

# Epigenase™ Type I PRMT Methyltransferase Activity/Inhibition Assay Kit (Colorimetric)

Base Catalog # P-3098

## PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

**Uses:** The Epigenase™ Type I PRMT Methyltransferase Activity/Inhibition Assay Kit (Colorimetric) is suitable for measuring activity or inhibition of total type I PRMT using nuclear extracts or purified enzymes such as PRMT1 to PRMT 4 from a broad range of species such as mammals, plants, fungi, and bacteria, in a variety of forms including cultured cells and fresh tissues. Nuclear extracts can be prepared by using your own successful method. For your convenience and the best results, EpiGentek offers a nuclear extraction kit (Cat. # OP-0002) optimized for use with this kit. Nuclear extracts can be used immediately or stored at  $-80^{\circ}\text{C}$  for future use. Purified enzymes can be active type I PRMTs from recombinant proteins or isolated from cells/tissues.

**Input Material:** Input materials can be nuclear extracts or purified type I enzymes such as PRMT1 to PRMT4. The amount of nuclear extracts for each assay can be  $1\ \mu\text{g}$  to  $20\ \mu\text{g}$  with an optimal range of  $5$  to  $10\ \mu\text{g}$ . The amount of purified enzymes can be  $10\ \text{ng}$  to  $500\ \text{ng}$ , depending on the purity and catalytic activity of the enzymes.

**Internal Control:** The PRMT assay standard (methylated histone H4-Arg 3) is provided in this kit for the quantification of type I PRMT enzyme activity. Because PRMT activity can vary from tissue to tissue and from normal and diseased states, it is advised to run replicate samples to ensure that the signal generated is validated.

**Precautions:** To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

## KIT CONTENTS

Component	48 Assays Cat. #P-3098-48	96 Assays Cat. #P-3098-96	Storage Upon Receipt
<b>WB</b> (10X Wash Buffer)	14 ml	28 ml	4°C
<b>PA1</b> (PRMT Assay Buffer)	4 ml	8 ml	4°C
<b>PA2</b> (PRMT Substrate, 50X)*	60 µl	120 µl	-20°C
<b>PA3</b> (Adomet, 50X)*	60 µl	120 µl	-20°C
<b>PAS</b> (PRMT Assay Standard, 20 µg/ml)*	10 µl	20 µl	-20°C
<b>PAC</b> (Capture Antibody, 1000X)*	5 µl	10 µl	4°C
<b>PAD</b> (Detection Antibody, 2000X)*	6 µl	12 µl	-20°C
<b>DS</b> (Developer Solution)	5 ml	10 ml	4°C
<b>SS</b> (Stop Solution)	5 ml	10 ml	RT
8-Well Assay Strips (With Frame)	6	12	4°C
Adhesive Covering Film	1	1	RT

\* Spin the solution down to the bottom prior to use.

## SHIPPING & STORAGE

The kit is shipped in three parts: the first part at ambient room temperature, and the second and third parts on frozen ice packs at 4°C. Upon receipt: (1) Store **PA2**, **PA3**, **PAS** and **PAD** at -20°C away from light; (2) Store **WB**, **PA1**, **PAC**, **DS**, and **8-Well Assay Strips** at 4°C away from light; and (3) Store remaining components (**SS** and **Adhesive Covering Film**) at room temperature away from light.

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

**Note:** (1) Check if **WB (10X Wash Buffer)** contains salt precipitates before use. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved; and (2) check if a blue color present in **DS (Developer Solution)**, which would indicate contamination of the solution and should not be used. To avoid contamination, transfer the amount of **DS** required into a secondary container (tube or vial) before adding **DS** into the assay wells.

## MATERIALS REQUIRED BUT NOT SUPPLIED

- Adjustable pipette or multiple-channel pipette
- Multiple-channel pipette reservoirs
- Aerosol resistant pipette tips
- Microplate reader capable of reading absorbance at 450 nm
- 1.5 ml microcentrifuge tubes
- Incubator for 37°C incubation

- Distilled water
- Nuclear extract or purified enzymes
- Parafilm M or aluminum foil

## GENERAL PRODUCT INFORMATION

**Quality Control:** Each lot of the Epigenase™ Type I PRMT Methyltransferase Activity/Inhibition Assay Kit (Colorimetric) is tested against predetermined specifications to ensure consistent product quality. EpiGentek guarantees the performance of all products in the manner described in our product instructions.

