

Certificate of Analysis

JMJD2B

Human jumonji domain containing 2B

(Recombinant enzyme expressed in *E.coli*)

Item # EPI030

Lot # 140428

Product Description: N-terminal 6His-tagged, recombinant, amino acids 1-363, human JMJD2B, expressed in *E.coli*. Purified using immobilised metal affinity chromatography. MW = 44.3kDa.

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

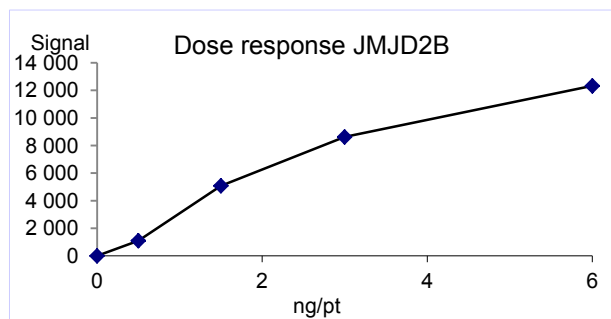
Storage and Stability: Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

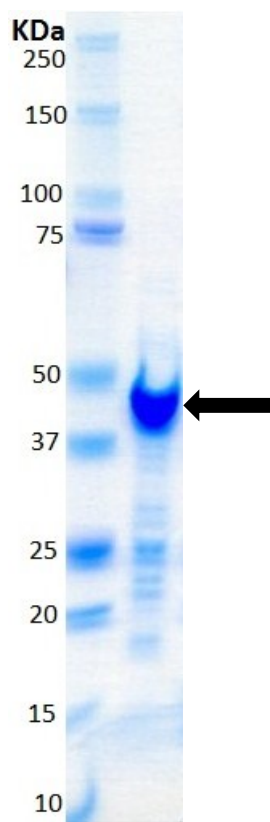
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Demethylase Assay: 0.5-6ng 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 JMJD2B.

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Demethylase Assay Protocol

Stock Solutions:

1. **Reaction buffer:** 56mM Hepes pH7.0, 0.0125% Tween 20, 6.25 μ M FAS, 125 μ M Ascorbic acid, 3.75 μ M 2-Oxoglutarate, 0.0125% BSA.
2. **JMJD2B:** Dilute with reaction buffer. Use 0.5-6ng 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 plate format):

1. Add 2 μ l of 5% DMSO per assay to wells.
2. Add 3 μ l (**0.5-6ng**) **JMJD2B**.
3. Add 5 μ l of Biotin-H3K9me3.
4. Incubate for 20 minutes at 22°C.
5. Add 5 μ l of STOP solution.
6. Incubate for 5 minutes at 22°C.
7. Add 5 μ l 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 JMJD2B.

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JMJD2B Sequence Information

Protein Human JMJD2B
Tags N-Terminal 6His
Accession number GenBank NP_055830.1

Recombinant JMJD2B amino acid sequence:

1 MHHHHHSSG VDLGTENLYF QSMGSEDHGA QNPSCKIMTF RPTMEEFKDF
51 NKYVAYIESQ GAHRAGLAKI IPPKEWKPRQ TYDDIDDVVI PAPIQQVVTG
101 QSGLFTQYNI QKKAMTVGEY RRLANSEKYC TPRHQDFDDL ERKYWKNLTF
151 VSPIYGADIS GSLYDDDVAQ WNIGSLRIL DMVERECGTI IEGVNTPLY
201 FGMWKTTFAW HTEDMDLYSI NYLHFGEPKS WYAIPEHGK RLERLAIGFF
251 PGSSQGDAF LRHKMTLISP IILKKGIPF SRITQEAGEF MITFPYGYHA
301 GFNHGFNCAE STNFATLRWI DYGKVATQCT CRKDMVKISM DVFVRILQPE
351 RYELWKQGKD LTVLDHTRPT ALTPELSSW SASRA

Reviewed and approved by site quality representative.

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