

Certificate of Analysis

SETD2

Human histone Lysine N-methyltransferase SETD2, active

(Recombinant enzyme expressed in *E.coli*)

Item # EPI055-K, EPI055

Lot # 212435

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal, 6His-tagged recombinant human SETD2, amino acids 843-1208, expressed in *E.coli*. Purified using immobilised metal affinity chromatography. Purity 94% by SDS-PAGE and Coomassie blue staining. MW= 44.1kDa.

Formulation: 0.21mg/ml of enzyme in 50mM Tris/HCl pH7.5, 500mM NaCl, 10% Glycerol. Frozen solution.

Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

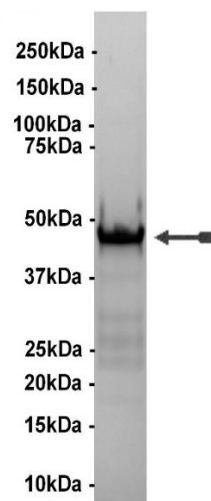
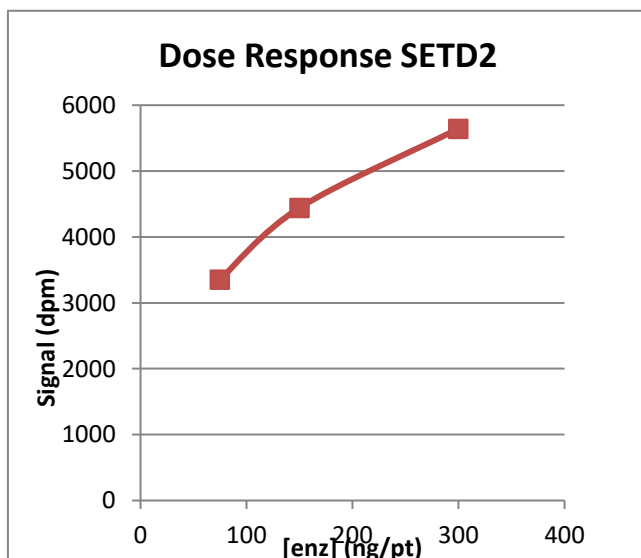
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

Quality Control Testing

HMT Assay: 75.0-300ng of this lot of enzyme transferred methyl groups from [3H] SAM to 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 SETD2.

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SETD2 Assay Protocol

Stock Solutions:

1. **Reaction buffer:** 50mM Tris/HCl pH9, 4mM DTT.
2. **SETD2, active:** Dilute with reaction buffer. Use 75.0-300ng per assay point.
3. **Nucleosome:** Dilute with reaction buffer to 2.5µg/ml.
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 wells.
2. Add 25µl of [3H] SAM.
3. Add 10µl **(75.0-300ng) SETD2, active.**
4. Add 10µl of Nucleosome.
5. Incubate for 10 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 SETD2.

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SETD2 Sequence Information

<u>Protein</u>	Human SETD2
<u>Tags</u>	N-Terminal 6His
<u>Accession number</u>	GenBank AAI17163.1

Recombinant SETD2 amino acid sequence:

1 MHHHHHSSG RENLYFQGSF FSDQSDKFL SLQKDKGSVQ APEISSNSIK
51 DTLAVNEKKD FSKNLEKNDI KDRGPLKKRR QEIESDSESD GELQDRKKVR
101 VEVEQGETSV PPGSALVGPS CVMDDFRDPQ RWKECAKQ GK MPCYFDLIEE
151 NVYLTERKKK KSHRDIKRMQ CECTPLSKDE RAQGEIACGE DCLNRLLMIE
201 CSSRCPNGDY CSNRRFQRKQ HADVEVILTE KKGWGLRAAK DLPSNTFVLE
251 YCGEVL DHKE FKARVKEYAR NKNIHYFMA LKNDEIIDAT QKGNC SRFMN
301 HSCEPNCETQ KWTVNGQLRV GFFTTKL VPS GSELTFDYQF QRYGKEAQKC
351 FCGSANC RGY LGGENRV SIR AAGGKMKKER SRK

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.