**Product Warranty:** If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

**Safety:** Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

**Product Updates:** EpiGentek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Thus, only use the User Guide that was supplied with the kit when using that kit.

**Usage Limitation:** The Epigenase™ Type I PRMT Methyltransferase Activity/Inhibition Assay Kit (Colorimetric) is for research use only and is not intended for diagnostic or therapeutic application.

**Intellectual Property:** The Epigenase™ Type I PRMT Methyltransferase Activity/Inhibition Assay Kit (Colorimetric) and methods of use contain proprietary technologies by EpiGentek.

## A BRIEF OVERVIEW

Arginine histone methylation is one of the many important epigenetic marks, and is essential for the regulation of multiple cellular processes. Arginine methylation of histones H3 (Arg2, 17, 26) and H4 (Arg3) promotes transcriptional activation and is mediated by a family of protein arginine methyltransferases (PRMTs). There are 9 types of PRMTs found in humans but only 7 members are reported to methylate histones. They can mediate mono or dimethylation of arginine residues. These enzymes use S-adenosyl-methionine (SAM) as a methyl donor and transfer it to the guanidinium side chain of arginine. Based on the position of methyl group addition, the PRMTs can be classified into type I (PRMT1, PRMT2, PRMT3, CARM1/PRMT4, PRMT6, and PRMT8) and type II (PRMT5 and PRMT9).

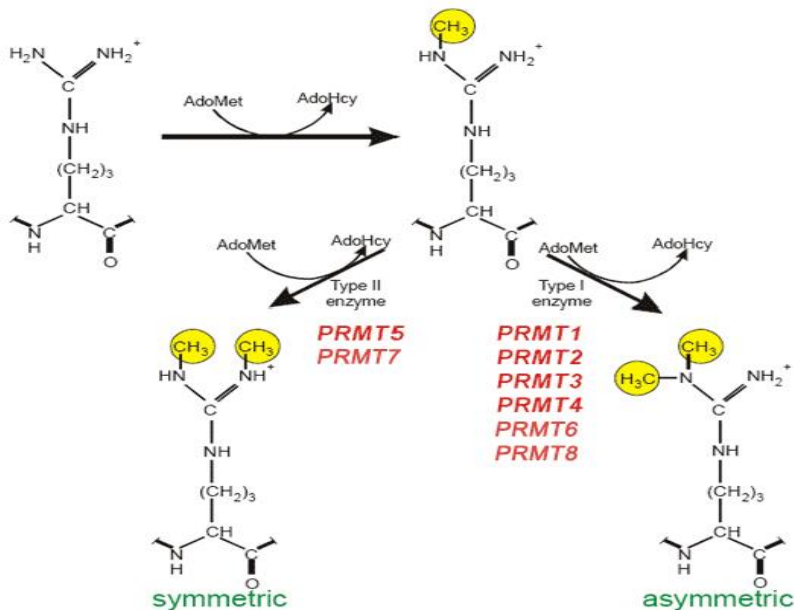


Fig. 1. Histone arginine methylation reaction catalyzed by PRMTs.

Type I PRMTs are found to be strongly implicated in diseases like cancer and immune disorders. For example, PRMT1 and PRMT4 were identified as an important oncoprotein that is frequently overexpressed in many tumors, including breast cancer, lung cancer, and colon cancer. PRMT1 was also recently found to methylate arginine in the SARS-CoV-2 N protein to promote viral replication. Detection of activity and inhibition of type I PRMTs would be important in elucidating mechanisms of epigenetic regulation of gene activation and silencing, benefiting cancer diagnostics and therapeutics as well as inhibiting viral life cycle including SARS-Cov-2 viral replication.

