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Data Sheet
PRMT4 Homogeneous Assay Kit
Catalog #52068
Size: 384 reactions

DESCRIPTION: The *PRMT4 Homogeneous Assay Kit* is designed to measure PRMT4 activity for screening and profiling applications. PRMT4 (CARM1) is a dual functional coregulator that facilitates transcription initiation by methylation of Arg17 and Arg26 of histone H3. The *PRMT4 Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with His-tagged PRMT4 substrate, primary antibody, methylation assay buffer, and purified PRMT4 for 384 enzyme reactions. The key to the *PRMT4 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 PRMT4 enzyme is incubated with the substrate. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

| Catalog # | Component | Amount | Storage | |
|-----------|---|--------|---------|---|
| 51047 | PRMT4/CARM1* | 60 µg | -80°C | Avoid freeze/ thaw cycles! |
| 52120 | 100 µM S-adenosylmethionine | 250 µl | -80°C | |
| 52140Z3 | Primary antibody 28 | 20 µl | -80°C | |
| | PRMT4 substrate | 300 µl | -80°C | |
| | 4x PRMT4 assay buffer (add 30 ul of 500 mM DTT before experiment) | 3 ml | -20°C | |
| | 4x Detection buffer 3D | 2 ml | -20°C | |

*The concentration of PRMT4 is lot-specific and will be indicated on the tube containing the enzyme

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

AlphaLISA® anti-rIgG acceptor beads, 5 mg/ml (PerkinElmer #AL104C)
AlphaScreen Nickel donor beads, 5 mg/ml (PerkinElmer #AS101D)
Oптиplate-384 (PerkinElmer #6007290)

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 (NaN₃) or metal ions (Fe²⁺, Fe³⁺, Cu²⁺, Zn²⁺ and Ni²⁺). 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.

REFERENCE(S): Dillon SC, *et al.* 2005. *Genome Biology* 6:227.

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

- 1) Add 30 μ l of 500 mM DTT to the 3-ml tube of **4x PRMT4 assay buffer**. Prepare **1x PRMT4 buffer** by adding 1 part of **4x PRMT4 Assay buffer** to 3 parts water (v/v).
- 2) Prepare the master mixture: N wells x (2.0 μ l **4x PRMT4 Assay buffer** + 0.5 μ l **100 μ M S-adenosylmethionine** + 0.75 μ l **PRMT4 substrate** + 1.75 μ l H₂O). Add 5 μ l to wells designated "Positive Control", "Test Sample", and "Blank".
- 3) Add 3 μ l of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 3 μ l of the same solution without inhibitor (Inhibitor buffer).
- 4) Thaw **PRMT4** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **PRMT4** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: PRMT4 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme. Depending on the nature of inhibitor, pre-incubation with the enzyme may be necessary.*
- 5) Dilute **PRMT4** in **1X PRMT4 Assay buffer** at 75 ng/ μ l (150 ng/2 μ l). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

| | Positive Control | Test Sample | Blank |
|----------------------------------|-----------------------------|-----------------------------|-----------------------------|
| 4x PRMT4 Assay buffer | 2 μ l | 2 μ l | 2 μ l |
| 100 μ M S-adenosylmethionine | 0.5 μ l | 0.5 μ l | 0.5 μ l |
| PRMT4 substrate | 0.75 μ l | 0.75 μ l | 0.75 μ l |
| H ₂ O | 1.75 μ l | 1.75 μ l | 1.75 μ l |
| Test Inhibitor/Activator | - | 3 μ l | - |
| Inhibitor Buffer (no inhibitor) | 3 μ l | - | 3 μ l |
| PRMT4 (75 ng/ μ l) | 2 μ l | 2 μ l | - |
| 1x PRMT4 Assay buffer | - | - | 2 μ l |
| Total | 10 μl | 10 μl | 10 μl |

- 6) To the wells designated as "Blank", add 2 μ l of 1x PRMT4 Assay buffer.
- 7) Initiate reaction by adding 2 μ l of diluted **PRMT4** enzyme to the wells designated "Positive Control", "Substrate Control", and "Test Sample". Seal the wells with the protective film. Incubate overnight with slow shaking at room temperature.
Protect your samples from direct exposure to light for steps 2 and 3!

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Step 2:

- 1) Dilute anti-Rabbit Acceptor beads (PerkinElmer #AL104C) 1:250-fold with **1x Detection buffer 3D** (made by diluting **4x Detection buffer 3D** 1:4 in distilled water). Add 5 μ l per well. Shake plate briefly.
- 2) Dilute **Primary antibody 28** 100-fold with **1x Detection buffer 3D**. Add 5 μ l per well. Shake plate. Incubate 30 min at room temperature.
(Alternatively, dilute anti-Rabbit Acceptor beads (1:500) and Primary antibody 28 (1:200) with 1x Detection buffer in one step. Add 10 μ L of acceptor beads/antibody mixture per well.)

Step 3:

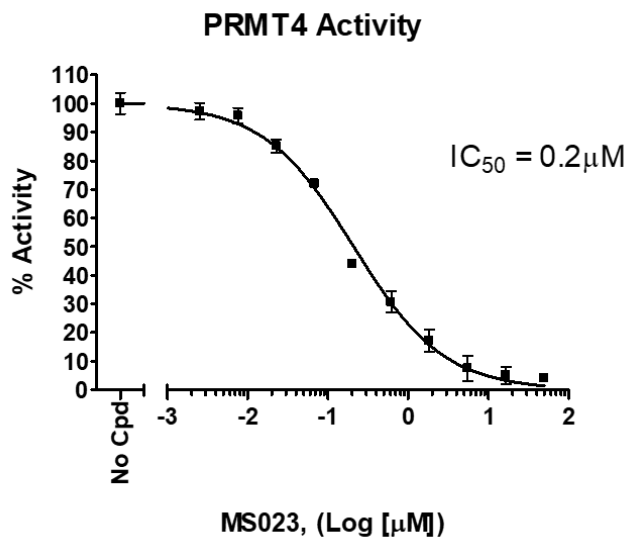
- 1) Dilute Nickel donor beads (PerkinElmer #AS101D) 125-fold with **1x Detection buffer 3D**. Add 10 μ l per well. Incubate for 60-90 min. at room temperature.
- 2) Read Alpha-counts.

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Examples of Assay Results:


PRMT4 enzyme activity, measured using the *PRMT4 Homogeneous Assay Kit*, BPS Bioscience Cat. #52068. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

RELATED PRODUCTS:

| | | |
|--|---------|---------------|
| PRMT1 Homogeneous Assay Kit | #52054 | 384 reactions |
| PRMT3 Homogeneous Assay Kit | #52055 | 384 reactions |
| PRMT6 Homogeneous Assay Kit | #52056 | 384 reactions |
| PRMT8 Homogeneous Assay Kit | #52058 | 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 |
| PRMT5 recombinant protein (HEK293) | #51045 | 20 μg |
| PRMT5/MEP50 recombinant protein (Sf9) | #51048 | 20 μg |
| PRMT1 recombinant protein (<i>E. coli</i>) | #51040 | 50 μg |
| PRMT1 recombinant protein (Sf9) | #51041 | 20 μg |
| PRMT3 recombinant protein | #51043 | 50 μg |
| PRMT4 (CARM 1) recombinant protein | #51047 | 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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