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Certificate of Analysis

JMJD2A
Human jumonji domain containing 2A, active
(Recombinant enzyme expressed in *E.coli*)
Item # EPI029
Lot # 139698

Product Description: *N*-terminal 6His-tagged, recombinant, amino acids 1-359, human JMJD2A, expressed in *E.coli*. Purified using immobilised metal affinity chromatography. MW = 44.3kDa.

Aliases: JHDM3A, KDM4A, KIAA0677

Formulation: 1mg/ml of enzyme in 25mM Hepes/NaOH pH 7.4, 250mM NaCl, 50% glycerol. Frozen solution.

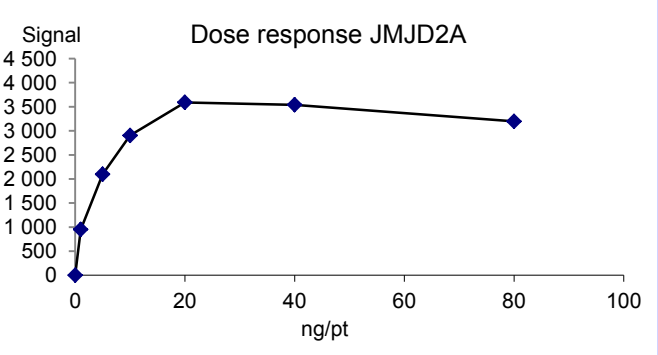
Storage and Stability: Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

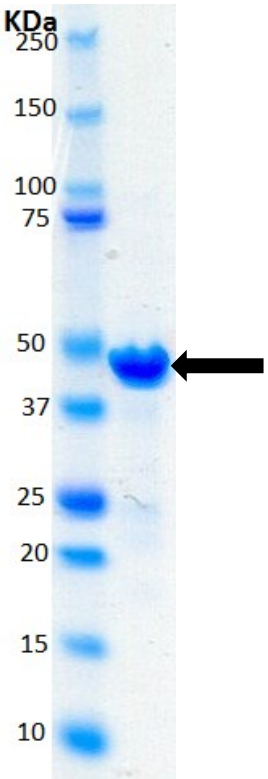
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Demethylase Assay: 1-80ng of this lot of protein bound 100nM 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 JMJD2A.

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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, 12.5µM 2-Oxoglutarate, 0.0125% BSA.
2. **JMJD2A, active:** Dilute with reaction buffer. Use 1-80ng per assay point.
3. **Biotin-H3K9me3:** Dilute with reaction buffer to 200nM.
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 **(1-80ng) JMJD2A, 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 JMJD2A.

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JMJD2A Sequence Information

Protein Human JMJD2A

Tags N-Terminal 6His

Accession number GenBank NP_055478.2

Recombinant JMJD2A amino acid sequence:

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  1 MHHHHHHSSG VDLGTENLYF QSMASESETL NPSARIMTFY PTMEEFRNFS
 51 RYIAYIESQG AHRAGLAKVV PPKEWKPRAS YDDIDDLVIP APIQQLVTGQ
101 SGLFTQYNIQ KKAMTVREFR KIANSDKYCT PRYSEFEELE RKYWKNLTFN
151 PPIYGADVNG TLYEKHVDEW NIGRLRTILD LVEKESGITI EGVNTPYLYF
201 GMWKTSFAWH TEDMDLYSIN YLHFGEPKSW YSVPPEHGKR LERLAKGFFP
251 GSAQSCEAFL RHKMTLISPL MLKKYGIPFD KVTQEAGEFM ITFPYGYHAG
301 FNHGFNCAES TNFATRRWIE YGKQAVLCSC RKDMVKISMD VFVRKFQPER
351 YKLWKAGKDN TVIDHTLPTP EAAEFLKESE L
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Reviewed and approved by site quality representative.

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