

## Certificate of Analysis

### SETD7

#### Human histone Lysine N-methyltransferase SETD7, active

(Recombinant enzyme expressed in *E.coli*)

Item # EPI056

Lot # 139699

**Product Description:** C-terminal, 6His-tagged recombinant human SETD7, amino acids 1-366, expressed in *E.coli*. Purified using immobilised metal affinity chromatography. MW = 41.5kDa.

**Formulation:** 1mg/ml of enzyme in 5mM Tris/HCl pH7.5, 250mM NaCl, 65% Glycerol, 0.6mM TCEP. Frozen solution.

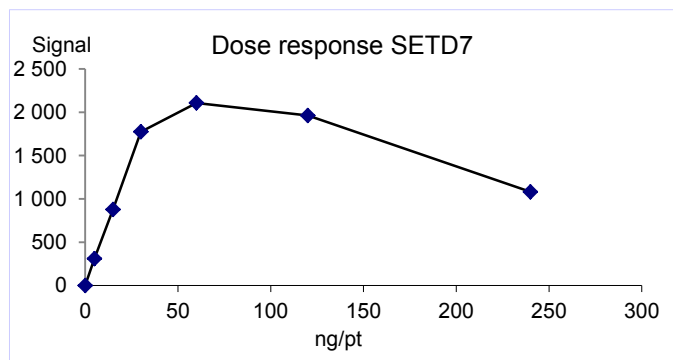
**Storage and Stability:** Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

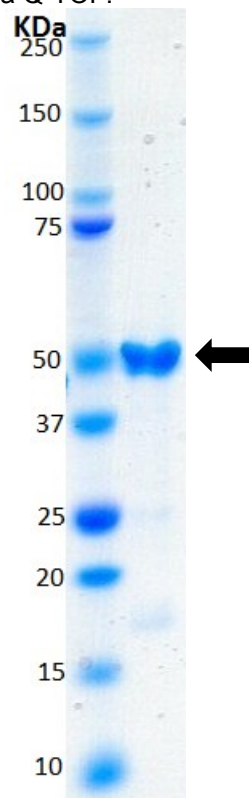
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**HMT Assay:** 5-240ng of this lot of enzyme transferred methyl groups from [3H] SAM to Histone H3 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 SETD7.

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### SETD7 Assay Protocol

#### Stock Solutions:

1. **Reaction buffer:** 50mM Tris/HCl pH9, 50mM NaCl, 5mM MgCl<sub>2</sub>, 4mM DTT.
2. **SETD7, active:** Dilute with reaction buffer. Use 5-240ng per assay point.
3. **H3 Histone:** Dilute with reaction buffer to 600nM.
4. **[3H] SAM:** Dilute with SAM solution (1530nM) to 170nM.
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 (**5-240ng**) **SETD7, active**.
4. Add 10µl of Histone H3.
5. Incubate for 30 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 SETD7.

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## SETD7 Sequence Information

<b><u>Protein</u></b>	Human SETD7
<b><u>Tags</u></b>	C-Terminal 6His
<b><u>Accession number</u></b>	GenBank NP_085151.1

***Recombinant SETD7 amino acid sequence:***

1 MDSDDDEVVEE AVEGHLDDDG LPHGFCTVTY SSTDRFEGNF VHGEKNGRGK  
51 FFFFDGSTLE GYYVDDALQG QGVVYTYEDGG VLQGTYYVDGE LNGPAQEYDT  
101 DGRLIFKGQY KDNIRHGVCW IYYPDGGSLV GEVNEDGEMT GEKIAYVYPD  
151 ERTALYGKFI DGEMIEGKLA TLMSTEEGRP HFELMPGNSV YHFDKSTSSC  
201 ISTNALLPDP YESERVYVAE SLISSAGEGL FSKVAVGPNT VMSFYNGVRI  
251 THQEVDSDRW ALNGNTLSLD EETVIDVPEP YNHVSKYCAS LGHKANHSFT  
301 PNCIYDMFVH PRFGPIKCIR TLRAVEADEE LTVAYGYDHS PPGKSGPEAP  
351 EWYQVELKAF QATQQKHHHH HH

Reviewed and approved by site quality representative.

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