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Certificate of Analysis

SETD8
Human histone Lysine N-methyltransferase SETD8, active
(Recombinant enzyme expressed in *E.coli*)
Item # EPI057
Lot # 140882

Product Description: recombinant human SETD8, amino acids 186-352, expressed in *E.coli*. Purified using immobilised metal affinity chromatography. MW = 19.2kDa.

Tag cleaved by thrombin protease.

Formulation: 1mg/ml of enzyme in 25mM Tris/HCl pH7.5, 250mM NaCl, 50% Glycerol. Frozen solution.

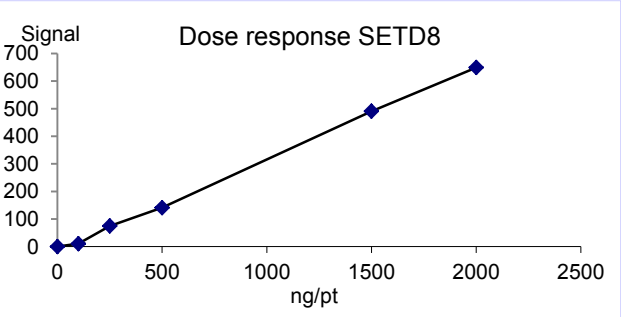
Storage and Stability: Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

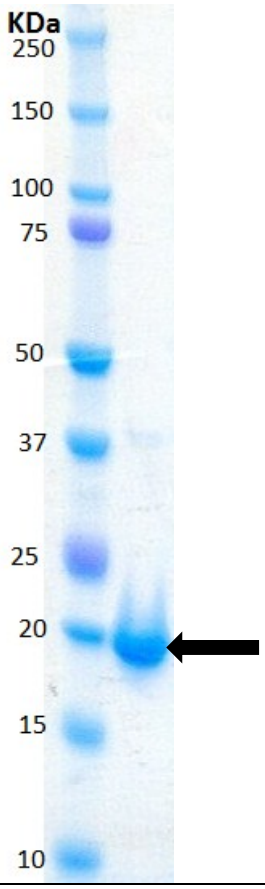
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

HMT Assay: 100-2000ng of this lot of enzyme transferred methyl groups from [3H] SAM to histone H4 full length 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 SETD8.

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SETD8 Assay Protocol

Stock Solutions:

1. **Reaction buffer:** 50mM Tris/HCl pH9, 5mM MgCl2, 50mM NaCl, 4mM DTT.
2. **SETD8, active:** Dilute with reaction buffer. Use 100-2000ng per assay point.
3. **Histone H4 full length:** Dilute with reaction buffer to 1250mM.
4. **[3H] SAM:** Dilute with SAM solution (1080mM) to 120nM.
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 **(100-2000ng) SETD8, active**.
4. Add 10µl of Histone H4 full length.
5. Incubate for 120 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 SETD8.

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SETD8 Sequence Information

Protein Human SETD8

Tags tag cleaved by thrombin protease

Accession number GenBank NP_065115.3

Recombinant SETD8 amino acid sequence:

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  1 GSPVRRSSRK SKAELQSEER KRIDELIESG KEEGMKIDLI DGKGRGVIAT
 51 KQFSRGDFVV EYHGDLIEIT DAKKREALYA QDPSTGCYMY YFQYLSKTYC
101 VDATRETNRL GRLINHSKCG NCQTKLHDID GVPHLILIAS RDIAAGEELL
151 YDYGDRSKAS IEAHPWLKH
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Reviewed and approved by site quality representative.