

Certificate of Analysis

SETD2

Human histone Lysine N-methyltransferase SETD2, active

(Recombinant enzyme expressed in *E.coli*)

Item # EPI055

Lot # 139693

Product Description: N-terminal, 6His-tagged recombinant human SETD2, amino acids 843-1208, expressed in *E.coli*. Purified using immobilised metal affinity chromatography. MW = 44.1kDa.

Formulation: 1mg/ml of enzyme in 5mM Tris/HCl pH7.5, 250mM NaCl, 50% Glycerol, 1.4mM DTT. Frozen solution.

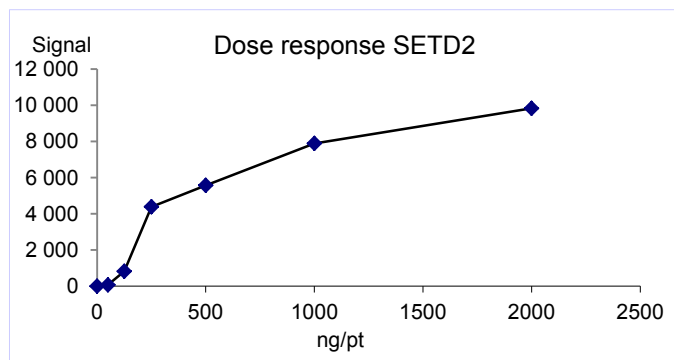
Storage and Stability: Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

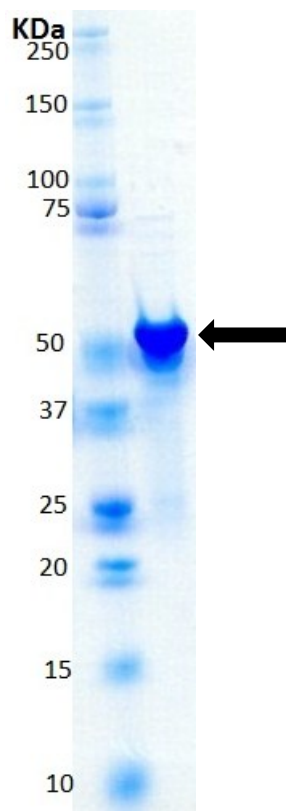
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

HMT Assay: 50-2000ng of this lot of enzyme transfer 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 50-2000ng 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 (**50-2000ng**) **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 FSDQSDKFLF SLQKDKGSVQ APEISSNSIK
51 DTLAVNEKKD FSKNLEKNDI KDRGPKKKRR QEIESDSESD GELQDRKKVR
101 VEVEQGETSV PPGSALVGPS CVMDDFRDPQ RWKECAKQGK MPCYFDLIEE
151 NVYLTERKKN KSHRDIKRMQ CECTPLSKDE RAQGEIACGE DCLNRLLMIE
201 CSSRCPNGDY CSNRRFQRKQ HADVEVILTE KKGWGLRAAK DLPSNTFVLE
251 YCGEVLDHKE FKARVKEYAR NKNIHYFFMA LKNDEIIDAT QKGNCSEFMN
301 HSCEPNCETQ KWTVNGQLRV GFFTTKLVPF GSELTFDYQF QRYGKEAQKC
351 FCGSANCRGY LGGENRVSIR AAGGKMKKER SRK

Reviewed and approved by site quality representative.

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