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Data Sheet

G9a Homogeneous Assay Kit

Catalog #52051

DESCRIPTION: The *G9a Homogeneous Assay Kit* is designed to measure G9a activity for screening and profiling applications. G9a is a histone methyltransferase that exhibits methylation activity toward H3-K9. The *G9a Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with biotinylated histone H3 peptide substrate, primary antibody, methylation assay buffer, and purified G9a for 384 enzyme reactions. The key to the *G9a Homogeneous Assay Kit* is a highly specific antibody that recognizes methylated substrate. With this kit, only three simple steps on a microtiter plate are required for methyltransferase detection. First, a sample containing G9a enzyme is incubated with the biotinylated substrate for two hours. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Catalog #	Components	Amount	Storage	Storage
51001	G9a enzyme	8 µg	-80°C	***(Avoid freeze/thaw cycles!)***
52120	100 µM S-adenosylmethionine	2 x 250 µl	-80°C	
52140E	Primary antibody 5	2 x 12.5 µl	-80°C	
	Biotinylated histone H3 peptide substrate (G9α)	10 µl	-80°C	
	4x G9a assay buffer 1A (add DTT before experiment)	3 ml	-20°C	
	4x Detection buffer 1	2 ml	-20°C	

MATERIALS REQUIRED BUT NOT SUPPLIED:
DTT (Dithiothreitol), 0.5M (Sigma, Cat. # D0632)
AlphaLISA anti-mIgG acceptor beads, 5 mg
AlphaLISA anti-mIgG acceptor beads, 5 mg/ml (PerkinElmer #AL105C)
AlphaScreen Streptavidin-conjugated donor beads, 5 mg/ml (PerkinElmer #6760002)
Optiplate -384 (PerkinElmer #6007290)
AlphaScreen microplate reader

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

CONTRAINDICATIONS: Green and blue dyes that absorb light in the AlphaScreen signal emission range (520-620 nm), such as Trypan Blue. Avoid the use of the potent singlet oxygen quenchers such as sodium azide (NaN_3) or metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} and Ni^{2+}). The presence of >1% RPMI 1640 culture medium leads to a signal reduction due to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen assays.

STABILITY: At least one year from date of receipt when stored as directed.

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REFERENCES: Dillon SC, *et al.* 2005. *Genome Biology* **6:**227.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Step 1:

1) Dilute **Biotinylated histone H3 peptide substrate** 40-fold with water. Dilute only the amount required for the assay. Discard any unused diluted **Biotinylated histone H3 peptide substrate** after use.

2) Add 125 µl 0.5M DTT (not provided) to 3-ml tube with **4x G9a Assay Buffer**. Prepare **1x G9A buffer** by adding 1 part of **4x G9A buffer** to 3 parts water (v/v).

3) Prepare the master mixture: N wells x (2 µl **4x G9A buffer** + 1 µl **S-adenosylmethionine** (100 µM) + 1 µl **Biotinylated substrate**). Add 4 µl to wells designated "Positive Control", "Test Sample", and "Blank". To wells labeled "Substrate Control", add 2 µl 4x G9A buffer + 1 µl Biotinylated substrate plus 1 µl water.

4) Add 3 µl of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 3 µl of the same solution without inhibitor (Inhibitor buffer).

5) Thaw **G9a** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **G9a** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **G9a** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme. Pre-incubation of enzyme with the inhibitor before starting the reaction may provide better results.*

6) Dilute **G9a** in **1x G9A assay buffer** at 0.3-0.9 ng/µl. Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

	Positive Control	Substrate Control	Test Sample	Blank
4x G9A assay buffer	2 µl	2 µl	2 µl	2 µl
100 µM S-adenosylmethionine	1 µl	–	1 µl	1 µl
Biotinylated substrate (diluted)	1 µl	1 µl	1 µl	1 µl
H_2O	1 µl	2 µl	1 µl	1 µl
Test Inhibitor/Activator	–	–	3 µl	–
Inhibitor Buffer (no inhibitor)	3 µl	3 µl		3 µl
G9a (0.3-0.9 ng/µl)	2 µl	2 µl	2 µl	–
1x G9A assay buffer	–	–	–	2 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

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7) To the wells designated as "Blank", add 2 µl of **1x G9A buffer**.

8) Initiate reaction by adding 2 µl of diluted **G9a** enzyme to the wells designated “Positive Control”, “Substrate Control”, and "Test Sample". Incubate for 2 hours at 30°C.

Protect your samples from direct exposure to light for steps 2 and 3!

Step 2:

1) Dilute anti-Mouse Acceptor beads (PerkinElmer #AL105C) 1:250-fold with **1x Detection buffer 1** (made by diluting **4x Detection buffer 1** 1:4 in distilled water). Add 5 µl per well. Shake plate briefly.

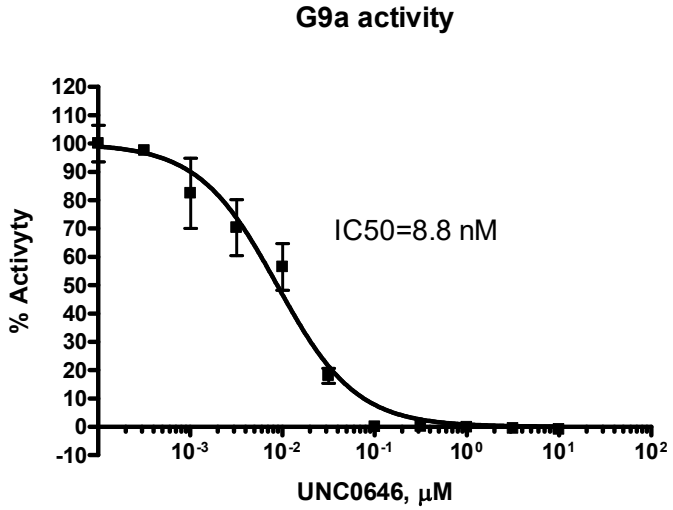
2) Dilute “**Primary antibody 5**” 100-fold with **1x Detection buffer 1**. Add 5 µl per well. Shake plate. Incubate 30 min at room temperature.
(Alternatively, dilute anti-Mouse Acceptor beads (1:500) and Primary antibody 5 (1:200) with 1x Detection buffer in one step. Add 10 µL of acceptor beads/antibody mixture per well.)

Step 3:

1) Dilute Streptavidin-conjugated donor beads (PE #6760002) 125-fold with **1x Detection buffer 1**. Add 10 µl per well. Incubate for 10-15 min. at room temperature.

2) Read Alpha-counts. The “Blank” value is subtracted from all other values.

Example of Assay Results:



G9a inhibition by UNC0646, measured using the G9a Homogeneous Assay Kit, BPS Bioscience Cat. #52051. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:	**Cat #:**	**Size:**
G9a Chemiluminescent Assay Kit	52001L	96 reactions
G9a recombinant protein (insect)	51001	20 µg
G9a recombinant protein (*E. coli*)	51000	50 µg
SUV39H1 recombinant protein	51070	50 µg
SUV39H1, full length recombinant protein	51071	5 µg
SUV39H2 recombinant protein	51080	50 µg
SUV39H1 Chemiluminescent Assay Kit	52006L	96 reactions
SUV39H2 Chemiluminescent Assay Kit	52008	96 reactions
Histone H3(K9) Universal Methyltransferase Assay Kit	52072	96 reactions