TECHNICAL DATASHEET

LOT: 001

HOLOGIC® Diagenode

NRF1 antibody

Cat. No. C15200013

Lot:	001
Size:	10 µg / 50 µg
Type:	Monoclonal, **ChIP-grade**, **ChIP-seq grade**, **CUT&Tag-grade**
Isotype:	IgG2a
Source:	Mouse
Concentration:	2.2 µg/µl

Specificity:	Human: 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 human NRF1 (Nuclear Respiratory Factor 1), using a recombinant protein.

Applications

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

*Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-10 µg per IP.

Target description

NRF1 (UniProt/Swiss-Prot entry Q16656) is a transcription factor which activates the expression of some key metabolic genes regulating cellular growth and nuclear genes required for respiration, heme biosynthesis, and mitochondrial DNA transcription and replication. NRF1 has also been associated with the regulation of neurite outgrowth.

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Last update: September, 2025

Results

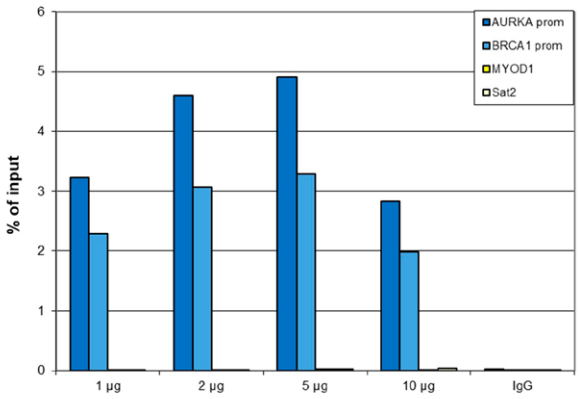

Figure 1: ChIP results obtained with the monoclonal antibody directed against NRF1

ChIP was performed using K562 cells, the monoclonal antibody against NRF1 (cat. No. C15200013) and optimized PCR 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, 5 and 10 µg per ChIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control. Quantitative PCR was performed with primers for the BRCA1 and AURKA promoters, used as positive controls, and for the coding region of the inactive 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).

