

## Certificate of Analysis

### JMJD2D

#### Human jumonji domain containing 2D

(Recombinant enzyme expressed in *E.coli*)

Item # EPI032

Lot # 139701

**Product Description:** N-terminal 6His-tagged, recombinant, amino acids 1-358, human JMJD2D, expressed in *E.coli*. Purified using immobilised metal affinity chromatography. MW = 43.8kDa.

**Aliases:** KDM4D

**Formulation:** 1mg/ml of enzyme in 25mM Hepes/NaOH pH 7.5, 250mM NaCl, 50% glycerol. Frozen solution.

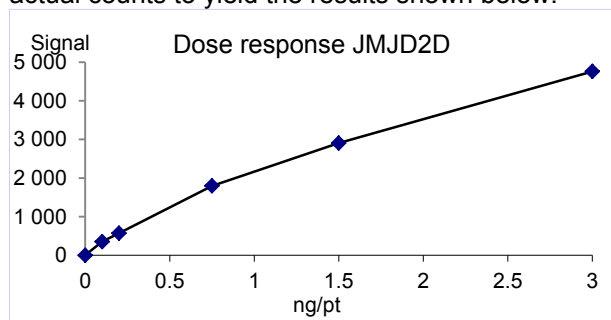
**Storage and Stability:** Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

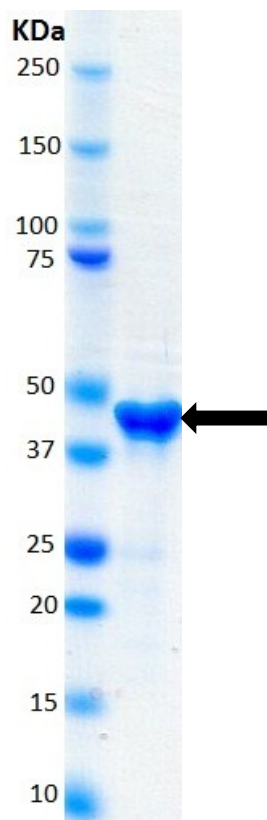
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Demethylase Assay:** 0.1-3ng 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 JMJD2D.

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### Demethylase Assay Protocol

#### Stock Solutions:

1. **Reaction buffer:** 56mM Hepes/Tris pH7.4, 0.0125% Tween 20, 6.25µM FAS, 125µM Ascorbic acid, 3.75µM 2-Oxoglutarate, 0.0125% BSA.
2. **JMJD2D:** Dilute with reaction buffer. Use 0.1-3ng 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 (**0.1-3ng**) **JMJD2D**.
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 JMJD2D.

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### JMJD2D Sequence Information

<b><u>Protein</u></b>	Human JMJD2D
<b><u>Tags</u></b>	N-Terminal 6His
<b><u>Accession number</u></b>	GenBank NP_060509.2

#### ***Recombinant JMJD2D amino acid sequence:***

1 MHHHHHSSG VDLGTENLYF QSMETMKSKA NCAQNPNONI MIFHPTKEEF  
51 NDFDKYIAYM ESQGAHRAGL AKIIPPKEWK ARETYDNISE ILIATPLQQV  
101 ASGRAGVFTQ YHKKKKAMTV GEYRHLANSK KYQTPPHQNF EDLERKYWKN  
151 RIYNSPIYGA DISGSLFDEN TKQWNLGHLG TIQDLLEKEC GVIEGVNTP  
201 YLYFGMWKTT FAWHTEDMDL YSINYLHLGE PKTWYVVPPE HGQRLERLAR  
251 ELFPGSSRGC GAFLRHKVAL ISPTVLKENG IPFNTRITQEA GEFMVTFPYG  
301 YHAGFNHGFN CAEAINFATP RWIDYGKMAS QCSCGEARVT FSMDAFVRIL  
351 QPERYDLWKR GQDRAVVDHM EPRVPASQEL

Reviewed and approved by site quality representative.

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