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Certificate of Analysis

G9a
Euchromatic histone-lysine N-methyltransferase 2
(Recombinant enzyme expressed in *E.coli*)
Item # EPI022
Lot # 139715

Product Description: recombinant human G9a, amino acids 913-1193, expressed in *E.coli*. Purified using immobilised metal affinity chromatography.
MW = 32.6kDa.

Tag cleaved y Thrombin.

Aliases : BAT8, EHMT2, HLA-B, NG36

Formulation: 1mg/ml of enzyme in 5mM Tris/HCl pH7.5, 250mM NaCl, 50% glycerol. Frozen solution.

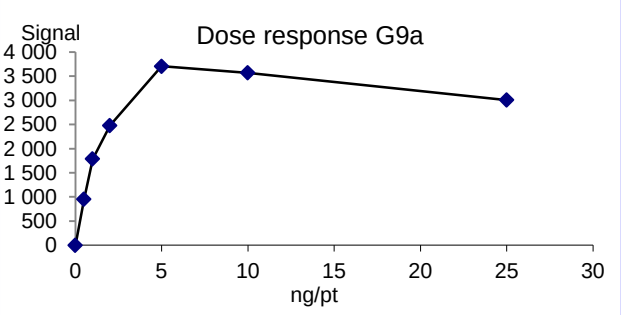
Storage and Stability: Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

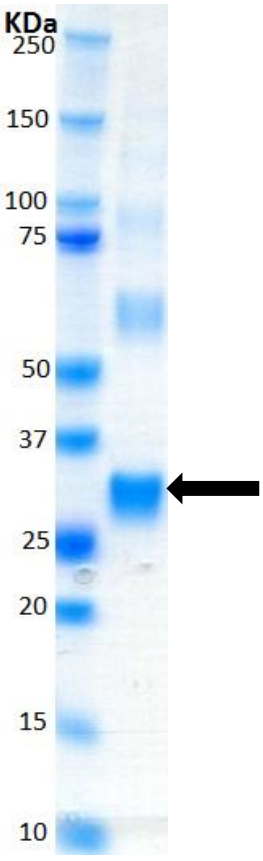
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

HMT Assay: 0.5-25ng of this lot of enzyme transferred methyl groups from [3H] SAM to H3 Histone 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 G9a.

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G9a Assay Protocol

Stock Solutions:

1. **Reaction buffer:** 50mM Tris/HCl pH9.0, 50mM NaCl, 5mM MgCl2, 4mM DTT.
2. **G9a:** Dilute with reaction buffer. Use 0.5-25ng per assay point.
3. **H3 Histone:** Dilute with reaction buffer to 25nM.
4. **[3H] SAM:** Dilute with reaction buffer to 50nM.
5. **Filtration Buffer :** 33mM Citric acid pH2.2

Assay Procedure (96 well plate format):

1. Add 5µl of 10% DMSO per assay to each well.
2. Add 25µl of [3H] SAM.
3. Add 10µl **(0.5-25ng) G9a**.
4. Add 10µl of H3 Histone.
5. Incubate for 120 minutes at 22°C.
6. Stop the reaction by adding 500µl of citric acid, then filter on a GF/B Filter. Wash 4 times with filtration buffer.
7. Dry and add scintillation cocktail.
8. Read in a scintillation counter. Compare the signal of enzyme samples with that of a background sample that contains all assay components except the enzyme G9a.

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G9a Sequence Information

Protein Human G9a

Tags *Tag cleaved by Thrombin*

Accession number GenBank NP_006700.2

Recombinant G9a amino acid sequence:

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  1 GSNRAIRTEK IICRDVARGY ENVPIPCVNG VDGEPCPEDY KYISENCETS
 51 TMNIDRNITH LQHCTCVDDC SSSNCLCGQL SIRCWYDKDG RLLQEFNKIE
101 PPLIFECNQA CSCWRNCKNR VVQSGIKVRL QLYRTAKMGW GVRALQTIPQ
151 GTFICEYVGE LISDAEADVR EDDSYLFDLD NKDGEVYCID ARYYGNISRF
201 INHLCDPNII PVRVFMLHQD LRFPRIAFFS SRDIRTGEEL GFDYGDRFWD
251 IKSKYFTCQC GSEKCKHSAE AIALEQSRLA RLD
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Reviewed and approved by site quality representative.