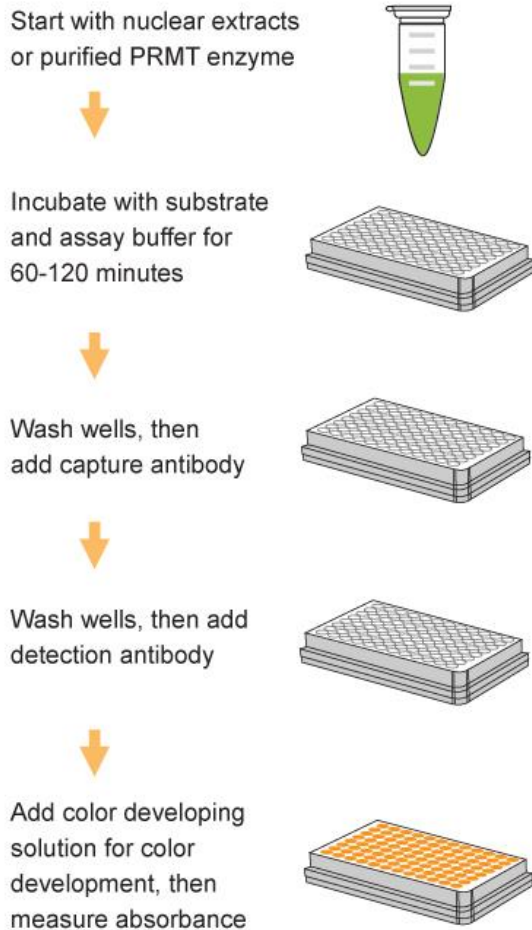
The Epigenase™ Type I PRMT Methyltransferase Activity/Inhibition Assay Kit (Colorimetric) is designed to detect type I PRMTs including PRMT1-4 and PRMT6 and PRMT8 activity and inhibition. This kit has the following advantages:

- 3-hour colorimetric procedure in a 96 stripwell microplate format allows for either manual or high throughput analysis.
- Directly measures type I PRMT activity via a straightforward detection of PRMT-converted methylated products.
- Both cell/tissue extracts and purified type I PRMT can be used, which allows for the detection of inhibitory effects of type I PRMT inhibitors *in vivo* and *in vitro*.
- Sensitive detection limit can be as low as 10 ng of purified PRMT6 enzyme.
- Methylated H4-Arg3 standard is included, allowing for specific activity of type I PRMT to be quantified.
- Accurate, reliable, and consistent with extremely low background signals.

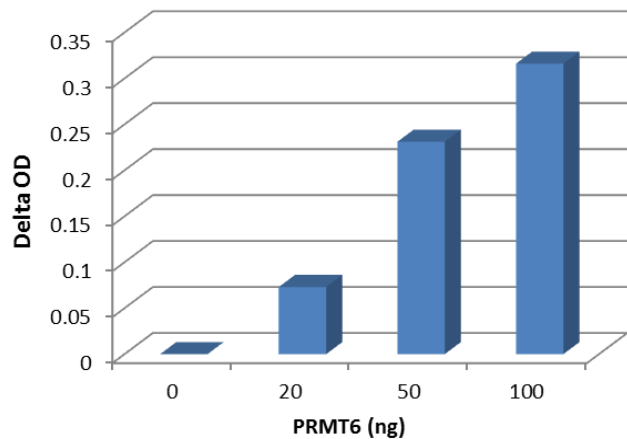
## PRINCIPLE & PROCEDURE

In this assay, a type I PRMT substrate is stably coated onto microplate wells. Active type 1 PRMTs such as PRMT1-4 or PRMT6 and PRMT 8 bind to the substrate and transfer a methyl group from Adomet to methylate the substrate. The methylated products can be recognized with a specific antibody. The ratio or amount of methylated products, which is proportional to enzyme activity, can then be colorimetrically measured by reading the absorbance in a microplate spectrophotometer at a

