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Data Sheet **DNMT1 Direct Activity Assay Kit** Catalog # 52050L

DESCRIPTION: The *DNMT1 Direct Activity Assay Kit* is designed to measure DNMT1 activity using purified DNMT1 or cell extracts containing DNMT1. The *DNMT1 Direct Activity Assay Kit* comes in a convenient format, with a 96-well plate precoated with DNMT substrate, the antibody against 5-methylcytosine, the secondary HRP-labeled antibody, S-adenosylmethionine, DNMT1 assay buffer, and purified DNMT1 for 100 enzyme reactions. The key to the *DNMT1 Direct Activity Assay Kit* is a highly specific antibody that recognizes 5-methylcytosine of the substrate. With this kit, only three simple steps on a microtiter plate are required for DNMT1 detection. First, S-adenosylmethionine is incubated with a sample containing assay buffer and DNMT1 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 any DNA methyltransferase may be responsible for the activity measured.*

COMPONENTS:

	Cat. #		Amount	Storage
LotXXX (Avoid freeze/thaw cycles!)	51101	DNMT1	20 µg	-80 °C
	52120	400 µM S-adenosylmethionine	500 µl	-80 °C
		Anti-5-methylcytosine antibody	25 µl	-80 °C
	52130H	Secondary HRP-labeled antibody 1	10 µl	-80 °C
	52200	4x DNMT assay buffer 1	2.5 ml	-20 °C
	52100	Blocking buffer	50 ml	+4 °C
		HRP chemiluminescent substrate (2 components)	6 ml each	+4 °C
		Black plate precoated with DNMT1 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.

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:

1. Svedruzic ZM. *Curr. Med. Chem.* 2008; **15**(1):92-106.

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 DNMT1 on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot DNMT1 into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: DNMT1 enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

3) Dilute DNMT1 in 1X DNMT assay buffer at 10 ng/ μ l (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
DNMT1 (10 ng/ μ l)	20 μ l	20 μ l	20 μ l	–
4x DNMT assay buffer 1	12.5 μ l	12.5 μ l	12.5 μ l	12.5 μ l
400 μ M S-adenosylmethionine	5 μ l	5 μ l	–	5 μ l
Test Inhibitor/Activator	–	X μ l	–	–
H ₂ O	12.5 μ l	12.5 - X μ l	17.5 μ l	32.5 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 37°C for 1 hour.

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.

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Step 2:

- 1) Dilute "Anti-5-methylcytosine antibody" 400-fold with Blocking buffer.
- 2) Add 100 μ l per well. Incubate 1 hour at room temperature with slow shaking.
- 3) Wash plate three times with TBST buffer and incubate in Blocking buffer as in steps 1-6 and 1-7.

Step 3:

- 1) Dilute "Secondary HRP-labeled antibody 1" 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 three times with TBST buffer and incubate in Blocking buffer as in steps 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:

Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

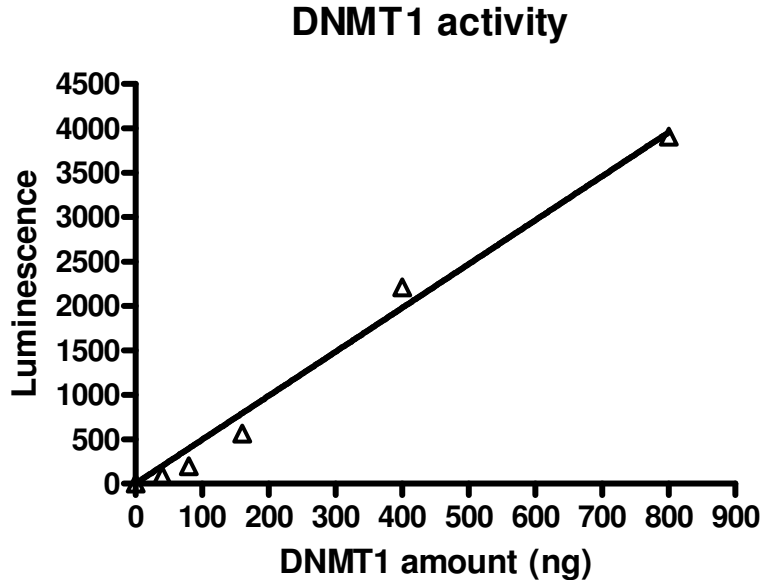
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Example of Assay Results:



DNMT1 enzyme activity, measured using the DNMT1 Assay Kit, BPS Bioscience #52050. 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

DNMT1	#51101	10 µg
DNMT2	#51102	10 µg
DNMT3a	#51103	10 µg
4x DNMT Assay Buffer 1	#52200	30 ml
EZH2 Assay Kit	#52009	100 reactions
G9a Assay Kit	#52001	100 reactions
MLL Assay Kit	#52008	100 reactions
PRMT1 Assay Kit	#52004	100 reactions
PRMT3 Assay Kit	#52005	100 reactions
PRMT5 Assay Kit	#52002	100 reactions
SET7/9 Assay Kit	#52003	100 reactions
SUV39H1 Assay Kit	#52006	100 reactions
SUV39H2 Assay Kit	#52007	100 reactions

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	DNMT1 enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (DNMT1, BPS Bioscience #51101). 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 (DNMT1, BPS Bioscience #51101) to create a standard curve.

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