

Certificate of Analysis

JMJD3

Human jumonji domain containing 3

(Recombinant enzyme expressed in *E.coli*)

Item # EPI034

Lot # 139705

Product Description: recombinant, amino acids 1141-1641, human JMJD3, expressed in *E.coli*. Purified using immobilised metal affinity chromatography. MW = 57.2kDa.

Tag cleaved by TEV protease.

Aliases: KIAA0346, KDM6B

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

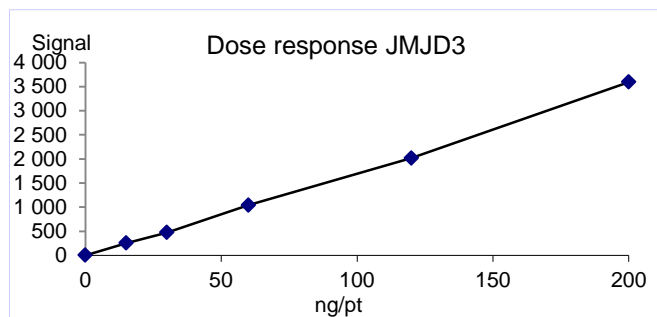
Storage and Stability: Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

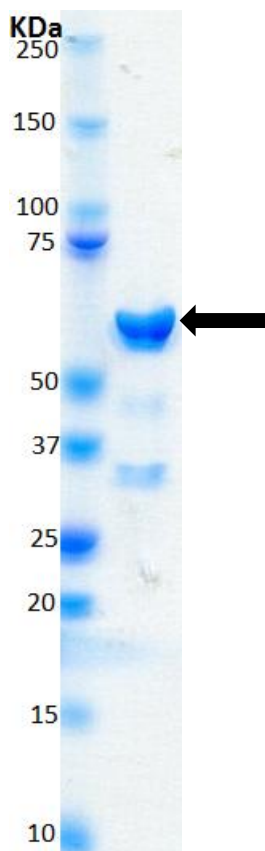
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Demethylase Assay: 15-200ng of this lot of protein bound 200nM biotin-H3K27me3 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 JMJD3.

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Demethylase Assay Protocol

Stock Solutions:

1. **Reaction buffer:** 20mM Tris/HCl pH7.5, 150mM NaCl, 0.01% Tween 20, 50µM FAS, 2mM Ascorbic acid, 5µM 2-Oxoglutarate.
2. **JMJD3:** Dilute with reaction buffer. Use 15-500ng per assay point.
3. **Biotin-H3K27me3:** Dilute with reaction buffer to 400nM.
4. **STOP solution:** 4mM EDTA.
5. **Detection Mix:** Dilute Eu-anti-methyl histone H3K27me2 and Ulight™-Streptavidine in detection buffer to 8nM and 200nM respectively.

Assay Procedure (384 well white plate format):

1. Add 2µl of 5% DMSO per assay to wells.
2. Add 3µl (**15-500ng**) **JMJD3**.
3. Add 5µl of Biotin-H3K27me3.
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 JMJD3.

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JMJD3 Sequence Information

Protein Human JMJD3

Tags Cut by TEV

Accession number GenBank NP_001073893.1

Recombinant JMJD3 amino acid sequence:

1 SMDVVRASRN AKVKGKFRES YLSPAQSVKP KINTEEKLPK EKLNPPTPSI
51 YLESKRDAFS PVLLQFCTDP RNPITVIRGL AGSLRLNLGL FSTKTLVEAS
101 GEHTVEVRTQ VQQPSDENWD LTGTRQIWPC ESSRSHTTIA KYAQYQASSF
151 QESLQEEKES EDEESEEPDS TTGTPPSSAP DPKNHIIKF GTNIDLSDAK
201 RWKPQLQELL KLPAFMRVTS TGNMLSHVGH TILGMNTVQL YMKVPGSRTP
251 GHQENNNFCS VNINIGPGDC EWFVHEHYW ETISAFCDRH GVDYLTGSWW
301 PILDDLASN IPVYRFVQRP GDLVWINAGT VHWVQATGWC NNIAWNVGGL
351 TAYQYQLALE RYEWNEVKNV KSIVPMIHVS WNVARTVKIS DPDLFKMIKF
401 CLLQSMKHCQ VQRESLVRAG KKIAYQGRVK DEPAYCNEC DVEVFNILFV
451 TSENGSRNTY LVHCEGCARR RSAGLQGVV LEQYRTEELA QAYDAFTLVR
501 ARR

Reviewed and approved by site quality representative.

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