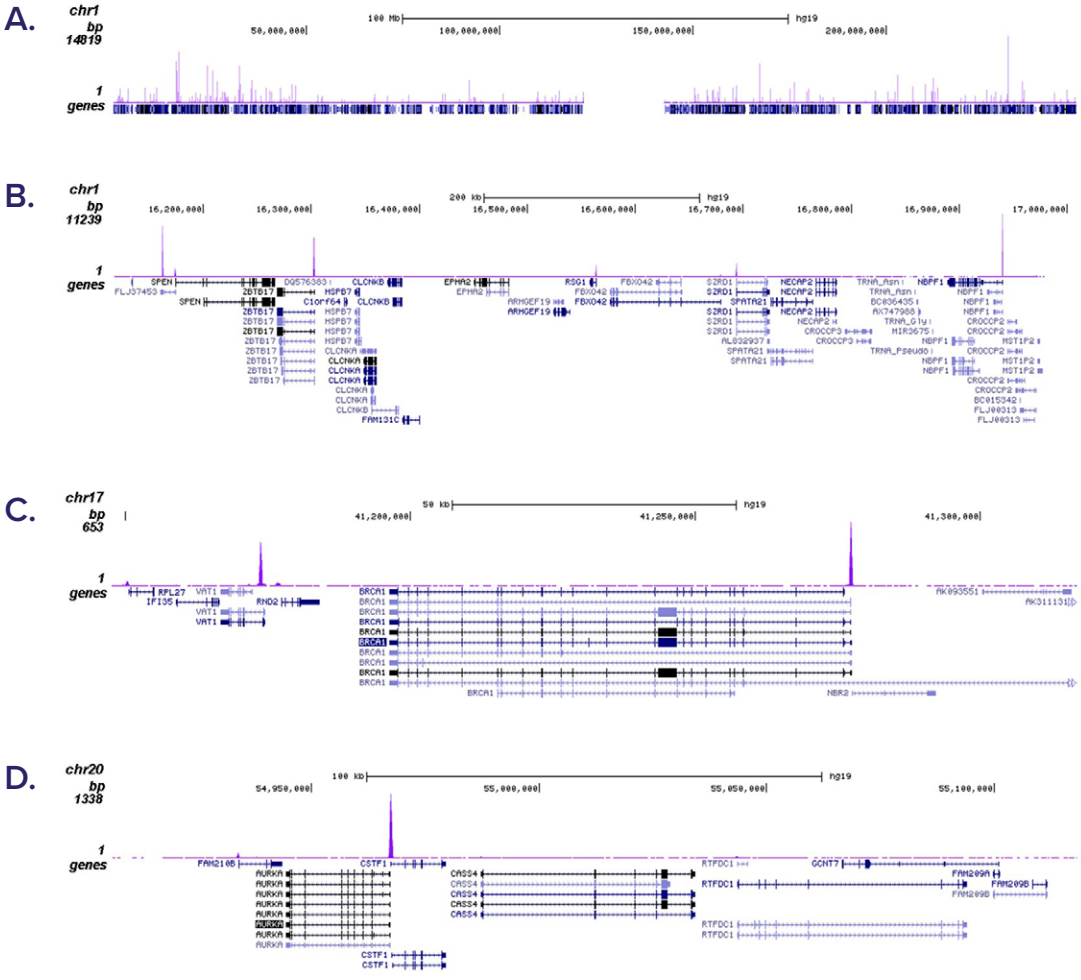

Figure 2: ChIP-seq results obtained with the monoclonal antibody directed against NRF1

ChIP was performed with 2 µg of the antibody against NRF1 (cat. No. C15200013) on sheared chromatin from 4 million K562 cells as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq 2000. 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 peak distribution along the complete sequence and a 700 kb region of chromosome 1 (figure 2A and B) and in two regions surrounding the BRCA1 and AURKA positive control genes (figure 2C and D).

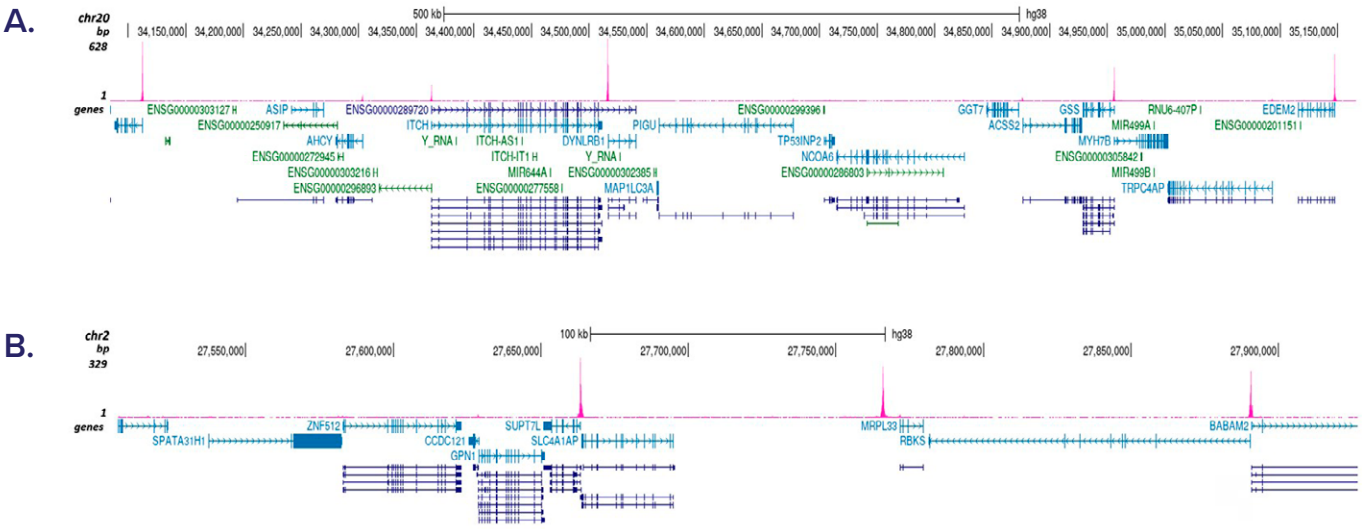

Figure 3: Cut&Tag results obtained with the antibody directed against NRF1

CUT&TAG was performed on 300,000 K562 cells using 1 µg of the monoclonal antibody against NRF1 (cat. No. C15200013), the pA-Tn5 transposase (C01070001) and the Universal Cut&Tag kit (cat. No. C01070024). The libraries were subsequently analysed 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 2 genomic regions on chromosome 20 and 2 (figure 3A and B, respectively).

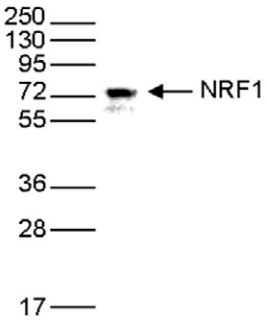

Figure 4: Western blot analysis using the monoclonal antibody directed against NRF1

Whole cell extracts from K562 cells were analysed by Western blot using the antibody against NRF1 (cat. No. C15200013) diluted 1:1,000 in TBST containing 5% milk powder. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.