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Certificate of Analysis

WHSC1 (NSD2)
Human Wolf-Hirschhorn syndrome candidate 1
(Recombinant enzyme expressed in *E.coli*)
Item # EPI073
Lot # 142157

Product Description: Recombinant human NSD2, Amino acids 825-1208, expressed in *E.coli*. Purified using immobilised metal affinity chromatography.
MW = 44.2kDa.

Tag cleaved by TEV protease.

Formulation: 0.765 mg/ml of enzyme in 25mM Tris/HCl pH8, 100mM NaCl, 2.5mM TCEP, 2.5 mM imidazole, 50% Glycerol. Frozen solution.

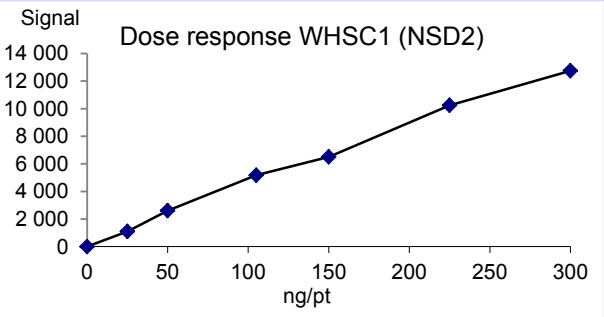
Storage and Stability: Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

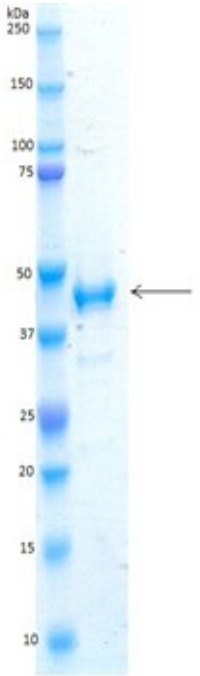
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

HMT Assay: 25-300ng of this lot of enzyme transferred methyl groups from [3H] SAM to core histone 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 WHSC1 (NSD2)

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WHSC1 (NSD2) Assay Protocol

Stock Solutions:

1. **Reaction buffer:** 50mM Tris/HCl pH8, 5mM $MgCl_2$, 50mM NaCl, 4mM DTT.
2. **WHSC1 (NSD2):** Dilute with reaction buffer. Use 25-300ng per assay point.
3. **Core Histone:** Dilute with reaction buffer to 7500nM.
4. **[3H] SAM:** Dilute with reaction buffer to 500nM.
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 **(25-300ng/pt) WHSC1 (NSD2)**.
4. Add 10µl of Core Histone.
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 WHSC1 (NSD2).

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WHSC1 (NSD2) Sequence Information

Protein Human WHSC1 (NSD2)

Tags *tag cleaved by TEV protease*

Accession number GenBank NP_579877.1

Recombinant WHSC1 (NSD2) amino acid sequence:

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  1 GHHAHVNVSW CFVCSKGGSL LCCESCPAAF HPDCLNIEMP DGSWFCNDCR
 51 AGKKLHFQDI IWVKLGNYRW WPAEVCHPKN VPPNIQKMKH EIGEFPVFFF
101 GSKDYYWTHQ ARVFPYMEGD RGSRYQGVRG IGRVFKNALQ EAEARFREIK
151 LQREARETQE SERKPPPYKH IKVNKPYGKV QIYTADISEI PKCNCKPTDE
201 NPCGFDSECL NRMLMFECHP QVCPAGEFCQ NQCFTKRQYP ETKIIKTDGK
251 GWGLVAKRDI RKGEFVNEYV GELIDEEECM ARIKHAHEND ITHFYMLTID
301 KDRIIDAGPK GNYSRFMNHS CQPNCETLKW TVNGDTRVGL FAVCDIPAGT
351 ELTFNYNLDC LGNEKTVCRC GASNCSGFLG DRPKT
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Reviewed and approved by site quality representative.