# GLP SAM-Screener™ Assay Kit

Item No. 600570

**Customer Service** 800.364.9897 * **Technical Support** 888.526.5351

**www.caymanchem.com**

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# GENERAL INFORMATION

## Materials Supplied

Kit will arrive packaged as a -80°C kit. For best results, remove components and store as stated below.

| Item Number | Item | 384 wells Quantity/ Size | 1,920 wells Quantity/ Size | Storage |
|---|---|---|---|---|
| 600491 | SAM-Binding Site Assay Buffer (10X) | 1 vial/2 ml | 1 vial/10 ml | -20°C |
| 600572 | GLP (human recombinant) Assay Enzyme | 1 vial/125 µl | 5 vials/125 µl | -80°C |
| 600493 | SAM-Binding Site Probe* | 1 vial | 5 vials | -20°C |
| 600494 | SAM-Binding Site Positive Control | 1 vial/40 µg | 5 vials/40 µg | -20°C |
| 400093 | 384-Well Solid Plate (low volume; black) | 1 plate | 5 plates | RT |
| 400023 | Foil Plate Covers | 1 cover | 5 covers | RT |

**United States Patent 9,120,820*

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

**WARNING:** THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

## Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

## Precautions

**Please read these instructions carefully before beginning this assay.**

The reagents in this kit have been tested and formulated to work exclusively with Cayman's SET7/9 SAM-Screener™ Assay Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

## If You Have Problems

**Technical Service Contact Information**

**Phone:** 888-526-5351 (USA and Canada only) or 734-975-3888
**Fax:** 734-971-3641
**Email:** techserv@caymanchem.com
**Hours:** M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## Storage and Stability

This kit will perform as specified in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

## Materials Needed But Not Supplied

1. A plate reader capable of fluorescence polarization measurements using sulforhodamine 101 as the fluorophore (see page 13 for details).
2. Adjustable pipettes and a multichannel pipettor.



# INTRODUCTION

## Background

The alteration of chromatin structure through the posttranslational modification of histones provides a key regulatory step for all processes that act upon DNA. Among the numerous covalent modifications, histone lysine methylation has a central role in the epigenetic regulation of the genome.[1] With the exception of Dot1, all histone lysine methyltransferases contain a conserved domain (SET) that utilizes S-adenosyl-L-methionine (SAM or AdoMet) as a co-factor to catalyze the methylation of the epsilon amino group of lysine.[1] G9a-like protein (GLP) is a SET domain-containing methyltransferase that specifically mono- and di-methylates histone H3 at lysine 9 (H3K9).[2,3] GLP and G9a share 80% sequence identity in their SET domains.[2] Together, GLP and G9a function as major euchromatic H3K9me1 and H3K9me2 histone methyltransferases and therefore multiple biological roles for these enzymes have been assigned.[4] In addition, GLP and G9a have been found to methylate several nonhistone substrates, including p53(K372).[4] Human recombinant GLP in this assay kit is an N-terminal truncation containing residues 1,004-1,298.

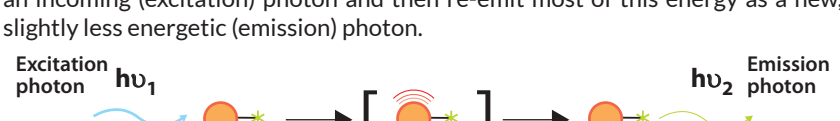
## About This Assay

This fluorescence polarization assay is based upon a proprietary small molecule fluorescent probe that binds to the SAM binding pocket in GLP. Binding of the small molecule probe to GLP induces an increase in fluorescence polarization. Binding of the probe can be competed with the endogenous cofactor SAM or by the inhibitor sinefungin, but is unaffected by the histone H3 peptide substrate. The GLP SAM-Screener™ Assay is robust (Z' >0.5) and exhibits a greater than 100 mP shift over a range of 0-250 nM GLP. The assay is suitable for high-throughput screening in the provided 384-well plate or can be scaled to higher density plate formats (*e.g.*, 1,536-well) if desired. The assay is stable at room temperature for several hours and in the presence of less than 2% DMSO.

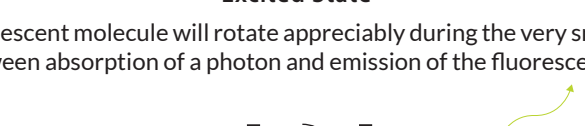
## Introduction to FP

Fluorescence polarization (FP) assays are homogeneous, single-step assays ideally suited for high-throughput screening (HTS) of large numbers of samples. All FP assays employ a large molecular species, or binding partner (BP) in conjunction with a small, low molecular weight fluorescent analyte (FA).

Fluorescence is by definition the ability of a molecule to absorb the energy of an incoming (excitation) photon and then re-emit most of this energy as a new, slightly less energetic (emission) photon.




