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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 strip 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	
51101	DNMT1	10 µg	-80°C	(Avoid freeze/thaw cycles!)
51106	DNMT3A/3L Complex	10 µg	-80°C	
51109	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*	5 ml	-20°C	
52100	Blocking buffer 4	50 ml	+4°C	
	HRP chemiluminescent substrate (2 components)	6 ml each	+4°C	
	8-well strip plate precoated with DNMT substrate	1	+4°C	

*Add 10 µl of 0.5 M DTT before use

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, 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 μ l 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) Add **10 μ l of 0.5 M DTT** to **4x DNMT assay buffer 2**. Dilute each **DNMT** in **1x DNMT assay buffer 2** 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
DNMT (5-10 ng/ μ l)	20 μ l	20 μ l	20 μ l	-
4x DNMT assay buffer 2	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 μ l	15 - X μ l	17.5 μ l	35 μ l
Total	50 μl	50 μl	50 μl	50 μl

- 5) Add the entire reaction mixture (50 μ l) to the substrate-coated wells. Incubate at 37°C for 1-2 hours.
- 6) Wash the wells three times with TBST buffer. Blot dry onto clean paper towels.
- 7) Add 100 μ l of **Blocking buffer 4** 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 4**.
- 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 4** as in steps 1-6 and 1-7.

Step 3:

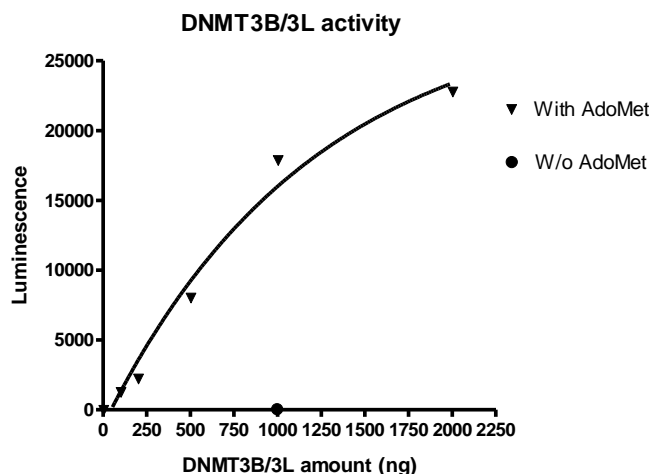
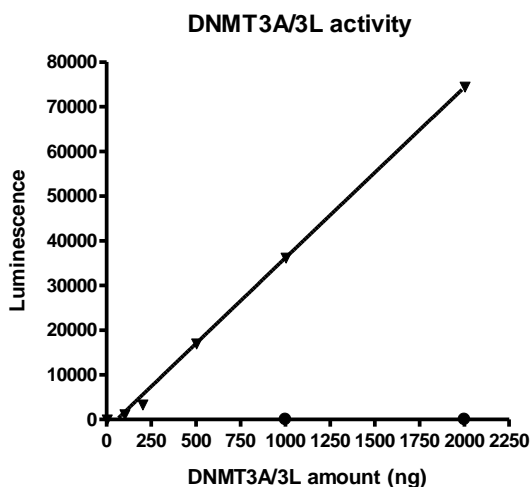
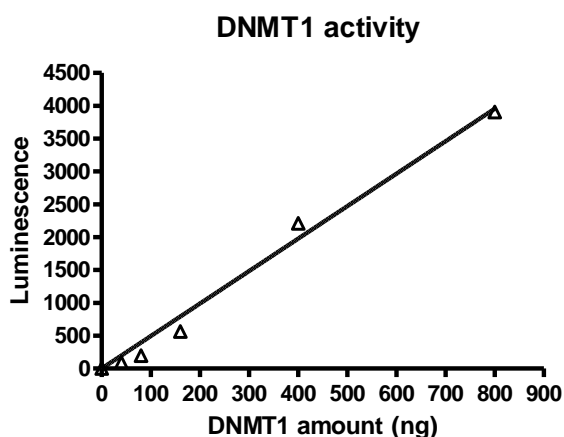
- 1) Dilute "**Secondary HRP-labeled antibody 1**" 1,000-fold with **Blocking buffer 4**.
- 2) Add 100 µl per well. Incubate for 30 min. at room temperature with slow shaking.
- 3) Wash plate three times with TBST buffer and incubate in **Blocking buffer 4** as in steps 1-6 and 1-7.
- 4) Just before 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

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