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# Data Sheet

## *PRMT8 Homogeneous Assay Kit*

**Catalog #52058**
**Size: 384 reactions**

**DESCRIPTION:** The *PRMT8 Homogeneous Assay Kit* is designed to measure PRMT8 activity for screening and profiling applications. PRMT8 is a histone methyltransferase that exhibits methylation activity toward histones H2A and H4. The *PRMT8 Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with a 384-well plate, biotinylated histone H4 peptide substrate, primary antibody, methylation assay buffer, and purified PRMT8 for 384 enzyme reactions. The key to the *PRMT8 Homogeneous Assay Kit* is a highly specific antibody that recognizes methylated histone substrate. With this kit, only three simple steps on a microtiter plate are required for methyltransferase detection. First, a sample containing PRMT8 enzyme is incubated with the biotinylated substrate for one hour. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

**COMPONENTS:**

| Catalog # | Components | Amount | Storage | |
|---|---|---|---|---|
| 51052 | PRMT8 | 50 µg | -80°C | ***Avoid freeze/ thaw cycles!*** |
| 52120 | 100 µM S-adenosylmethionine | 250 µl | -80°C | |
| 52150-3 | Primary antibody 4-3 | 400 µl | -80°C | |
| | Biotinylated histone H4 peptide substrate | 400 µl | -80°C | |
| 52170-A | 4x HMT Assay Buffer 2A | 3 ml | -20°C | |
| 52301 | 4x Detection Buffer | 2 ml | -20°C | |

**MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:**
AlphaLISA® anti-rIgG acceptor beads, 5 mg/ml (Perkin Elmer #AL104C)
AlphaScreen® Streptavidin-conjugated donor beads, 5 mg/ml (Perkin Elmer # 6760002)
OptiPlate-384 (Perkin Elmer #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:** One year from date of receipt when stored as directed.

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**REFERENCE(S):** Yang, Y., Bedford, M.T. 2013. Nat Rev Cancer. 13(1):37-50.

**ASSAY PROTOCOL:**

All samples and controls should be tested in duplicate.

**Step 1:**

1) Prepare the master mixture: N wells x (2.5 µl **4x HMT Assay Buffer 2A** + 0.5 µl **S-adenosylmethionine** (100 µM) + 1.0 µl **Biotinylated substrate** + 0.5 µl water). Add 4.5 µl to wells designated "Positive Control", "Test Sample", and "Blank". To wells labeled "Substrate Control", add 2.5 µl **4x HMT Assay Buffer 2A** + 1.0 µl **Biotinylated substrate** + 1.0 µl water.

2) Add 2.5 µl of Inhibitor solution of each well labeled as "Test Inhibitor." For the "Positive Control," "Substrate Control," and "Blank," add 2.5 µl of 4% DMSO in Water (inhibitor buffer).

3) Prepare **1x HMT Buffer 2** by adding 1 part of **4x HMT Assay Buffer 2A** to 3 parts water (v/v).

4) Thaw **PRMT8** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **PRMT8** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -70°C. *Note: PRMT8 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme*.

5) Dilute **PRMT8** in **1X HMT Assay Buffer 2A** at 25-40 ng/µl. Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

| | Positive Control | Test Sample | Substrate Control | Blank |
|---|---|---|---|---|
| 4x HMT Assay Buffer 2A | 2.5 µl | 2.5 µl | 2.5 µl | 2.5 µl |
| 100 µM S-adenosylmethionine | 0.5 µl | 0.5 µl | – | 0.5 µl |
| Biotinylated substrate | 1 µl | 1 µl | 1 µl | 1 µl |
| $H_2O$ | 0.5 µl | 0.5 µl | 1 µl | 3.5 µl |
| Test Inhibitor/Activator | – | 2.5 µl | – | – |
| 4% DMSO in Water (inhibitor buffer) | 2.5 µl | – | 2.5 µl | 2.5 µl |
| PRMT8 (25-40 ng/µl) | 3 µl | 3 µl | 3 µl | – |
| **Total** | **10 µl** | **10 µl** | **10 µl** | **10 µl** |

6) To the wells designated as "Blank," add 3 µl of water.