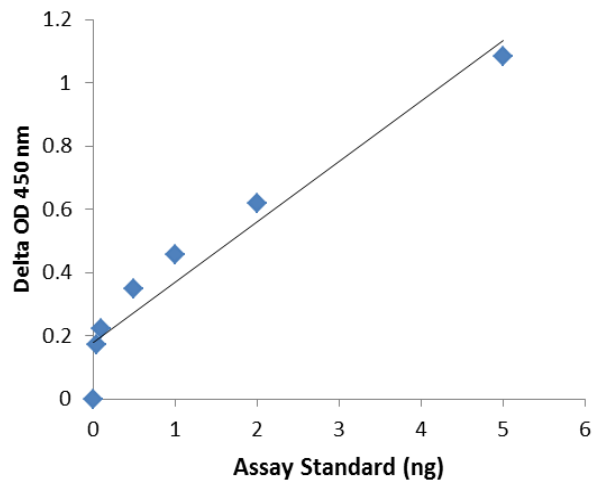
wavelength of 450 nm. The activity of the type I PRMT enzymes is, in turn, proportional to the optical density intensity measured.



Schematic procedure of the Epigenase Type I PRMT Methyltransferase Activity/Inhibition Assay Kit (Colorimetric)



Demonstration of sensitivity of the type I PRMT activity assay achieved by using recombinant PRMT1 with the Epigenase Type I PRMT Methyltransferase Activity/Inhibition Assay Kit (Colorimetric).



Illustrated standard curve generated with type I PRMT assay standard.

## PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

### Starting Materials

*Input Amount:* The amount of nuclear extracts for each assay can be between 1 µg and 20 µg with an optimal range of 5 to 10 µg. The amount of purified enzymes can be between 10 ng and 500 ng, depending on the purity and catalytic activity of the enzymes.

*Nuclear Extraction:* You can use your method of choice for preparing nuclear extracts. EpiGentek also offers a nuclear extraction kit (Cat # OP-0002) optimized for use with this kit.

Nuclear extracts or PRMT enzyme should be stored in aliquots at –80°C until use.

### 1. Working Buffer and Solution Preparation

- a. Prepare **Diluted WB (1X Wash Buffer)**:

48-Assay Kit: Add 13 ml of **WB (10X Wash Buffer)** to 117 ml of distilled water and adjust pH to 7.2-7.5.

96-Assay Kit: Add 26 ml of **WB (10X Wash Buffer)** to 234 ml of distilled water and adjust pH to 7.2-7.5.

This **Diluted WB (1X Wash Buffer)** can now be stored at 4°C for up to six months.

- b. Prepare **Diluted PAC (Capture Antibody)** solution:

Dilute **PAC (Capture Antibody)** with **Diluted WB (1X Wash Buffer)** at a ratio of 1:1000 (i.e., add 1 µl of **PAC** to 1000 µl of **Diluted WB**). 50 µl of **Diluted PAC** will be required for each assay well.

- c. Prepare **Diluted PAD (Detection Antibody)** Solution:

Dilute **PAD (Detection Antibody)** with **Diluted WB (1X Wash Buffer)** at a ratio of 1:2000 (i.e., add 1 µl of **PAD** to 2000 µl of **Diluted WB**). 50 µl of **Diluted PAD** will be required for each assay well.

- d. Prepare **Diluted PAS (PRMT Assay Standard)** solution:

Suggested Standard Curve Preparation: First, dilute **PAS (PRMT Assay Standard)** with **PA1 (PRMT Assay Buffer)** to 5 ng/µl by adding 3 µl of **PAS** to 9 µl of **PA1**. Then, further prepare five concentrations by combining the 5 ng/µl **Diluted PAS** with **PA1** into final concentrations of 0.05, 0.1, 0.5, 1, 2, 5 ng/µl according to the following dilution chart:

Tube	Diluted PAS (5 ng/µl)	PA1	Resulting PAS Concentration
1	0.5 µl	49.5 µl	0.05 ng/µl
2	1.0 µl	49.5 µl	0.1 ng/µl
3	1.0 µl	9.0 µl	0.5 ng/µl
4	1.0 µl	4.0 µl	1 ng/µl
5	2.0 µl	3.0 µl	2 ng/µl
6	4.0 µl	0.0 µl	5 ng/µl

**Note:** Keep each of the diluted solutions except **Diluted WB (1X Wash Buffer)** on ice until use. Any remaining diluted solutions other than **Diluted WB** should be discarded if not used within the same day.

