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Certificate of Analysis

JMJD2C
Human jumonji domain containing 2C
(Recombinant enzyme expressed in *E.coli*)
Item # EPI031
Lot # 140454

Product Description: recombinant human JMJD2C, amino acids 1-366, expressed in *E.coli*. Purified using immobilised metal affinity chromatography. MW = 42.6kDa.

Tag cleaved by TEV protease.

Aliases: bA146B14.1, FLJ25949, GASC1, JHDM3C, KDM4C, KIAA0780

Formulation: 0.8mg/ml of enzyme in 25mM Hepes/NaOH pH 7.5, 250mM NaCl, 50% glycerol. Frozen solution.

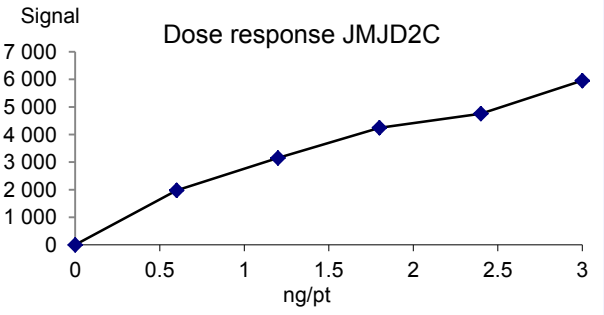
Storage and Stability: Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

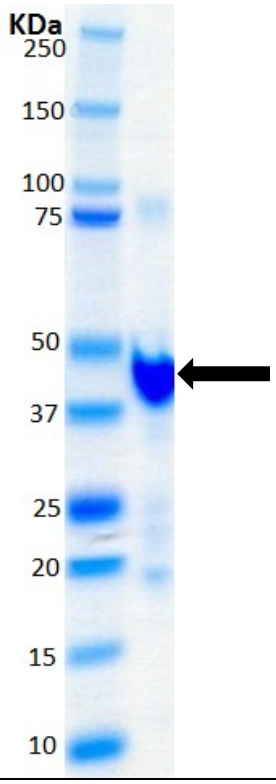
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Demethylase Assay: 0.6-3ng of this lot of protein bound with 150nM 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 JMJD2C.

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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. **JMJD2C:** Dilute with reaction buffer. Use 0.6-3ng per assay point.
3. **Biotin-H3K9me3:** Dilute with reaction buffer to 300nM.
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 **(0.6-3ng) JMJD2C**.
3. Add 5µl of Biotin-H3K9me3.
4. Incubate for 15 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 JMJD2C.

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JMJD2C Sequence Information

Protein Human JMJD2C

Tags *tag cleaved by TEV protease*

Accession number GenBank NP_055876.2

Recombinant JMJD2C amino acid sequence:

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  1 SMEVAEVESP LNPSCKIMTF RPSMEEFREF NKYLAYMESK GAHRAGLAKV
 51 IPPKEWKPRQ CYDDIDNLLI PAPIQQMVTG QSGLFTQYNI QKKAMTVKEF
101 RQLANSGKYC TPRYLDYEDL ERKYWKNLTF VAPIYGADIN GSIYDEGVDE
151 WNIARLNTVL DVVEEECGIS IEGVNTPYLY FGMWKTTFAW HTEDMDLYSI
201 NYLHFGEPKS WYAIPPEHGK RLERLAQGFF PSSSQGCDAF LRHKMTLISP
251 SVLKKYGIPF DKITQEAGEF MITFPYGYHA GFNHGFNCAE STNFATVRWI
301 DYGKVAKLCT CRKDMVKISM DIFVRKFQPD RYQLWKQGKD IYTIDHTKPT
351 PASTPEVKAW LQRRRKV
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Reviewed and approved by site quality representative.