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Data Sheet
DNMT3B Direct Activity Assay Kit
Catalog #52034
Size: 96 reactions

DESCRIPTION: The *DNMT3B Direct Activity Assay Kit* is designed to measure DNMT3B activity for screening and profiling applications. The *DNMT3B Direct Activity Assay Kit* comes in a convenient format, with a 96-well plate precoated with DNA substrate, an antibody against 5-methylcytosine, a secondary HRP-labeled antibody, S-adenosylmethionine, DNMT assay buffer, and purified DNMT3B/3L complex for 100 enzyme reactions. The key to the *DNMT3B 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 detection of DNMT3B activity. First, S-adenosylmethionine is incubated with a sample containing assay buffer and DNMT3B/3L 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 then be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
51109	DNMT3B/3L	2 x 20 µg	-80°C	<i>Avoid freeze/ thaw cycles!</i>
52120	400 µM S-adenosylmethionine	2 x 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 A (translucent bottle)	6 ml	+4°C	
	HRP chemiluminescent substrate B (brown bottle)	6 ml	+4°C	
	Plate pre-coated with DNA substrate	1	+4°C	

MATERIALS OR INSTRUMENTS 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

APPLICATIONS: Useful for screening enzyme inhibitors.

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(S): 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 (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 **S-adenosylmethionine** on ice. Upon first thaw, briefly spin tube containing S-adenosylmethionine to recover full content of the tube. Aliquot S-adenosylmethionine into single use aliquots. Store remaining S-adenosylmethionine in aliquots at -80°C immediately. *Note: S-adenosylmethionine is very sensitive to freeze/thaw cycles. Avoid multiple freeze-thaw cycles.*
- 3) Prepare the master mixture: N wells × (7.5 µl **4x DNMT assay buffer 2** + 5 µl **400 µM S-adenosylmethionine** + 12.5 µl H₂O)
- 4) Add 25 µl of master mixture to each well designated for the "Positive Control" and "Test Inhibitor". For the "Substrate Control", add 7.5 µl **4x DNMT assay buffer 2** + 17.5 µl H₂O. For the "Blank", add 12.5 µl **4x DNMT assay buffer 2** + 5 µl **400 µM S-adenosylmethionine** + 27.5 µl H₂O.

Component	Positive Control	Test Sample	Substrate Control	Blank
4x DNMT assay buffer 2	7.5 µl	7.5 µl	7.5 µl	12.5 µl
400 µM S-adenosylmethionine	5 µl	5 µl	–	5 µl
H ₂ O	12.5 µl	12.5 µl	17.5 µl	27.5 µl
DNMT3B/3L (20 ng/µl)	20 µl	20 µl	20 µl	–
Inhibitor	–	5 µl	–	–
Inhibitor Buffer (no inhibitor)	5 µl	–	5 µl	5 µl
Total	50 µl	50 µl	50 µl	50 µl

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- 5) Add 5 μ l of inhibitor solution of each well designated "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 5 μ l of the same solution without inhibitor (inhibitor buffer).
- 6) Thaw **DNMT3B/3L** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **DNMT3B/3L** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C . *Note: **DNMT3B/3L** enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
- 7) Dilute **DNMT3B/3L** in **1x DNMT assay buffer 2** at 20 ng/ μ l (400 ng/20 μ l). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.
- 8) Initiate reaction by adding 20 μ l of diluted **DNMT3B/3L** (prepared as described above). Incubate at 37°C for 2 hours.
- 9) Remove the supernatant from the wells and wash the plate three times with 200 μ l TBST buffer. Blot dry onto clean paper towels.
- 10) Add 100 μ l of **Blocking buffer 4** to every well. Shake on a rotating platform for 10 min. Remove supernatant as described above.

Step 2:

- 1) Dilute "Anti-5-methylcytosine antibody" 400-fold with **Blocking buffer 4**.
- 2) Add 100 μ l per well. Incubate 1 hour at room temperature with slow shaking.
- 3) Remove the supernatant from the wells and wash plate three times with 200 μ l TBST buffer and incubate in **Blocking buffer 4** as described in steps 1-9 and 1-10.

Step 3:

- 1) Dilute "Secondary HRP-labeled antibody 1" 1,000-fold with **Blocking buffer 4**.
- 2) Add 100 μ l per well. Incubate for 30 min. at room temperature with slow shaking.
- 3) Remove the supernatant from the wells and wash plate three times with TBST buffer and incubate in **Blocking buffer 4** as described in steps 1-9 and 1-10.

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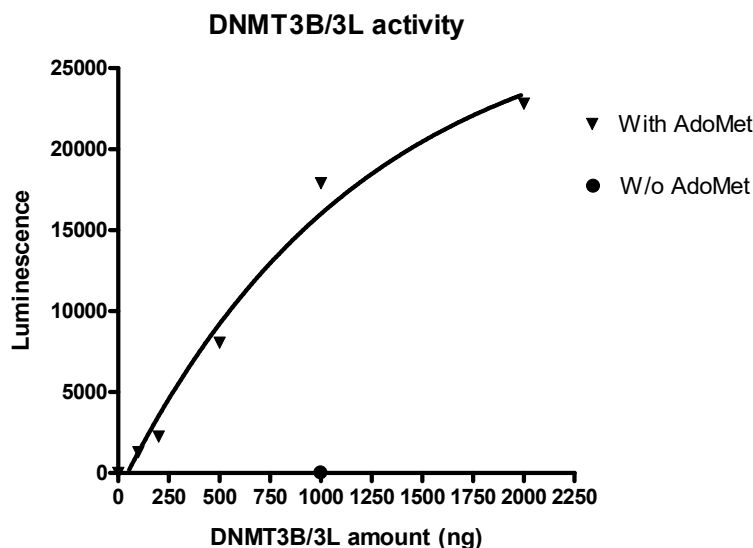
- 4) Just before use, mix on ice 50 μ l **HRP chemiluminescent substrate A** and 50 μ l **HRP chemiluminescent substrate B** and add 100 μ l per well. Discard any unused chemiluminescent reagent after use.
- 5) Immediately read sample in a luminometer or microtiter-plate reader capable of reading chemiluminescence. Blank value is subtracted from all readings.

Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example of Assay Results:



DNMT3B/3L enzyme activity, measured using the *DNMT3B Direct Activity Assay Kit*, BPS Bioscience #52034. 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:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
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
4x DNMT Assay Buffer 1	#52200	30 ml
4x DNMT Assay Buffer 2	#52201	30 ml
MECP2	#50250	50 µg

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	DNMT3B/3L enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (DNMT3B/3L, BPS Bioscience #51109). 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. See section on "Reading Chemiluminescence" above.
	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	Be sure to include blocking steps after wash steps. 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 (DNMT3B/3L, BPS Bioscience #51109, to create a standard curve.

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