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*7)* Initiate reaction by adding 3 µl of diluted **PRMT8** enzyme to the wells designated "Positive Control," "Substrate Control," and "Test Sample." Incubate at room temperature for 1 hour.

**Step 2:**

***Note: Protect your samples from direct exposure to light!***

1) Dilute anti-Rabbit Acceptor beads (Perkin Elmer #AL104C) 1:250-fold with **1x Detection Buffer**. Add 5 µl per well. Shake plate briefly.

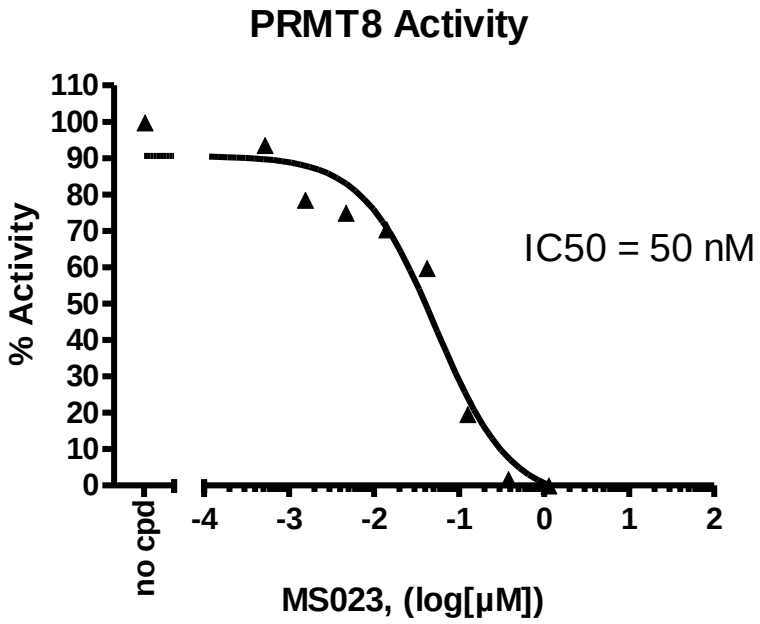
2) Dilute **Primary antibody 4** 10-fold with **1x Detection Buffer**. Add 5 µl per well. Shake plate. Incubate 30 min. at room temperature.

**Step 3:**

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

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

**Example of Assay Results:**




PRMT8 enzyme screening vs. S-Adenosyl-L-homocysteine, measured using the *PRMT8 Homogeneous Assay Kit*, BPS Bioscience Cat. #52058. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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**RELATED PRODUCTS:**

| | | |
|---|---|---|
| PRMT1 Homogeneous Assay Kit | #52054 | 384 reactions |
| PRMT3 Homogeneous Assay Kit | #52055 | 384 reactions |
| PRMT5 Homogeneous Assay Kit | #52052 | 384 reactions |
| PRMT6 Homogeneous Assay Kit | #52056 | 384 reactions |
| PRMT1 Chemiluminescent Assay Kit | #52004L | 96 reactions |
| PRMT3 Chemiluminescent Assay Kit | #52005L | 96 reactions |
| PRMT4 Chemiluminescent Assay Kit | #52041L | 96 reactions |
| PRMT5 Chemiluminescent Assay Kit | #52002L | 96 reactions |
| PRMT6 Chemiluminescent Assay Kit | #52046 | 96 reactions |
| PRMT1 recombinant protein (*E. coli*) | #51040 | 50 µg |
| PRMT1 recombinant protein (Sf9) | #51041 | 20 µg |
| PRMT3 recombinant protein | #51043 | 50 µg |
| PRMT4 (CARM 1) recombinant protein | #51047 | 20 µg |
| PRMT5 recombinant protein (HEK293) | #51045 | 20 µg |
| PRMT5/MEP50 recombinant protein (Sf9) | #51048 | 20 µg |
| PRMT6 recombinant protein | #51049 | 20 µg |
| PRMT7 recombinant protein | #51054 | 20 µg |
| PRMT8 recombinant protein | #51052 | 20 µg |
| PRMT9 recombinant protein | #51053 | 20 µg |

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