Anti-Human NRF1, monoclonal (clone R157.1.3D4)

Recommended name: Nuclear respiratory factor 1 (Short name = NRF-1);
Alternative name(s): Alpha palindromic-binding protein (Short name = Alpha-pal)

Cat. No.	**m13-005**	**Quantity:**	**100 µg**
Lot. No.	**20140415.DNF**	**Storage:**	**-20˚C**

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Uniprot / NCBI Summary

UniProt
Primary Accession # Q16656
Secondary Accession # Q15305

NCBI
GI # 93141039
GeneID 4899
Accession # NP_001035199
GenBank Nucleotide # NM_001040110.1

Molecular Weight 53,541 Da (503 aa)

Transcription factor that activates the expression of the EIF2S1 (EIF2-alpha) gene. Links the transcriptional modulation of key metabolic genes to cellular growth and development. Implicated in the control of nuclear genes required for respiration, heme biosynthesis, and mitochondrial DNA transcription and replication.

Subunit structure: Homodimer. Binds DNA as a dimer. Interacts with PPRC1. Ref.12 Ref.13

Subcellular location: Nucleus.

Continued on page 2.

Physical Characteristics

Quantity: 100 µg

Concentration: 1.0 mg/ml

Host / Isotype: mouse IgG2b

Clonality: monoclonal; ID R157.1.3D4

Immunogen: recombinant protein corresponding to amino acids 59-490 of human NRF1

Purification: affinity-chromatography using Protein G

Formulation: 30% glycerol, 1x PBS, 0**.02% sodium azide**

Specificity: monospecific for human NRF1; see microarray analysis below

Reactivity: human

Stability/Storage: 12 months long term: -20˚C; short term: 4˚C; avoid freeze-thaw cycles; aliquot as required

Handling Notes: small volumes of antibody may occasionally become entrapped in the seal of the product vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the container cap.

Tested Research Applications

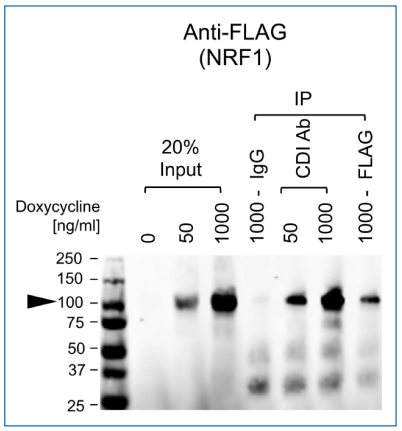
Western Immunoblotting: tested on cells transfected with a construct encoding NRF1; utility on native cells under evaluation

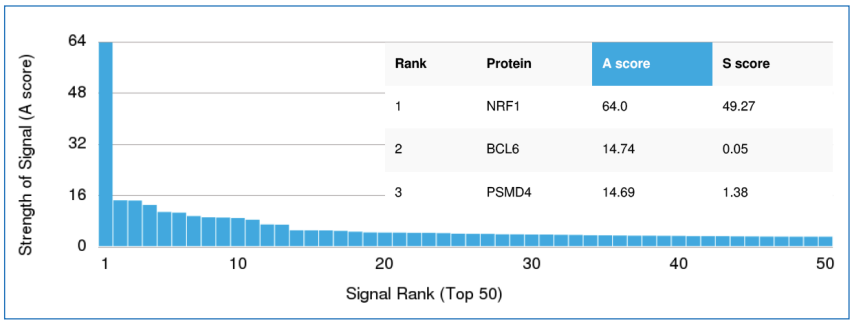
Immunoprecipitation: recommended; see below

ChIP-Seq: recommended; see page 2

Quality Assurance

IP Analysis:
Tet-ON HeLa cells were transfected with construct encoding NRF1 with an N-terminal fusion of FLAG, YFP (Venus) and V5 tags under a tet-inducible promoter. These cells were stimulated with 0, 50 or 1000 ng/ml doxycycline. Immunoprecipitation (IP) was carried out using 5µg of either IgG, CDI mAb Anti-NRF1 (cloneID# R157.1.3D4) or 1 µg of FLAG-M2. Immunoblotting was performed using rabbit Anti-FLAG (1:1000, Cell Signaling #2368).





Specificity Analysis with HuProt™ Human Proteome Microarray: Anti Human NRF1 (clone R157.1.3D4) was analyzed using the CDI HuProt™ Human Proteome Microarray.

For more information on A/S scores and how they relate to specificity, see page 2.

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Lot-specific Datasheet version tracker: v2.0.0

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Uniprot / NCBI Summary

Continued from page 1.

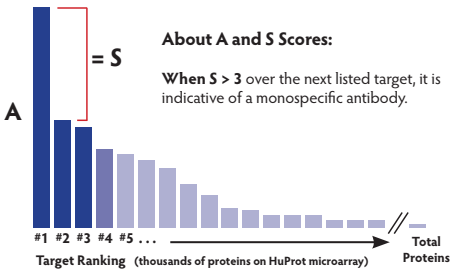
Tissue specificity: Ubiquitously expressed with strongest expression in skeletal muscle. Post-translational modification: Phosphorylation enhances DNA binding. Sequence similarities: Belongs to the NRF1/Ewg family.

