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Certificate of Analysis

NSD1
Human Nuclear receptor-binding SET-domain protein 1, active
(Recombinant enzyme expressed in *E.coli*)
Item # EPI041
Lot # 139682

Product Description: Recombinant human NSD1, Amino Acids 1810-2120, expressed in *E.coli*. Purified using immobilised metal affinity chromatography.
MW = 35.6 kDa.

Aliases : ARA267, KMT3B, SOTOS1

Tag cleaved by TEV protease.

Formulation: 1mg/ml of enzyme in 25mM Tris/HCl pH7.5, 125mM NaCl, 1.3mM TCEP, 50% glycerol. Frozen solution.

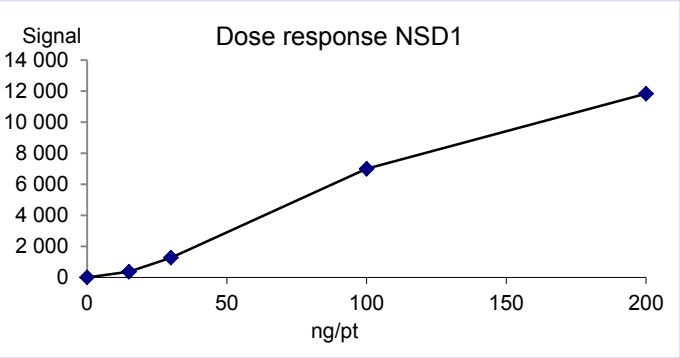
Storage and Stability: Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

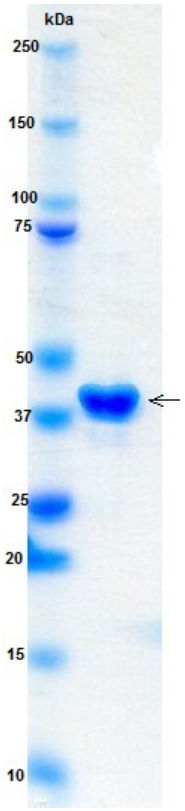
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

HMT Assay: 15-200ng of this lot of enzyme transferred methyl groups from [3H] SAM to poly nucleosome 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 NSD1.

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NSD1 Assay Protocol

Stock Solutions:

1. **Reaction buffer:** 50mM Tris/HCl pH9, 0.01% Triton.
2. **NSD1:** Dilute with reaction buffer. Use 15-200ng per assay point.
3. **Polynucleosome:** Dilute with reaction buffer to 15µg/ml.
4. **[3H] SAM:** Dilute with reaction buffer to 100nM.
5. **Filtration Buffer :** 33mM Citric acid pH2.2

Assay Procedure (96 well plate format):

1. Add 5µl of 10% DMSO per assay to each well.
2. Add 25µl of [3H] SAM.
3. Add 10µl **(15-200ng) NSD1**.
4. Add 10µl of polynucleosome.
5. Incubate for 15 minutes at 22°C.
6. Stop the reaction by adding 500µl of citric acid, then filter on a GF/B Filter. Wash 3 times with filtration buffer.
7. Dry and add scintillation cocktail.
8. Read in a scintillation counter. Compare the signal of enzyme samples with that of a background sample that contains all assay components except the enzyme NSD1.

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NSD1 Sequence Information

Protein Human NSD1

Tags *tag cleaved by TEV protease*

Accession number GenBank NP_071900.2

Recombinant NSD1 amino acid sequence:

```
  1 GARVFPYMEG DVSSKDKMGK GVDGTYKKAL QEAAARFEEL KAQKELRQLQ
 51 EDRKNDKKPP PYKHIKVNRP IGRVQIFTAD LSEIPRCNCK ATDENPCGID
101 SECINRMLLY ECHPTVCPAG GRCQNQCFSK RQYPEVEIFR TLQRGWGLRT
151 KTDIKKGEFV NEYVGELIDE EECRARIRYA QEHDITNFYM LTLDKDRIID
201 AGPKGNYARF MNHCCQPNCE TQKWSVNGDT RVGLFALSDI KAGTELTFNY
251 NLECLGNGKT VCKCGAPNCS GFLGVRPKNQ PIATEEKSKK FKKKQQGKRR
301 TQGEITKERE DE
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Reviewed and approved by site quality representative.