

Certificate of Analysis

sirtuin 1

Human NAD-dependent protein deacetylase sirtuin-1, isoform a
(Recombinant protein expressed in *E.coli*)

Item # EPI059

Lot # 140434

Product Description: N-terminal 6His-tagged, recombinant, amino acids 193-741, human sirtuin 1, expressed in *E.coli*. Purified using immobilised metal affinity chromatography.

MW = 63.8kDa.

Alias: SIRT1

Formulation: 1mg/ml of protein in 25mM Hepes/NaOH pH 7.4, 125mM NaCl, 50% glycerol. Frozen solution.

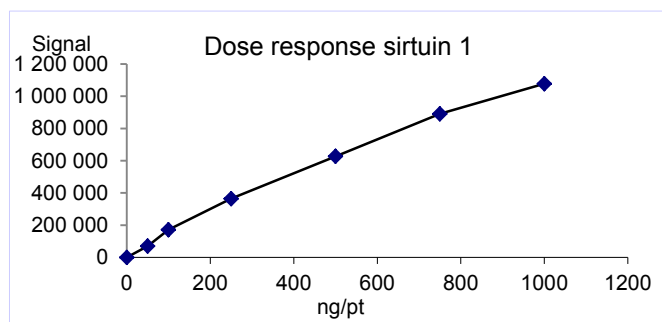
Storage and Stability: Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and store at -70°C.

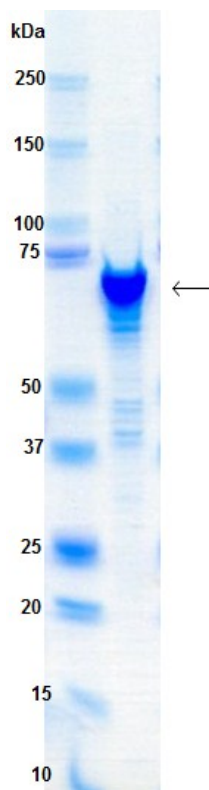
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Enzymatic Assay: 50-1000ng of this lot of enzyme deacetylated 200µM Fluorogenic HDAC substrate in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS: Size was confirmed by mass spectrometry using a Q-TOF.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 4µg of sirtuin 1.

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Sirtuin 1 Assay Protocol

Stock Solutions:

1. **Reaction buffer:** 50mM Tris/HCl 8, 137mM NaCl, 2.7mM KCl, 1mM MgCl₂, 0.2% BSA.
2. **Sirtuin 1:** Dilute with reaction buffer. Use 50-1000ng per assay point.
3. **Mix β -NAD / Fluorogenic HDAC Substrate:** Dilute with reaction buffer to 1mM and 400 μ M respectively.

Assay Procedure (384 well plate format):

1. Add 2 μ l of H₂O per assay to each well.
2. Add 8 μ l (**50-1000ng**) **sirtuin 1**.
3. Incubate for 5 minutes at 22°C.
4. Add 10 μ l of Fluorogenic HDAC substrate/ β -NAD.
5. Incubate for 20 minutes at 22°C.
6. Add 20 μ l of HDAC Developer.
7. Incubate for 15 minutes at 22°C.
8. Read at 460nm after an excitation flash at 355nm. Compare the signal of enzyme samples with that of a background sample that contains all assay components except the enzyme sirtuin 1.

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Sirtuin 1 Sequence Information

Protein Human sirtuin 1
Tags N-terminal 6His
Accession number GenBank NP_036370.2

Recombinant Sirtuin 1 amino acid sequence:

1 MHHHHHSSG VDLGTENLYF QSMIGTDPRT ILKDLLPETI PPELDDMTL
51 WQIVINILSE PPKRKKRKDI NTIEDAVKLL QECKKIIVLT GAGVSVSCGI
101 PDFRSRDIY ARLAVDFPDL PDPQAMFDIE YFRKDPRPFF KFAKEIYPGQ
151 FQPSLCHKFI ALSDKEGKLL RNYTQNIDTL EQVAGIQRII QCHGSFATAS
201 CLICKYKVDC EAVRGDIFNQ VVPRCPRCPA DEPLAIMKPE IVFFGENLPE
251 QFHRAKMYDK DEVDLLIVIG SSLKVRPVAL IPSSIPHEVP QILINREPLP
301 HLHFDVELLG DCDVIINELC HRLGGEYAKL CCNPVKLSEI TEKPPRTQKE
351 LAYLSELPPT PLHVSEDSSS PERTSPPDSS VIVTLLDQAA KSNDDL DVSE
401 SKGCMEEKPQ EVQTSRNVES IAEQMPNDL KNVGSSTGEK NERTSVAGTV
451 RKCWPNRVAK EQISRRLDGN QYLFLPPNRY IFHGAEVYSD SEDDLSSSS
501 CGSNSDSGTC QSPSLEEPME DESEIEEFYN GLEDEPDVPE RAGGAGFGTD
551 GDDQEAINEA ISVKQEV TDM N

Reviewed and approved by site quality representative.

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