A small fluorescent molecule will rotate appreciably during the very small interval of time between absorption of a photon and emission of the fluorescence photon.




If the excitation light is polarized, rotation of a small molecule freely tumbling in solution will result in complete randomization of the plane of the emitted light. Thus, small fluorescent molecules depolarize an excitation pulse of polarized light (see Figure 1, below).

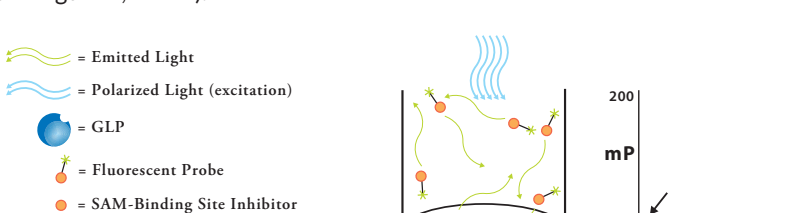



**Figure 1. Small fluorescent molecules rotate rapidly and depolarize the excitation light**

Large fluorescent molecules (MW >100,000) rotate much more slowly in solution than the small molecules discussed above. These molecules will therefore emit light that retains some of the polarization of the polarized excitation light. This polarization is determined by measuring the parallel and perpendicular fluorescence emitted from an assay sample. The polarization is quantified as milli-polarization units (mP) and can be calculated with the equation below.

$$FP = 1{,}000 \times \frac{(P - S \times G)}{(P + S \times G)}$$

Where FP: Fluorescence polarization, mP

P: Parallel fluorescence intensity

S: Perpendicular fluorescence intensity

G: An instrument specific correlation factor

When a small fluorescent molecule becomes tightly bound to a molecule of substantially larger mass, as in the binding of the SAM-Binding Site Probe to the GLP enzyme, the rotational speed of the small molecule is abruptly reduced to that of the entire complex (see Figure 2, below).

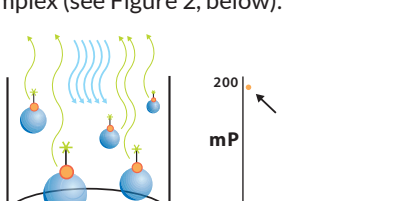



**Figure 2. Large fluorescent molecules rotate slowly and emit light that retains some of the polarization of the excitation light**

Therefore, when the SAM-Binding Site Probe is bound to GLP, the probe displays a dramatic increase in FP as compared to the free probe. Cayman's GLP SAM-Screener™ Assay is designed to characterize molecules that bind to the SAM binding site of GLP. Small molecules such as the positive control compound sinefungin compete directly with the SAM-Binding Site Probe for SAM binding on the GLP enzyme.

Displacement of the SAM-Binding Site Probe by a small molecule will cause a detectable loss of FP in the assay in a concentration-dependent manner (see Figure 3, below). Plotting mP *versus* competitor molecule allows for the calculation of $IC_{50}$ values.

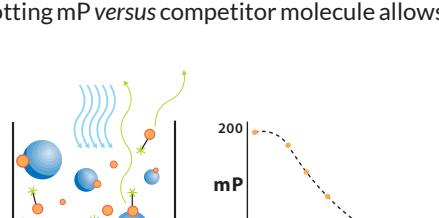



**Figure 3. Loss of fluorescent polarization due to displacement of the fluorescent SAM-Binding Site Probe by the competing small molecule**

# PRE-ASSAY PREPARATION

*NOTE: Water used to prepare all reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for FP. UltraPure water may be purchased from Cayman Chemical (Item No. 400000).*

## Buffer Preparation

**2 ml vial SAM-Binding Site Assay Buffer (10X) (384-well kit; Item No. 600491):** Add 18 ml of UltraPure water to the vial. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.* Store the diluted buffer at 4°C; it will be stable for approximately one month.

**OR**

**10 ml vial SAM-Binding Site Assay Buffer (10X) (1,920-well kit; Item No. 600491):** Dilute to a total volume of 100 ml with UltraPure water. Be certain to rinse the vial to remove any salts that may have precipitated. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.* Store the diluted buffer at 4°C; it will be stable for approximately one month.

## Sample Preparation

Samples may be prepared in organic solvents such as DMSO, DMF or short chain alcohols (*e.g.*, MeOH, EtOH), as long as the final concentration of organic solvents in the assay is <2%.



# ASSAY PROTOCOL

## Preparation of Assay-Specific Reagents

### GLP (human recombinant) Assay Enzyme

On ice, thaw one tube of GLP (human recombinant) Assay Enzyme (Item No. 600572) per 384-well plate and briefly centrifuge the tube before opening. For each 384-well plate, dilute 100 µl of GLP to a final volume of 4 ml in 1X SAM-Binding Site Assay Buffer. Mix gently (do not vortex) and keep on ice. Long-term storage of the diluted enzyme is not recommended.