General References:

Virbasius CA, Virbasius JV, Scarpulla RC (1993) NRF-1, an activator involved in nuclear-mitochondrial interactions, utilizes a new DNA-binding domain conserved in a family of developmental regulators. *Genes Dev* **7**:2431-2445. [PubMed]

Gugneja S, Scarpulla RC (1997) Serine phosphorylation within a concise amino-terminal domain in nuclear respiratory factor 1 enhances DNA binding. *J Biol Chem* **272**:18732-18739. [PubMed]

Vercauteren K, Pasko RA, Gleyzer N, Marino VM, Scarpulla RC (2006) PGC-1-related coactivator: immediate early expression and characterization of a CREB/NRF-1 binding domain associated with cytochrome c promoter occupancy and respiratory growth. *Mol Cell Biol* **26**:7409-7419. [PubMed]

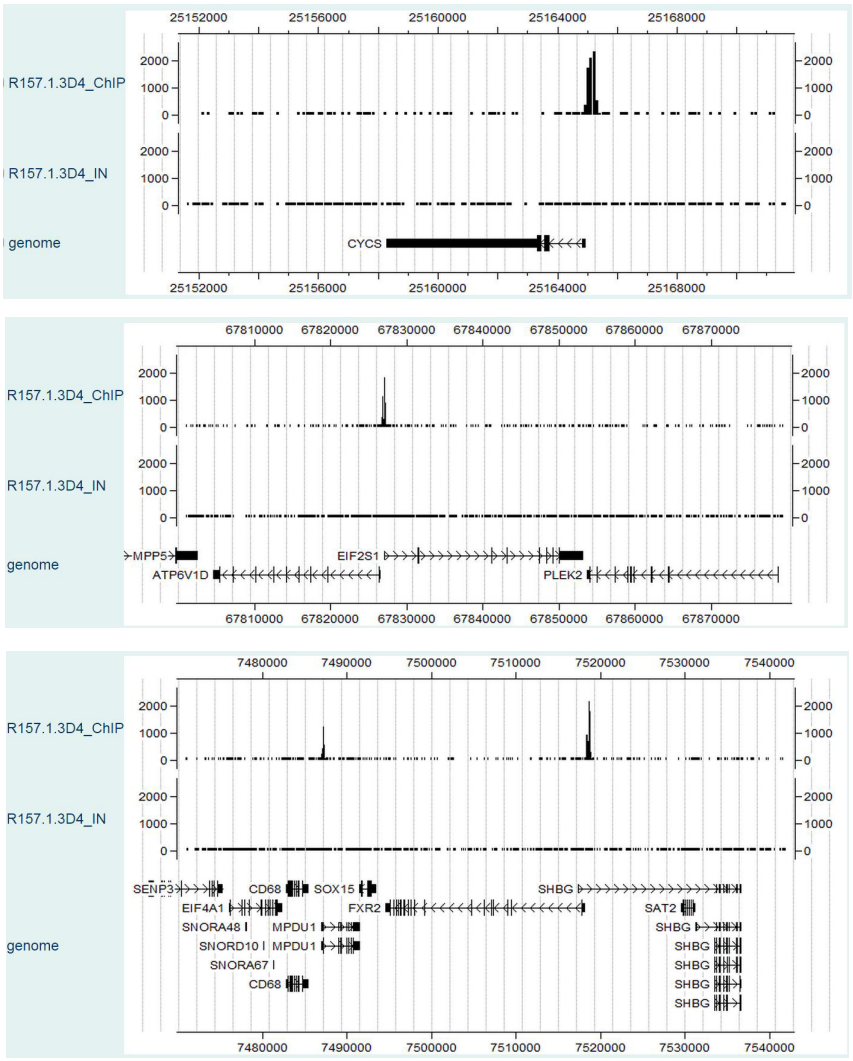


Statistical Analysis: Thousands of GenePix data points (from the microarray) are analyzed in terms of signal strength and ranked accordingly.

SUMMARY: The A-score indicates the number of standard deviations above background seen for the mean signal bound by the target antigen The S-score represents the difference between the A-score of the target antigen and the next best hit on the array. S-scores **greater than 3 standard deviations over the next listed target** are deemed statistically significant and indicate **highly specific antibodies.** More info at cdi-lab.com/HighSpec.html

Tested Research Applications

ChIP-Seq: Recommended



The ChIP was performed with chromatin from 10 million K562 cells and 3 µg of Anti-NRF1 (cloneID #R157.1.3D4) antibody. The ChIP DNA was sequenced on an Illumina HiSeq platform and read counts were calculated at consecutive 100 bp bins across the human genome hg19. Normalized read-count levels for ChIP-seq of NRF1 (R157.1.3D4_ChIP) and control (R157.1.3D4_IN) around the CYCS, EIF2S1 and FXR2 loci are displayed in the CisGenome browser.

The development of this antibody was supported by the National Institutes of Health Protein Capture Reagent Program under award U54HG06434 to CDI Laboratories and Johns Hopkins University.

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