

Discovery Services

Certificate of Analysis

EHMT1 Euchromatic histone-lysine N-methyltransferase 1, active (Recombinant enzyme expressed in *E.coli*) Item # EPI020 Lot # 139719

Product Description: recombinant human EHMT1, amino acids 951-1235, expressed in *E.coli*. Purified using immobilised metal affinity chromatography. MW = 32.9kDa.

Formulation: 1mg/ml of enzyme in 5mM Tris/HCl pH7.4, 250mM NaCl, 50% Glycérol. 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.

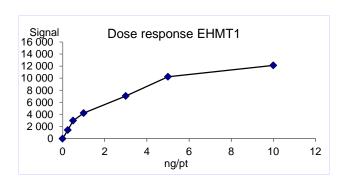
Tag cleaved y Thrombin

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.

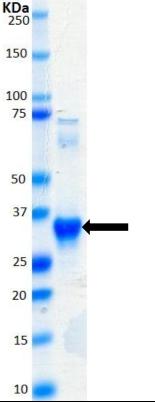
FOR IN VITRO RESEARCH USE ONLY NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

<u>HMT Assay</u>: 0.25–10ng 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.



<u>MS:</u> Size was confirmed by mass spectrometry using a Q-TOF.



<u>SDS-PAGE and Coomassie</u> <u>Stain</u>: Purity was assessed by SDS-PAGE and Coomassie blue staining using 4µg of active EHMT1.

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EHMT1 Assay Protocol

Stock Solutions:

- 1. Reaction buffer: 50mM Tris/HCl pH9, 50mM NaCl, 5mM MgCl2, 4mM DTT.
- 2. **EHMT1:** Dilute with reaction buffer. Use 0.25–10ng per assay point.
- 3. H3 Histone: Dilute with reaction buffer to 50nM.
- 4. [3H] SAM: Dilute with reaction buffer to 50nM.
- 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 (0.25-10ng) EHMT1.
- 4. Add 10µl of Histone H3.
- 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 EHMT1.



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EHMT1 Sequence Information

<u>Protein</u>	Human EHMT1		
<u>Tags</u>	Tag cleaved by Thrombin		

Accession number GenBank AAM09024.1

Recombinant EHMT1 amino acid sequence:

1	GSNSQVWSAL	QMSKALQDSA	PDRPSPVERI	VSRDIARGYE	RIPIPCVNAV
51	DSEPCPSNYK	YVSQNCVTSP	MNIDRNITHL	QYCVCIDDCS	SSNCMCGQLS
101	MRCWYDKDGR	LLPEFNMAEP	PLIFECNHAC	SCWRNCRNRV	VQNGLRARLQ
151	LYRTRDMGWG	VRSLQDIPPG	TFVCEYVGEL	ISDSEADVRE	EDSYLFDLDN
201	KDGEVYCIDA	RFYGNVSRFI	NHHCEPNLVP	VRVFMAHQDL	RFPRIAFFST
251	RLIEAGEQLG	FDYGERFWDI	KGKLFSCRCG	SPKCRHS	

Reviewed and approved by site quality representative.

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