### SAM-Binding Site Probe

For each 384-well plate, add 2 ml of 1X SAM-Binding Site Assay Buffer into a vial containing the SAM-Binding Site Probe (Item No. 600493) and vortex briefly. The probe is a dried film on the bottom of the container and may not be visible to the human eye. Keep the solution in the dark to prevent photobleaching. Unused solutions may be stored at -20°C for approximately two weeks.

### SAM-Binding Site Positive Control

For each 384-well plate, add 250 µl of 1X SAM-Binding Site Assay Buffer into a tube containing the SAM-Binding Site Positive Control (Item No. 600494) and vortex briefly. Unused solutions may be stored at -20°C for approximately two weeks.

## Performing the Assay

**Pipetting Hints**

- Use different tips to pipette each reagent.
- Do not expose the pipette tip to the reagent(s) already in the well.
- Avoid introducing bubbles into the wells.

*Follow the steps below to accurately measure mP in the assay. Allow all reagents except for the GLP enzyme to equilibrate to room temperature prior to performing the assay. Keep the GLP enzyme on ice until just prior to use. NOTE: Volumes indicated below are for a 384-well plate format with a 20 µl final assay volume. The customer may scale as needed for higher or lower density plate formats.*

### 1. Inhibitor Samples

Dilute inhibitor samples in 1X SAM-Binding Site Assay Buffer to a concentration that is 4X the desired final concentration (*e.g.*, if 1 µM is desired, prepare a 4 µM solution). This solution may contain up to 8% of an organic solvent (*e.g.*, DMSO). Add 5 µl of this dilution to the desired wells. For best results, perform the assay in duplicate.

It is recommended that inhibitor compounds be tested in a concentration-response format with at least eight independent concentrations that span approximately a 1,000-fold range around the expected $IC_{50}$ value of the inhibitor.

### 2. Positive and Negative Control Samples

For positive (inhibitor control) control wells, add 5 µl of the reconstituted SAM-Binding Site Positive Control to the desired wells.

For negative (no inhibition) control wells, add 5 µl of 1X SAM-Binding Site Assay Buffer to the desired wells. If inhibitor samples from Step 1 contain organic solvent, add an equivalent amount of the solvent into the assay in this step.

### 3. GLP Assay Enzyme

Add 10 µl of the diluted GLP Assay Enzyme to every well of the 384-well plate.

### 4. Pre-incubation (optional)

If desired, incubate the control and sample wells for 15 minutes at room temperature to allow pre-equilibration of the inhibitor and control compounds with the GLP enzyme.

### 5. SAM-Binding Site Probe

Add 5 µl of the reconstituted SAM-Binding Site Probe to every well.

### 6. Incubation of the Plate

Seal the plate with the provided adhesive aluminum seal and incubate at room temperature for 30 minutes. For automation purposes, the plate does not have to be sealed, but it must remain in the dark to prevent photobleaching.

### 7. Reading the Plate

Read the plate(s) with excitation and emission wavelengths of 575 nm and 620 nm, respectively. The plate reader used at Cayman Chemical also employs a dichroic filter at 595 nm. Some instruments may not utilize this type of filter. The measurements are taken with the G-factor set to 0.87, however this is instrument specific.

| Well Type | SAM-Binding Site Positive Control (µl) | 1X Assay Buffer (µl) | Test Sample (µl) | GLP (µl) | SAM-Binding Site Probe (µl) |
|---|---|---|---|---|---|
| Positive Control | 5 | - | - | 10 | 5 |
| Negative Control | - | 5* | - | 10 | 5 |
| Experimental Samples | - | - | 5 | 10 | 5 |

**Table 1. Pipetting summary**

*If an organic solvent is used at concentrations >2% in the test samples, include it in the negative control wells at the same concentration as the sample wells to control for solvent effects.

## Effects of Solvents

Samples may be prepared in organic solvents such as DMSO, DMF, or short chain alcohols (*e.g.*, MeOH, EtOH), as long as the final concentration of organic solvents in the assay is <2%. If conditions require different solvents or higher concentrations, additional assays may be required to assess solvent interference. Simply prepare a 2-fold serial dilution of the solvent in the 1X SAM-Binding Site Assay Buffer. Add reagents to the plate as described above and measure the polarization in the presence of the undiluted solvent and for each of the dilutions. As a control, measure polarization using only 1X SAM-Binding Site Assay Buffer with the SAM-Binding Site Probe.



# ANALYSIS

## Calculations

The sinefungin positive control wells represent the basal level of polarization for the SAM-Binding Site Probe. Average this value from all replicate wells and subtract it from the test inhibitor and negative control wells to generate baseline-corrected FP values.

