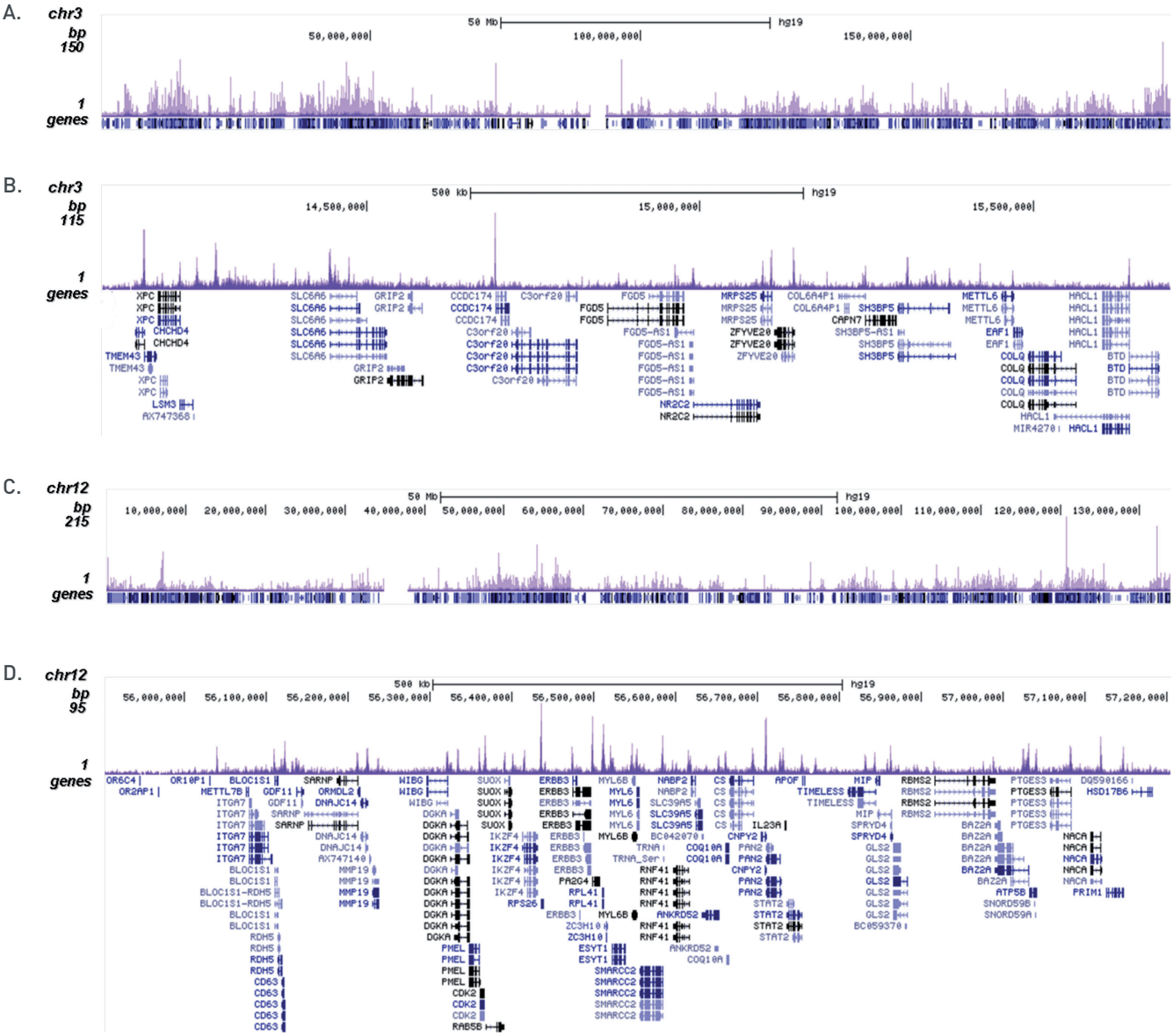

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against TARDBP

ChIP was performed on sheared chromatin from 5 million K562 cells using 2 µg of the Diagenode antibody against TARDBP (Cat. No. C15410266). 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 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 chromosomes 3 (fig 2A and B) and 12 (fig 2C and D), respectively.

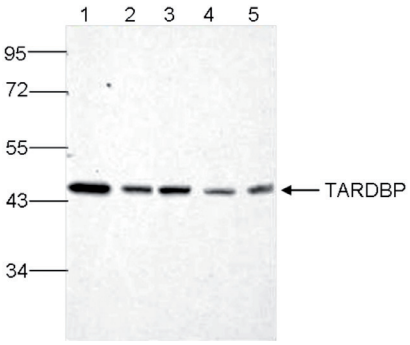

Figure 3. Western blot analysis using the Diagenode antibody directed against TARDBP

Whole cell extracts (30 µg) from Neuro2A (lane1), GL261 (lane 2), NIH3T3 (lane 3), BCL1 (lane 4) and Raw264.7 (lane 5) cells were analysed by Western blot using the Diagenode antibody against TARDBP (Cat. No. C15410266) diluted 1:3,000. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.