## **2. Enzymatic Reaction**

- a. Predetermine the number of strip wells required for your experiment. It is advised to run replicate samples (including blank and positive controls) to ensure that the signal generated is validated. Carefully remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).
- b. **Blank Wells:** Add 49 µl of **PA1 (PRMT Assay Buffer)** and 1 µl of **PA3 (Adomet)** to each blank well.
- c. **Standard Wells:** Add 49 µl of **PA1 (PRMT Assay Buffer)** and 1 µl of **Diluted PAS (PRMT Assay Standard)** at a different concentration between 0.05 and 5 ng/µl in duplicate for each concentration (based on the dilution chart in Step 1d; see [Table 2](#) as an example).
- d. **Sample Wells Without Inhibitor:** Add 44 to 47 µl of **PA1 (PRMT Assay Buffer)**, 1 µl of **PA2 (PRMT Substrate)**, 1 µl of **PA3 (Adomet)**, 1 to 4 µl of your nuclear extract or 1 to 4 µl of purified PRMT enzyme. Total volume should be 50 µl per well.
- e. **Sample Wells With Inhibitor:** Add 39 to 42 µl of **PA1 (PRMT Assay Buffer)**, 1 µl of **PA2 (PRMT Substrate)**, 1 µl of **PA3 (Adomet)**, 1 to 4 µl of your nuclear extract or 1 to 4 µl of purified PRMT enzyme, and 5 µl of inhibitor solution. Total volume should be 50 µl/well.

**Note:** (1) Follow the suggested well setup diagram in [Table 2](#); (2) It is recommended to use 2 µg to 10 µg of nuclear extract per well or 20 ng to 100 ng of purified enzyme per well; (3) The concentration of inhibitor to be added into the sample wells can be varied (e.g., 1 µM to 1000 µM). However, the final concentration of the inhibitors before adding to the wells should be prepared with **PA1 (PRMT Assay Buffer)** at a 1:10 ratio (e.g., add 0.5 µl of inhibitor to 4.5 µl of **PA1**), so that the original solvent of the inhibitor can be reduced to 1% of the reaction solution or less.

Tightly cover strip-well microplate with **Adhesive Covering Film** to avoid evaporation and incubate at 37°C for 60 to 120 min.

**Note:** (1) The incubation time may depend on intrinsic PRMT activity. However, 60-90 min incubation is generally suitable for active purified PRMT enzymes, and 90-120 min incubation is required for nuclear extracts. (2) The **Adhesive Covering Film** can be cut to the required size to cover the strips based on the number of strips to be used.

- f. Remove the reaction solution from each well. Wash each well three times with 150 µl of the **Diluted WB (1X Wash Buffer)** each time.

## **3. Antibody Binding**

- a. Add 50 µl of the **Diluted PAC (Capture Antibody)** to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 60 min.
- b. Remove the **Diluted PAC (Capture Antibody)** solution from each well.
- c. Wash each well three times with 150 µl of the **Diluted WB (1X Wash Buffer)** each time.

- d. Add 50 µl of the **Diluted PAD (Detection Antibody)** to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 30 min.
- e. Remove the **Diluted PAD (Detection Antibody)** solution from each well.
- f. Wash each well four times with 150 µl of the **Diluted WB (1X Wash Buffer)** each time.

**Note:** Ensure any residual wash buffer in the wells is removed as much as possible at each wash step.

#### 4. Signal Detection

- a. Add 100 µl of **DS (Developer Solution)** to each well and incubate at room temperature for 1 to 10 min away from light. Begin monitoring color change in the sample wells and control wells. The **DS** solution will turn blue in the presence of sufficient methylated products.
- b. Add 100 µl of **SS (Stop Solution)** to each well to stop enzyme reaction when the color in the positive control wells turns medium blue. The color will change to yellow after adding **SS**, and the absorbance should be read on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

**Note:** (1) Most microplate readers have the capability to carry out dual wavelength analysis and will automatically subtract reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, the plate can be read twice, once at 450 nm and once at 655 nm. Then, manually subtract the 655 nm ODs from 450 nm ODs; (2) If the strip well microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.

