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Data Sheet
DNMT Universal Assay Kit
Catalog #52035

DESCRIPTION: The *DNMT Universal Assay Kit* is designed to measure DNMT activity using purified enzymes. The *DNMT Universal Assay Kit* comes in a convenient format, with a 96-well plate precoated with DNMT substrate, an antibody against 5-methylcytosine, a secondary HRP-labeled antibody, S-adenosylmethionine, DNMT assay buffer, and purified DNMT1, DNMT3A/3L and DNMT3B/3L for 100 enzyme reactions. The key to the *DNMT Universal Assay Kit* is a highly specific antibody that recognizes 5-methylcytosine on the substrate. With this kit, only three simple steps on a microtiter plate are required for detection of DNMT activity. First, S-adenosylmethionine is incubated with a sample containing assay buffer and DNMT for two hours. 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 be measured using a chemiluminescence reader.

COMPONENTS:

	Cat. #		Amount	Storage
(Avoid freeze/thaw cycles!)	51101	DNMT1	10 µg	-80°C
	51106	DNMT3A/3L Complex	10 µg	-80°C
	51104	DNMT3B/3L Complex	10 µg	-80°C
	52120	400 µM S-adenosylmethionine	250 µl	-80°C
		Anti-5-methylcytosine antibody	25 µl	-80°C
	52130H	Secondary HRP-labeled antibody 1	10 µl	-80°C
	52201	4x DNMT assay buffer 2	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 DNMT 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

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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:
1. Svedruzic, Z.M. *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 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 DNMT enzymes on ice. Upon first thaw, briefly spin tubes containing enzymes to recover full content of the tubes. Aliquot DNMT enzymes into single use aliquots. Store remaining undiluted enzymes in aliquots at -80°C. *Note: All DNMT enzymes are very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

3) Dilute each DNMT in 1X DNMT assay buffer 2 at 5-10 ng/µl (100-200 ng/20 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**
DNMT (5-10 ng/µl)	20 µl	20 µl	20 µl	–
4x DNMT 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**

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5) Add the entire reaction mixture (50 µl) to the substrate-coated black plate. Incubate at 37ºC for 1-2 hours.

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.

Step 2:

1) Dilute "Anti-5-methylcytosine antibody" 400-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 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 µl 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 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. "Blank" value is subtracted from all readings.

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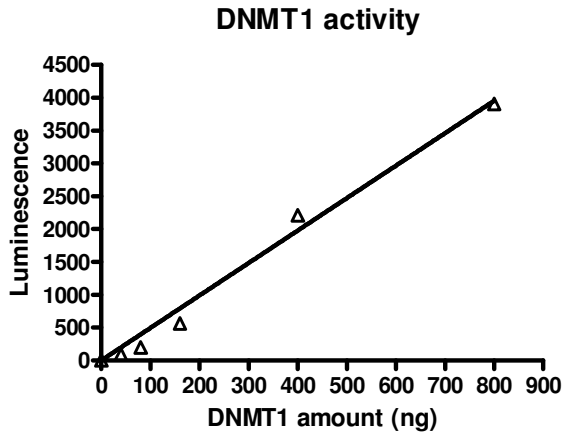
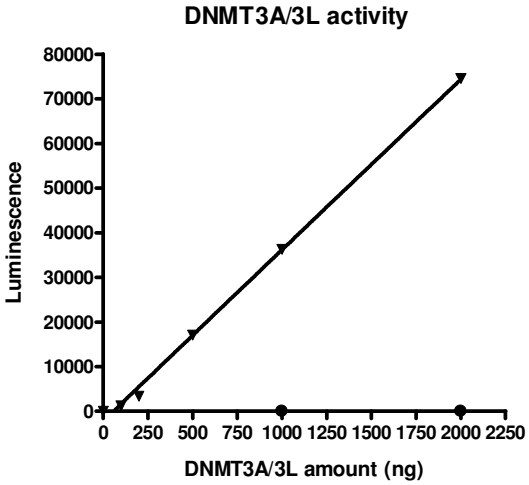
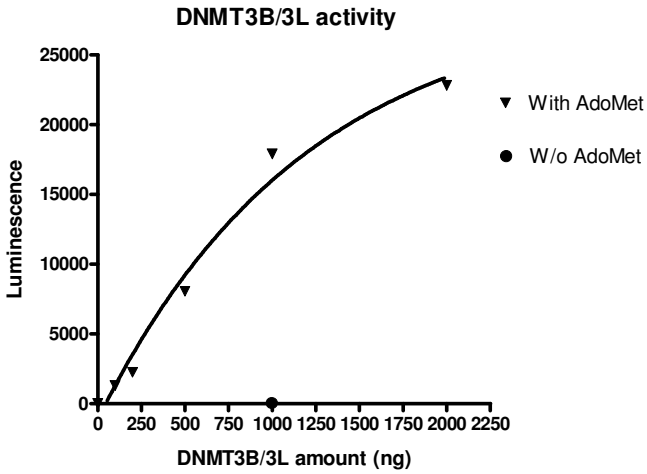
Example of Assay Results:







DNMT1, DNMT3A/3L and DNMT3B/3L enzyme activities, measured using the DNMT Universal Assay Kit, BPS Bioscience #52035. 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

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RELATED PRODUCTS

DNMT1	#51101	10 µg
DNMT2	#51102	10 µg
DNMT3A	#51103	10 µg
DNMT3A/DNMT3L	#51106	10 µg
DNMT3B/DNMT3L	#51104	10 µg
DNMT3B (murine)	#51105	10 µg
DNMT1 Assay Kit	#52050L	96 reactions
DNMT3A Assay Kit	#52033	96 reactions
DNMT3B Assay Kit	#52034	96 reactions
4x DNMT Assay Buffer 1	#52200	30 ml
4x DNMT Assay Buffer 2	#52201	30 ml
MECP2	#50250	50 µg