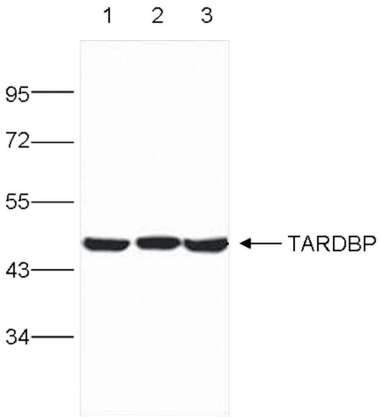

Figure 4. Western blot analysis using the Diagenode antibody directed against TARDBP

Whole cell extracts (30 µg) from A431 (lane1), H1299 (lane 2) and HeLa (lane 3),cells were analysed by Western blot using the Diagenode antibody against TARDBP (Cat. No. C15410266) diluted 1:1,000. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.

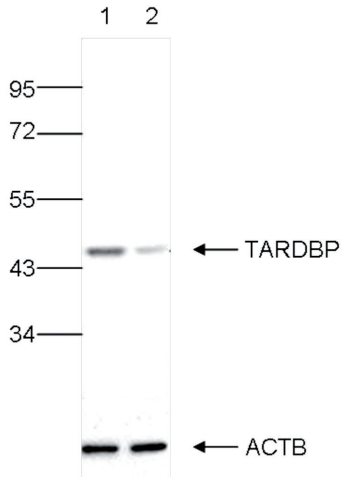

Figure 5. Western blot analysis using the Diagenode antibody directed against TARDBP

Whole cell extracts (30 µg) from HeLa cells transfected with sh-TARDBP (lane1), or a mock control (lane 2) were analysed by Western blot using the Diagenode antibody against TARDBP (Cat. No. C15410266) diluted 1:1,000. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left. The lower panel shows the signal obtained with an ACTB antibody, used as a loading control

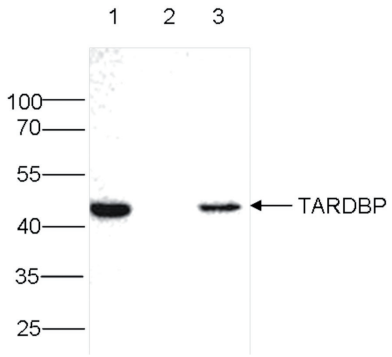

Figure 6. Immunoprecipitation using the Diagenode antibody directed against TARDBP

Immunoprecipitation was performed on whole cell extracts from HeLa cells using 2 µg of the Diagenode antibody against TARDBP (Cat. No. C15410266). An equal amount of rabbit IgG was used as a negative control. The immunoprecipitated TARDBP protein was detected by western blot with the TARDBP antibody diluted 1:1,000. The IP with the TARDBP antibody and with the IgG negative control are shown in lane 3 and lane 2, respectively. Lane 1 shows the input (40 µg of HeLa whole cell extract).

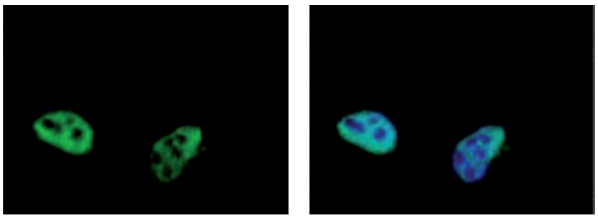

Figure 7. Immunofluorescence with the Diagenode antibody directed against TARDBP

HeLa cells were fixed with 4% formaldehyde for 15' at room temperature and stained with the Diagenode antibody against TARDBP (Cat. C15410266) diluted 1:200 (left). The right picture shows costaining with Hoechst 33342 nucleic acid stain.

A.

B.

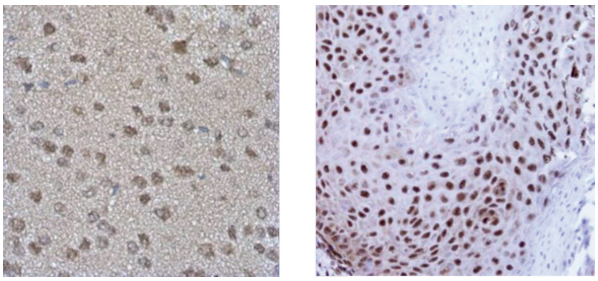

Figure 8. Immunohistochemistry using the Diagenode antibody directed against TARDBP

Formalin fixed paraffin embedded rat brain tissue (figure A) or Cal27 Xenograft (figure B) was stained with the Diagenode antibody against TARDBP (Cat. No. C15410266) diluted 1:500 and 1:100, respectively, followed by a peroxidase labelled goat anti-rabbit secondary antibody.

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Last update: September 16, 2015