

## Certificate of Analysis

### hPHF8

#### Human histone lysine demethylase PHF8, active

(Recombinant enzyme expressed in *E.coli*)

Item # EPI049

Lot # 139696

**Product Description:** N-terminal 6His-tagged, recombinant, amino acids 79-447, human PHF8, expressed in *E.coli*. Purified using immobilised metal affinity chromatography. MW = 56.8kDa.

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

**Aliases:** DKFZp686E0868, JHDM1F, KIAA1111, MRXSSD, ZNF422

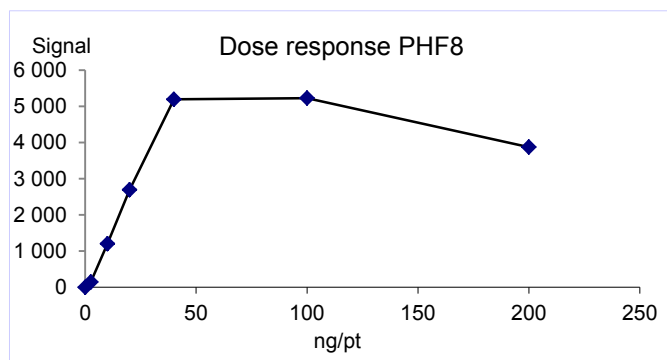
**Storage and Stability:** Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

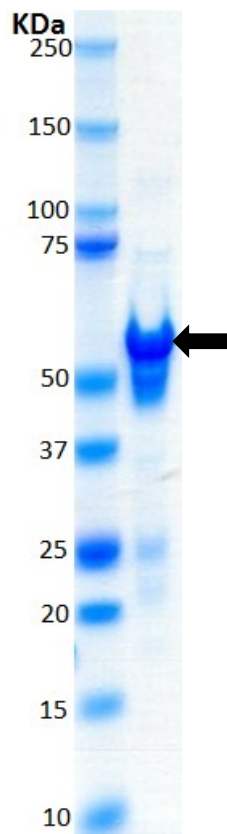
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Demethylase Assay:** 2.5-200ng of this lot of enzyme bound 15nM biotin-H3K9me1 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 PHF8.

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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, 3.8µM 2-Oxoglutarate, 0.0125% BSA.
2. **PHF8, active:** Dilute with reaction buffer. Use 2.5-200ng per assay point.
3. **Biotin-H3K9me1:** Dilute with reaction buffer to 30nM.
4. **STOP solution:** 4mM EDTA.
5. **Detection Mix:** Dilute Eu-anti-methyl histone H3K9/K27 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 each well.
2. Add 3µl (**2.5-200ng**) **PHF8 active**.
3. Add 5µl of Biotin-H3K9me1.
4. Incubate for 30 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 PHF8.

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## hPHF8 Sequence Information

<b><u>Protein</u></b>	Human PHF8
<b><u>Tags</u></b>	<i>N-Terminal 6His</i>
<b><u>Accession number</u></b>	<i>GenBank NP_055922.1</i>

**Recombinant PHF8 amino acid sequence:**

1 MHHHHHSSG MSDKIIHLTD DSFDTDVLKA DGAILVDFWA EWCGPCKMIA  
51 PILDEIADEY QGKLTVAKLN IDQNPGTAPK YGIRGIPTLL LFKNGEVAAT  
101 KVGALSKGQL KEFLDANLAG TENLYFQSMP VKTGSPTFVR ELRSRTFDSS  
151 DEVILKPTGN QLTVEFLEEN SFSVPILVLK KDGLGMTLPS PSFTVRDVEH  
201 YVGS DKEIDV IDVTRQADCK MKLGDFVKYY YSGKREKVLN VISLEFS DTR  
251 LSNLVETPKI VRKLSWVENL WPEECVFERP NVQKYCLMSV RDSYTFHID  
301 FGGTSVWYHV LKGEKIFYLI RPTNANLTLF ECWSSSSNQN EMFFGDQV DK  
351 CYKCSVKQGQ TLF IPTGWIH AVLTPVDCLA FGGN FLHSLN IEMQLKAYEI  
401 EKRLSTADLF RFPNFETICW YVGKHILDIF RGLRENRRHP ASYLVHGGKA  
451 LNLA FRAWTR KEALPDHEDE IPETVRTVQL IKDLAREIRL VEDIFQQN

Reviewed and approved by site quality representative.

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