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Data Sheet

PRMT3 Direct Activity Assay Kit

Catalog # 52005L

DESCRIPTION: The *PRMT3 Direct Activity Assay kit* is designed to measure PRMT3 activity for screening and profiling applications. The *PRMT3 Direct Activity Assay Kit* comes in a convenient format, with a 96-well plate precoated with specific substrate, the antibody against methylated substrate, the secondary HRP-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, and purified PRMT3 enzyme for 100 enzyme reactions. The key to the *PRMT3 Direct Activity 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, 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!)***	51043	PRMT3 human recombinant enzyme	1 µg	-80℃
	52120	400 µM S-adenosylmethionine	250 µl	-80℃
	52150	Primary antibody 4	100 µl	-80℃
	52131H	Secondary HRP-labeled antibody 2	10 µl	-80℃
	52170	4x HMT assay buffer 2	3 ml	-20℃
	52100	Blocking buffer	50 ml	+4℃
		HRP chemiluminescent substrate (2 components)	6 ml each	+4℃
		Black plate precoated with histone substrate	1	+4℃

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.

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

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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 µl 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 PRMT3 enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot PRMT3 enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: PRMT3 enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

3) Dilute PRMT3 enzyme in 1X HMT assay buffer to 300-500 pg pg/µl. 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
PRMT3 (300-500 pg/µl)	20 µl	20 µl	20 µl	–
4x HMT assay buffer 2	12.5 µl	12.5 µl	12.5 µl	12.5 µl
400 µM S-adenosylmethionine	2.5 µl	2.5 µl	–	2.5 µl
Test Inhibitor/Activator	–	X µl	–	–
H_2O	15 µl	15 - X µl	17.5 µl	35 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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

6) Wash the plate three times with TBST buffer. Blot dry onto clean paper towels.

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

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Step 2:

1) Dilute "Primary antibody 4" 100-fold with Blocking buffer.
2) Add 100 µl per well. Incubate 1 hour at room temperature with slow shaking.
3) Wash plate three times with TBST buffer as in step 1-4.

Step 3:

1) Dilute "Secondary HRP-labeled antibody 2" 1,000-fold with Blocking buffer.
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 as in step 1-6 and 1-7.
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.

Step 4:

Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence.

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BPS Bioscience

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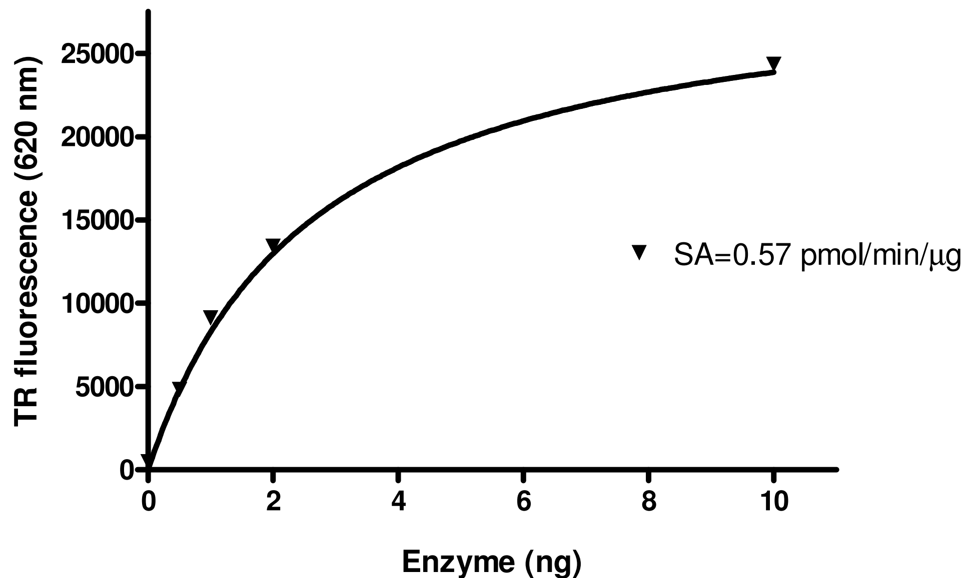
Example of Assay Results:



PRMT3 enzyme activity, measured using the PRMT3 Assay Kit, BPS Bioscience #52005L. 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
PRMT4 (expressed in Sf9 cells)	#51044	20 µg
PRMT5 (expressed in HEK293)	#51045	20 µg
PRMT5 (expressed in Sf9 cells)	#51048	20 µg
PRMT6 (expressed in HEK293)	#51046	20 µg
PRMT1 Assay Kit	#52004	100 reactions
PRMT5 Assay Kit	#52002	100 reactions

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	PRMT3 enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (PRMT3, BPS Bioscience #51043). 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	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 (PRMT3, BPS Bioscience #51043) to create a standard curve.

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