# Data Sheet

## *PRMT6 Direct Activity Assay Kit*

**Catalog #AMS.52046**

**Size: 96 reactions**

**DESCRIPTION:** The *PRMT6 Direct Activity Assay kit* is designed to measure PRMT6 activity for screening and profiling applications. The *PRMT6 Direct Activity Assay Kit* comes in a convenient format, with a 96-well plate precoated with histone H4 peptide substrate, the antibody against methylated arginine3 residue of Histone H4, the secondary HRP-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, and purified PRMT6 enzyme for 96 enzyme reactions. The key to the *PRMT6 Direct Activity Assay Kit* is a highly specific antibody that recognizes methylated R3 residue of Histone H4. With this kit, only three simple steps on a microtiter plate are required for methyltransferase detection. First, S-adenosylmethionine is incubated with a sample containing assay buffer and methyltransferase enzyme. Next, primary antibody is added. Finally, the plate is treated with an HRP-labeled secondary antibody followed by addition of the HRP substrate to produce chemiluminescence that can then be measured using a chemiluminescence reader.

**COMPONENTS:**

| Catalog # | Component | Amount | Storage | |
|---|---|---|---|---|
| AMS.51049 | PRMT6 human recombinant enzyme | 20 µg | -80°C | |
| AMS.52120 | 20 µM S-adenosylmethionine* | 250 µl | -80°C | |
| AMS.52150 | Primary antibody 4 | 100 µl | -80°C | |
| AMS.52131H | Secondary HRP-labeled antibody 2 | 10 µl | -80°C | |
| AMS.52192 | 4x HMT assay buffer 6** | 3 ml | -20°C | ***Avoid freeze/ thaw cycles!*** |
| AMS.52100 | Blocking buffer 4 | 50 ml | +4°C | |
| | HRP chemiluminescent substrate A (transparent bottle) | 6 ml | +4°C | |
| | HRP chemiluminescent substrate B (brown bottle) | 6 ml | +4°C | |
| | 96-well plate precoated with histone substrate | 1 plate | +4°C | |

**Decreasing S-adenosylmethionine concentration will make the assay more sensitive to the inhibitors.*

***Add 31 µl of 0.5 M DTT before use.*

**MATERIALS REQUIRED BUT NOT SUPPLIED:**

TBST buffer (1x TBS, pH 8.0, containing 0.05% Tween20)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips
Rotating or rocker platform

**APPLICATIONS:** Great for studying enzyme kinetics and HTS applications.

**CONTRAINDICATIONS:** DMSO >1%, strong acids or bases, ionic detergents, high salt.

**STABILITY:** One year from date of receipt when stored as directed.

**REFERENCE:** 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) Rehydrate the microwells by adding 150 µl of TBST buffer (1x TBS, pH 8.0, containing 0.05% Tween-20) to every well. Incubate 15 minutes at room temperature. Tap the plate onto clean paper towels to remove liquid.

2) Thaw **S-adenosylmethionine** on ice. Upon first thaw, briefly spin tube containing **S-adenosylmethionine** to recover full contents of the tube. Aliquot **S-adenosylmethionine** into single use aliquots and store at -80˚C. Note: **S-adenosylmethionine** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.

3) Add 31 µl of 0.5 M DTT before use. Prepare the master mixture: N wells × (7.5 µl **4x HMT assay buffer 6** + 2.5 µl 20 µM **S-adenosylmethionine** + 15 µl water). Add 25 µl of master mixture to all wells labeled “Positive Control”, “Test Sample” and “Blank”. For wells labeled “Substrate control”, add 7.5 µl **4x HMT assay buffer 6** + 17.5 µl water.

| | **Blank** | **Substrate Control** | **Positive Control** | **Test Sample** |
|---|---|---|---|---|
| 4x HMT assay buffer 6 | 7.5 µl | 7.5 µl | 7.5 µl | 7.5 µl |
| 20 µM S-adenosylmethionine | 2.5 µl | – | 2.5 µl | 2.5 µl |
| $H_2O$ | 15 µl | 17.5 µl | 15 µl | 15 µl |
| Test Inhibitor | – | – | – | 5 µl |
| Inhibitor buffer (no inhibitor) | 5 µl | 5 µl | 5 µl | |
| 1x HMT assay buffer 6 | 20 µl | – | – | – |
| Diluted PRMT6 (5-10 ng/µl) | | 20 µl | 20 µl | 20 µl |
| **Total** | **50 µl** | **50 µl** | **50 µl** | **50 µl** |

4) Add 5 µl of inhibitor solution of each well designated “Test Inhibitor”.

5) For the “Positive Control", “Substrate Control” and “Blank”, add 5 µl of the same solution without inhibitor (inhibitor buffer).

6) Thaw **PRMT6 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **PRMT6 enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: **PRMT6 enzyme** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

7) Dilute **PRMT6 enzyme** in **1x HMT assay buffer 6** at 5-10 ng/µl (100-200 ng/20 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use. *Note: Diluted enzyme may not be stable. Dilute the enzyme immediately before use.*

8) Add 20 µl of **1x HMT assay buffer 6** to the wells designated "Blank".

9) Initiate reaction by adding 20 µl of diluted **PRMT6 enzyme** to the wells designated “Positive Control”, "Substrate Control", and "Test Sample ". Incubate at room temperature for 1 hour.