#### 5. PRMT Activity Calculation

- a. Calculate the average duplicate readings for the sample wells and blank wells.
- b. Calculate PRMT activity or inhibition using the following formulas:

For simple calculation:

$$PRMT \text{ Activity (OD/min/mg)} = \frac{(\text{Sample OD} - \text{Blank OD})}{(\text{Protein Amount } (\mu\text{g}) * \text{min}^{**})} \times 1000$$

\* Protein amount (µg) added into the reaction at step 2d.

\*\* Incubation time (minutes) at step 2e.

Example calculation:

Average OD<sub>450</sub> of sample is 0.6

Average OD<sub>450</sub> of blank is 0.1

Protein amount is 5 µg

Incubation time is 120 minutes (2 hours)

$$PRMT \text{ activity} = \frac{(0.6 - 0.1)}{(5 \times 120)} \times 1000 = 0.83 \text{ OD/min/mg}$$



For accurate or specific activity calculation:

1. Generate a standard curve and plot OD value versus amount of **PAS (PRMT Assay Standard)** at each concentration point.
2. Determine the slope as OD/ng (you can use Microsoft Excel statistical functions for slope calculation), then calculate the amount of PRMT-converted methylated product using the following formulas:

$$\text{Methylated product (ng)} = \frac{(\text{Sample OD} - \text{Blank OD})}{\text{Slope}}$$

$$\text{PRMT Activity (ng/min/mg)} = \frac{\text{Methylated Product (ng)}}{(\text{Protein Amount } (\mu\text{g}) \times \text{min}^*)} \times 1000$$

\* Incubation time (minutes) at Step 2e.

For inhibition calculation:

$$\text{Inhibition \%} = \left[ 1 - \frac{\text{Inhibitor Sample OD} - \text{Blank OD}}{\text{No Inhibitor Sample OD} - \text{Blank OD}} \right] \times 100\%$$

## SUGGESTED BUFFER AND SOLUTION SETUP

**Table 1.** Approximate amount of required buffers and solutions for defined assay wells based on the protocol.

Reagents	1 well	1 strip (8 wells)	2 strips (16 wells)	6 strips (48 wells)	12 strips (96 wells)
Diluted WB	2.5 ml	20 ml	40 ml	120 ml	240 ml
PA1	50 $\mu$ l	400 $\mu$ l	800 $\mu$ l	2400 $\mu$ l	4800 $\mu$ l
PA2	1 $\mu$ l	8 $\mu$ l	16 $\mu$ l	50 $\mu$ l	120 $\mu$ l
PA3	1 $\mu$ l	8 $\mu$ l	16 $\mu$ l	50 $\mu$ l	120 $\mu$ l
PAS	N/A	N/A	4 $\mu$ L (optional)	8 $\mu$ l	8 $\mu$ l
Diluted PAC	50 $\mu$ l	400 $\mu$ l	800 $\mu$ l	2400 $\mu$ l	4800 $\mu$ l
Diluted PAD	50 $\mu$ l	400 $\mu$ l	800 $\mu$ l	2400 $\mu$ l	4800 $\mu$ l
Developer Solution	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml
Stop Solution	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml

## SUGGESTED STRIP WELL SETUP

**Table 2.** The suggested strip-well plate setup for PRMT activity assay in a 48-assay format (in a 96-assay format, Strips 7 to 12 can be configured as Sample). The controls and samples can be measured in duplicate.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	PAS 0.05 ng	PAS 0.05 ng	Sample	Sample	Sample	Sample
C	PAS 0.1 ng	PAS 0.1 ng	Sample	Sample	Sample	Sample
D	PAS 0.5 ng	PAS 0.5 ng	Sample	Sample	Sample	Sample
E	PAS 1 ng	PAS 1 ng	Sample	Sample	Sample	Sample
F	PAS 2 ng	PAS 2 ng	Sample	Sample	Sample	Sample
G	PAS 5 ng	PAS 5 ng	Sample	Sample	Sample	Sample
H	Sample	Sample	Sample	Sample	Sample	Sample

