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Data Sheet **PRMT6 Direct Activity Assay Kit** Catalog #52046

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 100 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 for one hour. 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. *Note: The kit is also suitable for use with cell extracts, but non-specific PRMT enzyme activity may be detected.*

COMPONENTS:

	Cat. #		Amount	Storage
Lot XXX (Avoid freeze/thaw cycles!)	51049	PRMT6	20 µg	-80 °C
	52120	400 µM S-adenosylmethionine	250 µl	-80 °C
	52150	Primary antibody 4	100 µl	-80 °C
	52131H	Secondary HRP-labeled antibody 2	10 µl	-80 °C
		4x HMT assay buffer 6	3 ml	-20 °C
	52100	Blocking buffer	50 ml	+4 °C
		HRP chemiluminescent substrate (2 components)	6 ml each	+4 °C
		Black plate precoated with histone substrate	1	+4 °C

MATERIALS REQUIRED BUT NOT SUPPLIED:

TBST buffer (1 x 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
Paper towels

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

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

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

REFERENCE: Dillon SC, Zhang X, Trievel RC, Cheng X. *Genome Biology* 2005; **6**:227.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

1) Rehydrate the microwells by adding 150 μ l of TBST buffer (1 x 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 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. *Note: PRMT6 enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

3) Dilute PRMT6 enzyme in 1X HMT assay buffer 6 at 5-10 ng/ μ l (100-200 ng/20 μ l). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

4) Using master mixes as much as possible, add the following reagents to the microwells, in duplicate:

	Positive Control	Test Sample	Substrate Control	Blank
PRMT6 (5-10 ng/ μ l)	20 μ l	20 μ l	20 μ l	–
4x HMT assay buffer 6	12.5 μ l	12.5 μ l	12.5 μ l	12.5 μ l
400 μ M S-adenosylmethionine	2.5 μ l	2.5 μ l	–	2.5 μ l
Test Inhibitor/Activator	–	X μ l	–	–
H ₂ O	15.0 μ l	15.0 – X μ l	17.5 μ l	35.0 μ l
Total	50 μl	50 μl	50 μl	50 μl

5) Add the entire reaction mixture (50 μ l) to the substrate-coated black plate. Incubate at room temperature for 1 hour.

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- 6) Wash the plate three times with TBST buffer. Blot dry onto clean paper towels.
- 7) Add 100 μ l of Blocking buffer 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.
- 2) Add 100 μ l per well. Incubate 1 hour at room temperature with slow shaking.
- 3) Wash plate with TBST buffer and Blocking buffer as in step 1-6 and 1-7.

Step 3:

- 1) Dilute "Secondary HRP-labeled antibody 2" 1,000-fold with Blocking buffer.
- 2) Add 100 μ l per well. Incubate for 30 min. at room temperature with slow shaking.
- 3) Wash plate with TBST buffer and Blocking buffer as in step 1-6 and 1-7.
- 4) Just before use, mix on ice 50 μ l HRP chemiluminescent substrate A and 50 μ l HRP chemiluminescent substrate B and add 100 μ l per well. Discard any unused chemiluminescent reagent after use.

Step 4:

- 1) Incubate plate for 10 min. at room temperature.
- 2) Read sample in a luminometer or microtiter-plate capable of reading chemiluminescence.

Example of Assay Results:

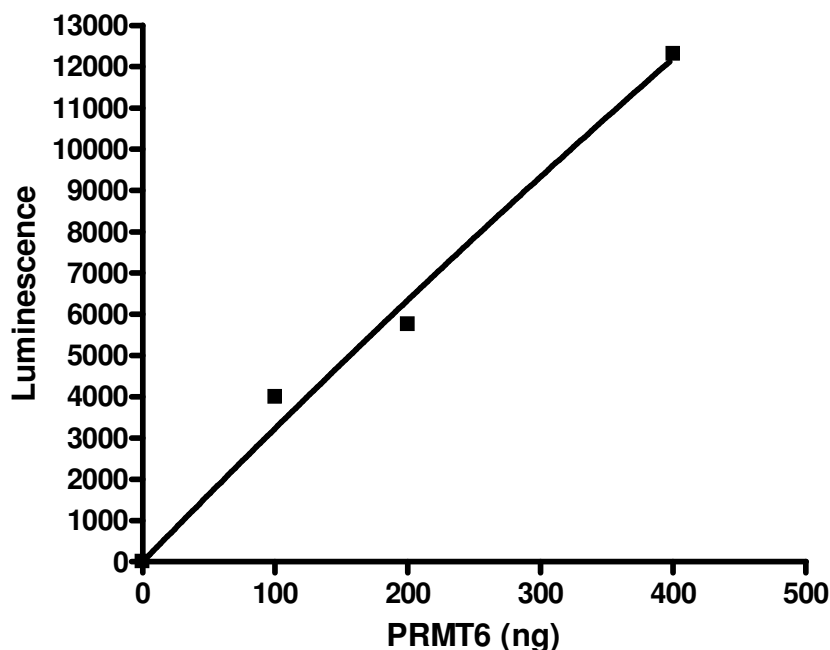
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PRMT6 activity



PRMT6 enzyme activity, measured using the PRMT6 Assay Kit, BPS Bioscience #52046. Luminescence was measured using a Bio-Tek fluorescent microplate reader.

Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

RELATED PRODUCTS

PRMT1 (expressed in E. coli)	#51040	50 µg
PRMT1 (expressed in Sf9 cells)	#51041	20 µg
PRMT3 (expressed in E. coli)	#51043	50 µg
PRMT4 (expressed in HEK293)	#51047	20 µg
PRMT5 (expressed in HEK293)	#51045	20 µg
PRMT5/MEP50 (expressed in Sf9 cells)	#51048	20 µg
PRMT6 (expressed in HEK293)	#51046	20 µg
PRMT1 Chemiluminescent Assay Kit	#52004L	100 reactions
PRMT3 Chemiluminescent Assay Kit	#52005L	100 reactions
PRMT1 Homogeneous Assay Kit	#52052	384 reactions
PRMT3 Homogeneous Assay Kit	#52055	384 reactions
PRMT5 Homogeneous Assay Kit	#52054	384 reactions

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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, BPS Bioscience #51049). Store enzyme in single-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. Use a multichannel pipettor. 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. Increase wash volume. 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%. Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of enzyme (PRMT6, BPS Bioscience #51049) to create a standard curve.

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