10) Remove the supernatant from the wells and wash the strip three times with 200 µl TBST buffer. Blot dry onto clean paper towels.

11) Add 100 µl of **Blocking buffer 4** to every well. Shake on a rotating platform for 10 min. Remove supernatant as above.

**Step 2:**

1) Dilute “**Primary antibody 4**” 100-fold with **Blocking buffer 4**.

2) Add 100 µl per well. Incubate 1 hour at room temperature with slow shaking.

3) Wash plate with TBST buffer and Blocking buffer as in step 1-10 and 1-11.

**Step 3:**

1) Dilute “**Secondary HRP-labeled antibody 2**” 1,000-fold with **Blocking buffer 4**.

2) Add 100 µl per well. Incubate for 30 min. at room temperature with slow shaking.

3) Wash plate with TBST buffer and **Blocking buffer 4** as in step 1-10 and 1-11.

4) Just before use, mix on ice 50 µl **HRP chemiluminescent substrate A** and 50 µl **HRP chemiluminescent substrate B** and add 100 µl per well. Discard any unused chemiluminescent reagent after use.

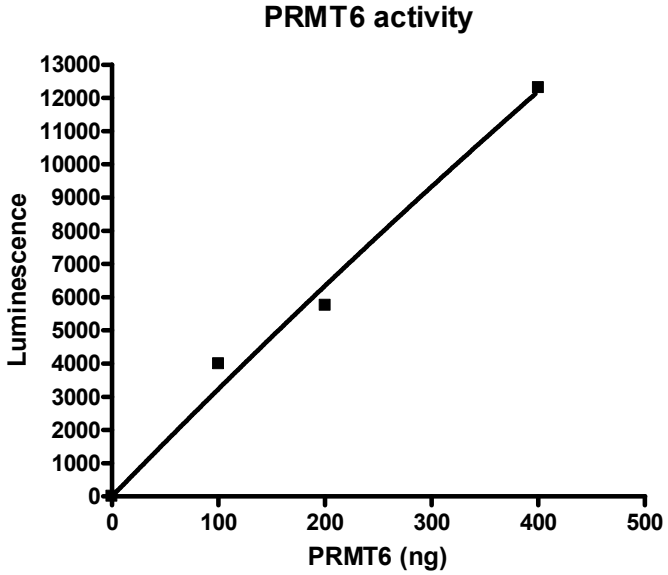
5) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. “Blank” value is subtracted from all other values.

**Reading Chemiluminescence:**

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the “hole” position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

**Example of Assay Results:**




PRMT6 enzyme activity, measured using the PRMT6 Chemiluminescent Assay Kit, #AMS.52046. Luminescence was measured using a Bio-Tek fluorescent microplate reader.
*Data shown is lot-specific.*

**RELATED PRODUCTS**

| Product | Catalog | Size |
|---|---|---|
| PRMT1 (expressed in E. coli) | AMS.51040 | 50 µg |
| PRMT1 (expressed in Sf9 cells) | AMS.51041 | 20 µg |
| PRMT3 (expressed in E. coli) | AMS.51043 | 50 µg |
| PRMT4 (expressed in HEK293) | AMS.51047 | 20 µg |
| PRMT5 (expressed in HEK293) | AMS.51045 | 20 µg |
| PRMT5/MEP50 (expressed in Sf9 cells) | AMS.51048 | 20 µg |
| PRMT6 (expressed in HEK293) | AMS.51046 | 20 µg |
| PRMT8 (expressed in Sf9 cells) | AMS.51052 | 20 µg |
| PRMT1 Chemiluminescent Assay Kit | AMS.52004L | 96 reactions |
| PRMT3 Chemiluminescent Assay Kit | AMS.52005L | 96 reactions |
| PRMT4 Chemiluminescent Assay Kit | AMS.52041L | 96 reactions |
| PRMT5 Chemiluminescent Assay Kit | AMS.52002 | 96 reactions |

**TROUBLESHOOTING GUIDE**

| Problem | Possible Cause | Solution |
|---|---|---|
| Luminescence signal of positive control reaction is weak | PRMT6 enzyme has lost activity | Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (PRMT6, #AMS.51049).Store enzyme insingle-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration. |
| | Antibody reaction is insufficient | Increase time for primary antibody incubation. Avoid freeze/thaw cycles of antibodies. |
| | Incorrect settings on instruments | Record light signals at 5 second intervals. Refer to instrument instructions for settings to increase sensitivity of light detection. |
| | Chemiluminescent reagents mixed too soon | Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed. |
| Luminescent signal is erratic or varies widely among wells | Inaccurate pipetting/technique | Run duplicates of all reactions.<br>Use a multichannel pipettor.<br>Use master mixes to minimize errors. |
| | Bubbles in wells | Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells. |
| Background (signal to noise ratio) is high | Insufficient washes | Increase number of washes.<br>Increase wash volume.<br>Increase Tween-20 concentration to 0.1% in TBST. |
| | Sample solvent is inhibiting the enzyme | Run negative control assay including solvent. Maintain DMSO level at <1%<br>Increase time of enzyme incubation. |
| | Results are outside the linear range of the assay | Use different concentrations of enzyme (PRMT6) to create a standard curve. |