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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

DNMT3A Direct Activity Assay Kit

Catalog # 52033

DESCRIPTION: The *DNMT3A Direct Activity Assay Kit* is designed to measure DNMT3A activity for screening and profiling applications. The *DNMT3A 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 DNMT3A/3L complex for 100 enzyme reactions. The key to the *DNMT3A 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 DNMT3A activity. First, S-adenosylmethionine is incubated with a sample containing assay buffer and DNMT3A/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:

Cat. #	Component	Amount	Storage	
51106	DNMT3A/3L Complex	20 µg	-80°C	***(Avoid freeze/thaw cycles!)***
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 (2 components)	6 ml each	+4°C	
	Plate pre-coated with DNA substrate	1	+4°C	

*Add 10 µl of 0.5 M DTT before use

MATERIALS REQUIRED BUT NOT SUPPLIED:
0.5 M DTT
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
STABILITY: One year from date of receipt when stored as directed.
REFERENCE: Svedruzic, Z.M. *Curr. Med. Chem.* 2008; **15**(1):92-106.

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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) Add 10 µl of 0.5 DTT in 4x DNMT assay buffer 2. Prepare the master mixture: N wells × (12.5 µl **4x DNMT assay buffer 2** + 5 µl **400uM S-adenosylmethionine** + 7.5 µl **H_2O**)

3) Thaw **DNMT3A/3L enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **DNMT3A/3L enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. Note: **DNMT3A/3L enzyme** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

4) Dilute **DNMT3A/3L enzyme** in **1x DNMT assay buffer 2** at 10 ng/µl (200 ng/20 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

5) Add 25 µl of mater mixture to each well designated for the "Positive Control", "Test Inhibitor", and "Blank". For the "Substrate Control", add 12.5 µl **4x DNMT assay buffer 2** + 12.5 µl **H_2O**

	Positive Control	Test Inhibitor	Substrate Control	Blank
4x DNMT assay buffer 2	12.5 µl	12.5 µl	12.5 µl	12.5 µl
400 µM S-adenosylmethionine	5 µl	5 µl	–	5 µl
H_2O	7.5 µl	7.5 µl	12.5 µl	7.5 µl
Test Inhibitor/Activator	–	5 µl	–	–
Inhibitor buffer (no inhibitor)	5 µl	–	5 µl	5 µl
1× DNMT assay buffer 2	–	–	–	20 µl
DNMT3A/3L (10 ng/µl)	20 µl	20 µl	20 µl	–
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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6) Add 5 µl of inhibitor solution of each well designated "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 5 µl of the same solution without inhibitor (inhibition buffer)

7) Add 20 µl of **1x DNMT buffer 2** to the well designated "Blank".

8) Initiate reaction by adding 20 µl of diluted **DNMT3A/3L** prepared as described above. Incubate at 37ºC for 2 hours.

9) Wash the plate three times with 200 µl TBST buffer. Blot dry onto clean paper towels.

10) 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 200 µl TBST buffer and incubate in **Blocking buffer** as in steps 1-8 and 1-9.

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 200 µl TBST buffer and incubate in **Blocking buffer** as in steps 1-8 and 1-9.

4) Just before use, mix on ice 50 µl **HRP chemiluminescent substrate A** and 50 µl **HRP chemiluminescent substrate B** and add 100 ul per well. Discard any unused chemiluminescent reagent after use.

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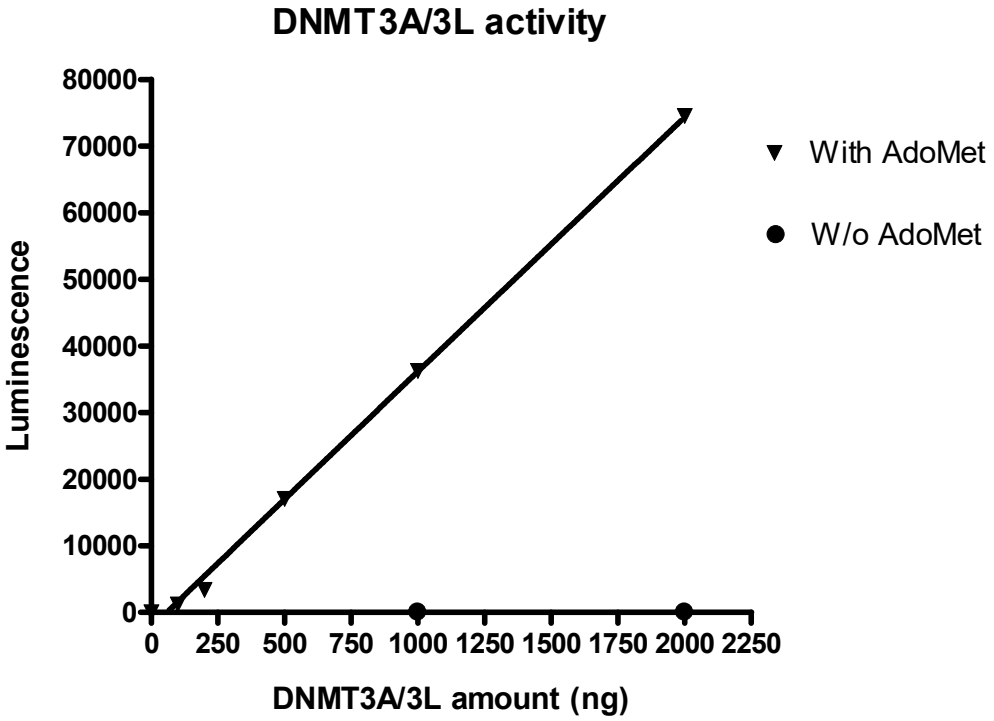
Step 4:

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

Example of Assay Results:



DNMT3A/3L enzyme activity, measured using the DNMT3A/3L Assay Kit, BPS Bioscience #52033. 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	#51109	10 µg
DNMT3B (murine)	#51105	10 µg
DNMT1 Assay Kit	#52050L	100 reactions
DNMT3B Assay Kit	#52034	100 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	DNMT3A/3L enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (DNMT3A/3L, BPS Bioscience #51106). 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 (DNMT3A/3L, BPS Bioscience #51106) to create a standard curve.