Eurofins Discovery Products France

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Certificate of Analysis

PRMT1

Protein arginine N-methyltransferase 1 (Recombinant enzyme expressed in *E.coli*)

Item # EPI053 Lot # 140440

Product Description: N-terminal, 6Histagged recombinant human PRMT1, amino acids 30-371, expressed in *E.coli*. Purified using immobilised metal affinity chromatography. MW = 41.8kDa.

Aliases: HMT2, HRMT1L2, IR1B4

Formulation: 0.54mg/ml of enzyme in 25mM Tris/HCl pH8.0, 75mM NaCl, 50% Glycerol. Frozen solution.

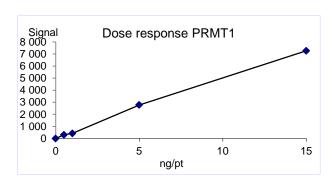
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

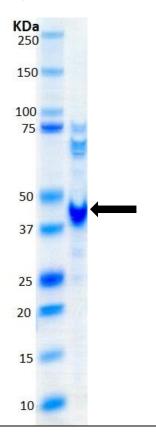
FOR IN VITRO RESEARCH USE ONLY NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

<u>HMT Assay</u>: 0.5-15ng of this lot of enzyme transfer methyl groups from [3H] SAM to Histone H4 full length human in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



 $\underline{\text{MS:}}$ 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 active PRMT1.

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PRMT1 Assay Protocol

Stock Solutions:

- Reaction buffer: 50mM Tris/HCl pH8.0, 50mM NaCl, 5mM MgCl2, 4mM DTT.
- 2. **PRMT1, active:** Dilute with reaction buffer. Use 0.5-15ng per assay point.
- 3. **Histone H4 full length human:** Dilute with reaction buffer to 125nM.
- 4. [3H] SAM: Dilute with reaction buffer 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 (0.5-15ng) PRMT1, active.
- 4. Add 10µl of Histone H4 full length human.
- 5. Incubate for 90 minutes at 22°C.
- Stop the reaction by adding 500μl of citric acid, then filtrate on a GF/B Filter. Wash 3 times with filtration buffer.
- 7. Dry and add scintillation cocktail.
- 8. Read in a scintillation counter. Compare signal of enzyme samples with that of a background that contain all assay components except enzyme PRMT1.

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PRMT1 Sequence Information

Protein Human PRMT1

<u>Tags</u> N-Terminal 6His

Accession number GenBank NP_001527.3

Recombinant PRMT1 amino acid sequence:

1 MHHHHHHSSG RENLYFQGME VSCGQAESSE KPNAEDMTSK DYYFDSYAHF

51 GIHEEMLKDE VRTLTYRNSM FHNRHLFKDK VVLDVGSGTG ILCMFAAKAG

101 ARKVIGIECS SISDYAVKIV KANKLDHVVT IIKGKVEEVE LPVEKVDIII

151 SEWMGYCLFY ESMLNTVLYA RDKWLAPDGL IFPDRATLYV TAIEDRQYKD

201 YKIHWWENVY GFDMSCIKDV AIKEPLVDVV DPKQLVTNAC LIKEVDIYTV

251 KVEDLTFTSP FCLQVKRNDY VHALVAYFNI EFTRCHKRTG FSTSPESPYT

301 HWKQTVFYME DYLTVKTGEE IFGTIGMRPN AKNNRDLDFT IDLDFKGQLC

351 ELSCSTDYRM R

Reviewed and approved by site quality representative.

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