A plot of baseline corrected mP versus inhibitor concentration on semi-log axes results in a sigmoidal dose-response curve typical of competitive binding assays. This data can be fit to a 4-parameter logistic equation.

## Performance Characteristics

### Z'-Factor:

Z'-factor is a term used to describe the robustness of an assay,[5] which is calculated using the equation below.

$$Z' = 1 - \frac{3\sigma_{c+} + 3\sigma_{c-}}{\left| \mu_{c+} - \mu_{c-} \right|}$$

Where c+: Positive control

c-: Negative control

The theoretical upper limit for the Z´-factor is 1.0. A robust assay has a Z´-factor >0.5. The Z´-factor for Cayman's GLP SAM-Screener™ Assay was determined to be 0.56.

### Sample Data

The data shown below and on page 18, are examples of the data typically produced with this kit; however, your results will not be identical to these. Do not use the data below to directly compare to your samples. Your results could differ substantially.

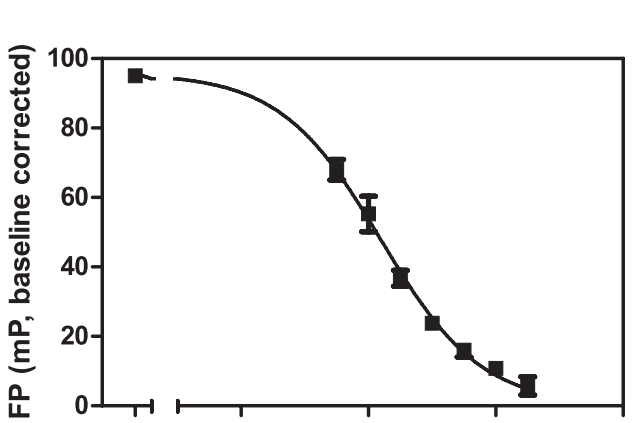



**Figure 4. Typical inhibition curves for the displacement of the SAM-Binding Site Probe from GLP by sinefungin.** Data are shown as the mean±SEM.

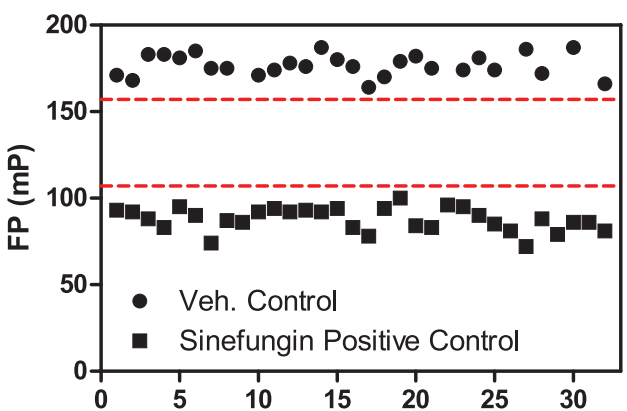



**Figure 5. Typical Z' data for the GLP SAM-Screener™ Assay.** Data are shown from 64 replicate wells of both positive and negative controls were prepared as described in the kit booklet. The calculated Z' from this experiment was 0.56. The red lines correspond to three standard deviations from the mean for each control value.



# RESOURCES

## Troubleshooting

| Problem | Possible Causes | Recommended Solutions |
|---|---|---|
| Erratic values; dispersion of duplicates | A. Bubble in the well<br>B. Poor pipetting/technique | A. Centrifuge the plate briefly |
| Little increase in mP in negative control wells | A. Fluorescent molecules in sample matrix<br>B. GLP enzyme handled improperly | A. Test sample matrix for interference before running samples in the assay<br>B. Keep the protein frozen at -80°C until ready to use; thaw protein and keep on ice until adding to assay |

## References

1. Bannister, A.J. and Kouzarides, T. Regulation of chromatin by histone modifications. *Cell Res.* **21**, 381-395 (2011).
2. Wu, H., Min, J., Lunin, V.V., *et al.* Structural biology of human H3K9 methyltransferases. *PLoS One* **5(1)**, e8570 (2010).
3. Patnaik, D., Chin, H.G., Estève, P.-O., *et al.* Substrate specificity and kinetic mechanism of mammalian G9a histone H3 methyltransferase. *J. Biol. Chem.* **279(51)**, 53248-58 (2004).
4. Shinkai, Y. and Tachibana, M. H3K9 methyltransferase G9a and the related molecule GLP. *Genes Dev.* **25**, 781-788 (2011).
5. Zhang, J.-H., Chung, T.D.Y., and Oldenburg, K.R. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J. Biomol. Screen.* **4(2)**, 67-73 (1999).

## NOTES

## Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.