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Certificate of Analysis

PRMT3
Human histone Lysine N-methyltransferase PRMT3, active
(Recombinant enzyme expressed in *E.coli*)
Item # EPI054-K,EPI054
Lot # 212438

Product Description: N-terminal, 6His-tagged recombinant human PRMT3, amino acids 211-531, expressed in *E.coli*. Purified using immobilised metal affinity chromatography. Purity 99% by SDS-PAGE and Coomassie blue staining.
MW = 38.2kDa.

Aliases : HRMT1L3

Formulation: 0.90mg/ml of enzyme in 50mM Tris/HCl pH7.4, 500mM NaCl, 10% glycerol. Frozen solution.

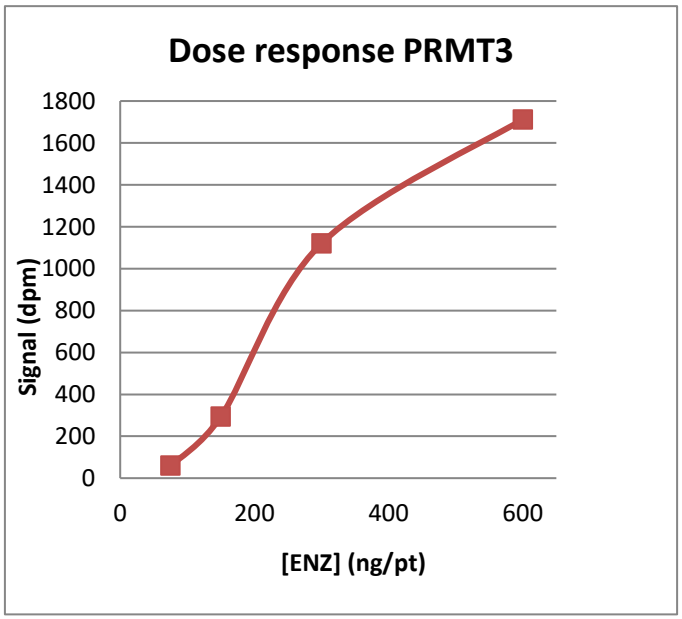
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

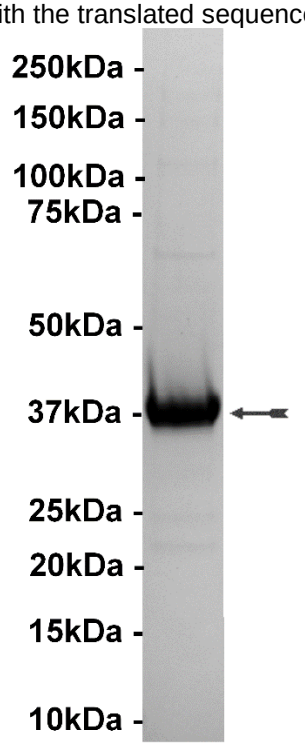
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

HMT Assay: 75.0-600ng of this lot of enzyme transferred methyl groups from [3H] SAM to Histone H4 in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic fingerprint: Confirmed identity as PRMT3 with the translated sequence listed on page two.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of PRMT3.

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PRMT3 Assay Protocol

Stock Solutions:

1. **Reaction buffer:** 50mM Tris/HCl pH9.0, 5mM MgCl2, 50mM NaCl, 4mM DTT.
2. **PRMT3, active:** Dilute with reaction buffer. Use 75.0-600ng per assay point.
3. **Histone H4:** Dilute with reaction buffer to 1250nM.
4. **[3H] SAM:** Dilute with a SAM solution (630nM) in reaction buffer to 70nM.
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 **(75.0-600ng) PRMT3, active**.
4. Add 10µl of Histone H4.
5. Incubate for 60 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 PRMT3.

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PRMT3 Sequence Information

Protein Human PRMT3

Tags N-Terminal 6His

Accession number GenBank NP_005779.1

Recombinant PRMT3 amino acid sequence:

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  1 MGSSHHHHHH SSGLVPRGSD LQEDEDGVYF SSYGHYGIHE EMLKDKIRTE
 51 SYRDFIYQNP HIFKDKVVLD VGCGTGILSM FAAKAGAKKV LGVDQSEILY
101 QAMDIIRLNK LEDTITLIKG KIEEVHLPVE KVDVIISEWM GYFLLFESML
151 DSVLYAKNKY LAKGGSVYPD ICTISLVAVS DVNKHADRIA FWDDVYGFKM
201 SCMKKAVIPE AVVEVLDPKT LISEPCGIKH IDCHTTSISD LEFSSDFTLK
251 ITRTSMCTAI AGYFDIYFEK NCHNRVVFST GPQSTKTHWK QTVFLLEKPF
301 SVKAGEALKG KVTVHKNKKD PRSLTVTLTL NNSTQTYGLQ
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Reviewed and approved by site quality representative.

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