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Certificate of Analysis

JMJD2E
Human jumonji domain containing 2E, active
(Recombinant enzyme expressed in *E.coli*)
Item # EPI033
Lot # 139704

Product Description: *N*-terminal 6His-tagged, recombinant, amino acids 1-336, human JMJD2E, expressed in *E.coli*. Purified using immobilised metal affinity chromatography. MW = 41.2kDa.

Aliases: KDM4E

Formulation: 1mg/ml of enzyme in 5mM Hepes/NaOH pH 7.4, 250mM NaCl, 50% glycerol. Frozen solution.

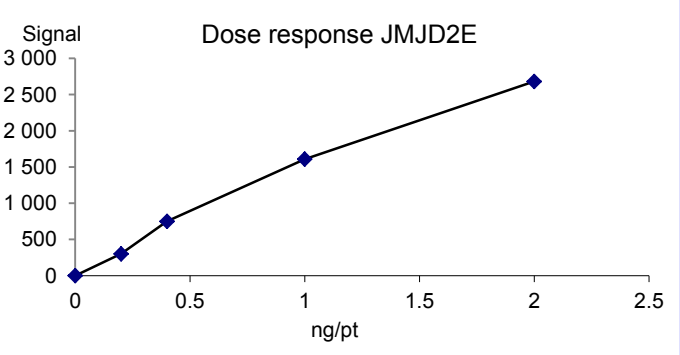
Storage and Stability: Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

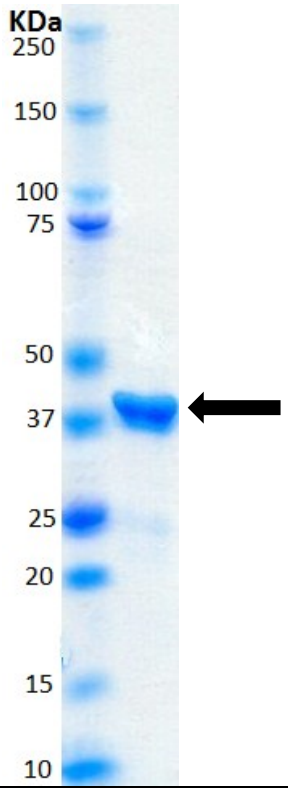
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Demethylase Assay: 0.2-2ng of this lot of protein bound 300nM biotin-H3K9me3 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 JMJD2E.

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Demethylase Assay Protocol

Stock Solutions:

1. **Reaction buffer:** 56mM Hepes pH7.0, 0.0125% Tween 20, 6.25µM FAS, 125µM Ascorbic acid, 3.75µM 2-Oxoglutarate, 0.0125% BSA.
2. **JMJD2E, active:** Dilute with reaction buffer. Use 0.2-2ng per assay point.
3. **Biotin-H3K9me3:** Dilute with reaction buffer to 600nM.
4. **STOP solution:** 4mM EDTA.
5. **Detection Mix:** Dilute Eu-anti-methyl histone H3K9me2 and Ulight™-Streptavidine in detection buffer to 4nM and 100nM respectively.

Assay Procedure (384 well white plate format):

1. Add 2µl of 5% DMSO per assay to wells.
2. Add 3µl **(0.2-2ng) JMJD2E, active**.
3. Add 5µl of Biotin-H3K9me3.
4. Incubate for 10 minutes at 22°C.
5. Add 5µl of STOP solution.
6. Incubate for 5 minutes at 22°C.
7. Add 5µl of Detection Mix.
8. Incubate for 60 minutes at 22°C
9. Excite at 320nm and read at 620/665nm. Calculate the HTRF ratio signal at 665nm / signal at 620nm x10000. Compare the signal of enzyme samples with that of a background sample that contains all assay components except the enzyme JMJD2E.

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JMJD2E Sequence Information

Protein Human JMJD2E

Tags N-Terminal 6His

Accession number GenBank NP_001155102

Recombinant JMJD2E amino acid sequence:

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  1 MHHHHHHSSG VDLGTENLYF QSMKSVHSSP QNTSHTIMTF YPTMEEFADF
 51 NTYVAYMESQ GAHQAGLAKV IPPKEWKARQ MYDDIEDILI ATPLQQVTSG
101 QGGVFTQYHK KKKAMRVGQY RRLANSKKYQ TPPHQNFADL EQRYWKSHPG
151 NPPIYGADIS GSLFEESTKQ WNLGHLGTIL DLLEQECGVV IEGVNTPYLY
201 FGMWKTTFAW HTEDMDLYSI NYLHFGEPKT WYVVPPEHGQ HLERLARELF
251 PDISRGCEAF LRHKVALISP TVLKENGIPF NCMTQEAGEF MVTFPYGYHA
301 GFNHGFNCAE AINFATPRWI DYGKMASQCS CGESTVTFSM DPFVRIVQPE
351 SYELWKHR
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Reviewed and approved by site quality representative.