

NDR, Active

Full-length human protein expressed in Sf9 cells

Catalog # S49-10G

Lot # W006-3

Product Description

Full-length recombinant human NDR1 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_007271](#).

Gene Aliases

NDR1, STK38

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

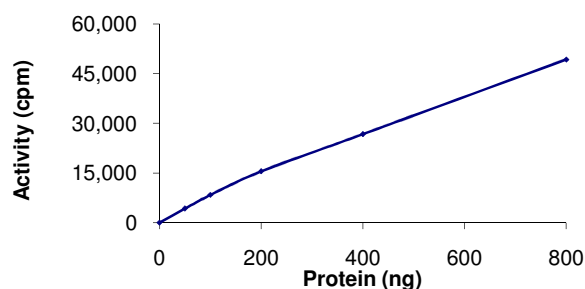
Scientific Background

NDR or Nuclear Dbf2-related protein is a serine-threonine kinase in the subgroup of the AGC kinase family that is regulated by Mob proteins (1). Fas and $\text{TNF}\alpha$ receptor stimulation activates human NDR by promoting phosphorylation at the hydrophobic motif. NDR acts as a novel pro-apoptotic kinase and key member of the RASSF1A/MST1 signaling cascade (2). NDR is crucial for the fidelity of mitotic chromosome alignment in mammalian cells and is incorporated into the HIV-1 particles and cleaved by the HIV-1 protease. NDR-driven centrosome duplication requires Cdk2 activity and Cdk2-induced centrosome amplification is affected upon reduction of NDR activity.

References

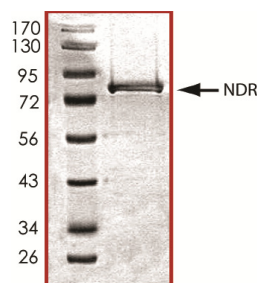
1. Millward, T. et.al: Molecular cloning and characterization of a conserved nuclear serine(threonine) protein kinase. Proc. Nat. Acad. Sci. 92: 5022-5026, 1995.
2. Vichalkovski A. et al: NDR kinase is activated by RASSF1A/MST1 in response to Fas receptor stimulation and promotes apoptosis. Curr Biol. 2008 Dec 9;18(23):1889-95.

Specific Activity



The specific activity of NDR was determined to be **4.1 nmol /min/mg** as per activity assay protocol.

Purity



The purity of NDR was determined to be **>90%** by densitometry, approx. MW **81kDa**.

NDR, Active

Full-length human protein expressed in Sf9 cells

Catalog Number	S49-10G
Specific Activity	4.1 nmol/min/mg
Specific Lot Number	W006-3
Purity	>90%
Concentration	0.1 µg/µl
Stability	1yr At -70°C from date of shipment
Storage & Shipping	Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: S49-10G)

Active NDR (0.1µg/µl) was diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active NDR for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA solution.

Kinase Assay Buffer (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM MgCl₂, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at -20°C.

Substrate (Catalog #: C01-58B)

Modified PKA-sub synthetic peptide substrate (modified-CGRTGRRNSI-amide) was diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active ADRBK1, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
 - Component 1.** 10µl of diluted Active NDR (Catalog #S49-10G)
 - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #C01-58B)
 - Component 3.** 5µl distilled H₂O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5.** Initiate the reaction by the addition of 5 µl [³³P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20 µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [³³P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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