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Certificate of Analysis

sirtuin 2

NAD-dependent protein deacetylase sirtuin-2 isoform 1 (Human)

(Recombinant protein expressed in *E.coli*)
Item # EPI060
Lot # 139684

Product Description: *N*-terminal 6His-tagged, recombinant, amino acids 50-356, human sirtuin 2, expressed in *E.coli*. Purified using immobilised metal affinity chromatography.
MW = 37.3kDa.

Formulation: 1mg/ml of protein in 25mM Hepes/NaOH pH7.4, 50mM NaCl, 50% glycerol. Frozen solution.

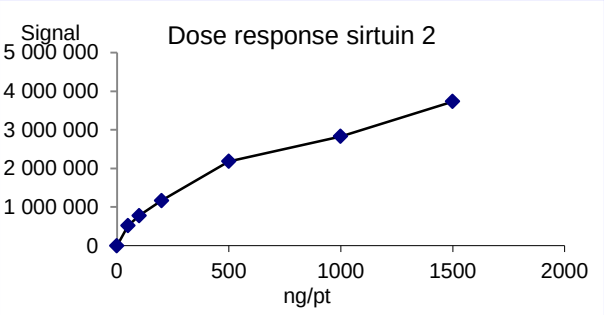
Storage and Stability: Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

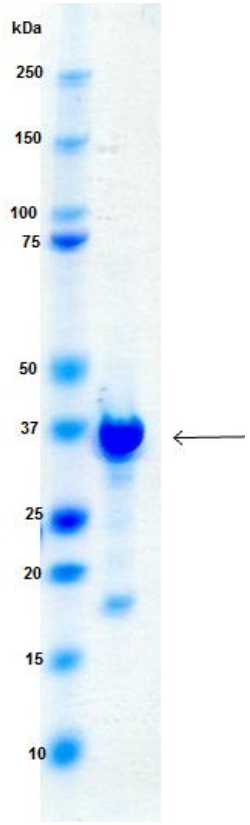
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Enzymatic Assay: 50-1500ng of this lot of enzyme deacetylated 150µM Fluor de Lys-SIRT2, deacetylase 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 2

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sirtuin 2 Assay Protocol

Stock Solutions:

1. **Reaction buffer:** 50mM Tris/HCl pH8, 137mM NaCl, 2.7mM KCl,1mM MgCl2, 0.2% BSA.
2. **sirtuin 2:** Dilute with reaction buffer. Use 50-1500ng per assay point.
3. **β-NAD:** Dilute with reaction buffer to 800µM.
4. **Fluor de Lys-SIRT2, Deacetylase Substrate:** Dilute with β-NAD solution to 300µM.

Assay Procedure (384 well plate format):

1. Add 2µl of H_2O per assay to each well.
2. Add 8µl **(50-1500ng) sirtuin 2.**
3. Add 10µl of Fluor de Lys-SIRT2, deacetylase substrate/β-NAD.
4. Incubate for 60 minutes at 22°C.
5. Add 20µl of HDAC Developer.
6. Incubate for 15 minutes at 22°C.
7. Read at 460nm after an excitation flash at 355nm. Compare signal of enzyme samples with value of Back Ground that contains all assay components except sirtuin 2.

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sirtuin 2 Sequence Information

Protein Human sirtuin 2

Tags *N*-terminal 6His

Accession number GenBank NP_036369.2

Recombinant sirtuin 2 amino acid sequence:

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  1 MHHHHHHSSG VDLGTENLYF QSMSLGSQKE RLLDELTLEG VARYMQSERC
 51 RRVICLVGAG ISTSAGIPDF RSPSTGLYDN LEKYHLPYPE AIFEISYFKK
101 HPEPFFALAK ELYPGQFKPT ICHYFMRLLK DKGLLLRCYT QNIDTLERIA
151 GLEQEDLVEA HGTFYTSHCV SASCRHEYPL SWMKEKIFSE VTPKCEDCQS
201 LVKPDIVFFG ESLPARFFSC MQSDFLKVDL LLVMGTSLQV QPFASLISKA
251 PLSTPRLLIN KEKAGQSDPF LGMIMGLGGG MDFDSKKAYR DVAWLGECDQ
301 GCLALAELLG WKKELEDLVR REHASIDAQS
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Reviewed and approved by site quality representative.