## TROUBLESHOOTING

Problem	Possible Cause	Suggestion
No signal or weak signal in both the positive control and sample wells	Reagents are added incorrectly.	Check if reagents are added in the proper order with the right amount, and if any steps in the protocol may have been omitted by mistake.
	The well is incorrectly washed before enzyme reaction.	Ensure the well is not washed prior to adding the positive control and sample.
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
	Incorrect absorbance reading.	Check if appropriate absorbance wavelength (450 nm) is used.
	Kit was not stored or handled properly.	Ensure all components of the kit were stored at the appropriate temperature and the cap is tightly closed after each opening or use.
No signal or weak signal in only the standard curve wells	The standard amount is insufficiently added to the well in Step 2c.	Ensure a sufficient amount of standard is added.

	The standard is degraded due to improper storage conditions.	Follow the Shipping & Storage guidance in this User Guide for storage of <b>PAS (PRMT Assay Standard)</b> .
High background present in the blank wells	Insufficient washing of wells.	Check if washing recommendations at each step is performed according to the protocol.
	Contaminated by sample or standard.	Ensure the well is not contaminated from adding sample or standard accidentally or from using contaminated tips.
	Incubation time with <b>Diluted PAD (Detection Antibody)</b> is too long.	The incubation time at Step 3d should not exceed 2 hours.
	Over-development of color.	Decrease the development time in Step 4a before adding <b>SS (Stop Solution)</b> in Step 4b.
No signal or weak signal only in sample wells	Protein sample is not properly extracted or purified.	Ensure your protocol is suitable for PRMT protein extraction. For the best results, it is advised to use EpiGenetek's Nuclear Extraction Kit (Cat. #OP-0002). Also, use fresh cells or tissues for protein extraction, as frozen cells or tissues could lose enzyme activity.
	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of purified enzymes or nuclear extracts is used as indicated in Step 2. The sample can be titrated to determine the optimal amount to use in the assay.
	Sample was not stored properly or has been stored for too long.	Ensure sample is stored in aliquots at – 80°C, with no more than 6 weeks for nuclear extracts and 6 months for purified enzymes. Avoid repeated freezing/thawing.
	Little or no activity of PRMT contained in the sample.	This problem may be a result of many factors. If the affecting factors cannot be determined, use new or re-prepared nuclear extracts or purified enzymes.
Uneven color development	Insufficient washing of the wells.	Ensure the wells are washed according to the guidance of washing and residual washing buffer is removed as much as possible.
	Delayed color development or delayed stopping of color development in the wells.	Ensure color <b>DS (Developer Solution)</b> or <b>SS (Stop Solution)</b> is added sequentially and is consistent with the order you added the other reagents (e.g., from well A to well H or from well 1 to well 12).

## RELATED PRODUCTS

### Nuclear Extract Preparation

OP-0002 EpiQuik™ Nuclear Extraction Kit

### Histone Demethylase Activity/Inhibition Assay

P-3078 Epigenase™ LSD1 Demethylase Activity/Inhibition Assay Kit (Colorimetric)  
P-3079 Epigenase™ LSD1 Demethylase Activity/Inhibition Assay Kit (Fluorometric)  
P-3081 Epigenase™ JMJD2 Demethylase Activity/Inhibition Assay Kit (Fluorometric)  
P-3082 Epigenase™ JARID Demethylase Activity/Inhibition Assay Kit (Colorimetric)  
P-3083 Epigenase™ JARID Demethylase Activity/Inhibition Assay Kit (Fluorometric)  
P-3088 Epigenase™ PRMT Methyltransferase (Type I-specific) Activity/Inhibition Assay Kit (Colorimetric)