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Data Sheet

Histone H4(R3) Universal Methyltransferase Assay Kit

Catalog #52074
96 reactions

DESCRIPTION: The *Histone H4(R3) Universal Methyltransferase Assay Kit* is designed for the detection of histone H4 (R3) methyltransferase (HMT) activity using purified enzymes or cell extracts. Histone H4 (R3) methyltransferases are enzymes that catalyze the transfer of a methyl group from the cofactor S-adenosylmethionine to arginine 3 residue of histone H4. The *Histone H4(R3) Universal Methyltransferase Assay Kit* comes in a convenient format, with a 96-well plate precoated with histone H4 peptide substrate, the antibody against methylated arginine residue of Histone H4, the secondary HRP-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, and purified PRMT1, PRMT3, and PRMT5 enzymes for 96 enzyme reactions. The key to the *Histone H4(R3) Universal Methyltransferase Assay Kit* is a highly specific antibody that recognizes methylated R3 residue of Histone H4. With this kit, only three simple steps on a microtiter plate are required for methyltransferase detection. First, S-adenosylmethionine is incubated with a sample containing assay buffer and methyltransferase enzyme for one hour. Next, primary antibody is added. Finally, the plates are 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. #	Components	Amount	Storage	
51040	PRMT1 human recombinant enzyme*	1 µg	-80°C	***Avoid freeze/ thaw cycles!***
51043	PRMT3 human recombinant enzyme	10 µg	-80°C	
51045	PRMT5 human recombinant enzyme	10 µg	-80°C	
	20 µM S-adenosylmethionine	250 µl	-80°C	
52150	Primary antibody 4	100 µl	-80°C	
52150-3	Primary antibody 4-3	100 µl	-80°C	
52131H	Secondary HRP-labeled antibody 2	10 µl	-80°C	
52170	4x HMT Assay Buffer 2	3 ml	-20°C	
52100	Blocking buffer 4	50 ml	+4°C	
	HRP chemiluminescent substrate (2 components)	6 ml each	+4°C	
	96-well plate precoated with histone substrate	1 plate	+4°C	

**We have provided additional material for ease of retrieval.*

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
TBST buffer (1 x TBS, pH 8.0, containing 0.05% Tween20)
Luminometer or microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips
Rotating or rocker platform

CONTRAINDICATIONS: DMSO >1%, strong acids or bases, ionic detergents, high salt

REFERENCE(S):
Dillon SC, Zhang X, Trievel RC, Cheng X. Genome Biology 2005; **6**:227.

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 **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 and store at -80°C. *Note: **S-adenosylmethionine** very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

3) Prepare master mix: N wells x (7.5 µl **4x HMT Assay Buffer 2** + 2.5 µl **S-adenosylmethionine** + 15 µl H_2O). Add 25 µl of master mixture to all wells labeled "Positive Control," "Test Inhibitor," and "Blank." For wells labeled "Substrate Control," add 7.5 µL **4x HMT Assay Buffer 2** + 17.5 µl H_2O.

4) Add 5 µl of inhibitor solution to each well designated "Test Inhibitor."

5) For the "Positive Control," "Substrate Control," and "Blank," add 5 µl of 10% DMSO in water (inhibitor buffer). *Note: Final DMSO concentration must be ≤1%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor dissolved in 100% DMSO at 10 µM, dilute 1 mM inhibitor with water to make a 100 µM inhibitor in 10% DMSO(aq). Then, add 5 µl of the 100 µM solution into the 50 µl assay to make a 1% DMSO concentration in the final reaction mixture.*

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6) Thaw **PRMT1, PRMT3,** and **PRMT5** enzymes on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **PRMT1**, **PRMT3**, and **PRMT5** enzymes into single use aliquots. Store remaining undiluted enzymes in aliquots at -80°C. *Note: All 3 enzymes are very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzymes.*

7) Dilute **PRMT1**, **PRMT3**, and **PRMT5** in **1x HMT Assay Buffer 2** to 0.1 – 0.5 ng/µl (2 – 10 ng/20 µl), 1 – 2.5 ng/µl (20 – 50 ng/20 µl), and 5 – 10 ng/µl (100 – 200 ng/20 µl) respectively. Keep diluted enzymes on ice until use. Discard any unused diluted enzyme after use.

8) Add 20 µl of **1x HMT Assay Buffer 2** to the well designated "Blank."

	Positive Control	**Test Sample**	**Substrate Control**	**Blank**
4x HMT assay buffer 2	7.5 µl	7.5 µl	7.5 µl	7.5 µl
20 µM S-adenosylmethionine	2.5 µl	2.5 µl	–	2.5 µl
H_2O	15 µl	15 µl	17.5 µl	15 µl
Test Inhibitor/Activator	–	5 µl	–	–
10%DMSO in water (Inhibitor buffer)	5 µl	–	5 µl	5 µl
1x HMT assay buffer 2	–	–	–	20 µl
PRMT1 (0.1 – 0.5 ng/µl) or PRMT3 (1– 2.5 ng/µl) or PRMT5 (5-10 ng/µl)	20 µl	20 µl	20 µl	–
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

9) Initiate reaction by adding 20 µl of diluted enzyme to the wells designated "Positive Control," Substrate Control," and "Test Inhibitor." Incubate at room temperature for 1 hour.

10) Remove the supernatant from the wells and wash three times with 200 µl TBST buffer. Blot dry onto clean paper towels.

11) Add 100 µl of **Blocking Buffer 4** to every well. Shake on a rotating platform for 10 min. Remove supernatant as above.

Step 2:

1) Dilute **Primary antibody*** 100-fold with **Blocking buffer 4**.

*(*For PRMT1 and PRMT3 catalyzed reactions use **Primary Antibody 4**. For PRMT5 catalyzed reactions use **Primary Antibody 4-3**)*

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2) Add 100 µl per well. Incubate 1 hour at room temperature with slow shaking.

3) Remove the supernatant from the wells and wash the strip three times with 200 µl TBST buffer and incubate in **Blocking Buffer 4** as described in steps 1-10 and 1-11.

Step 3:

1) Dilute **Secondary HRP-labeled antibody 2** 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 with TBST buffer and incubate in **Blocking buffer 4** as in steps 1-10 and 1-11.

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.

5) Immediately read sample in a luminometer or microtiter-plate 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 you are using your plate reader in a LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Make sure you don't have filter when emit the light (Synergy 2 BioTek: use "hole" position on filter wheel). Optics position –Top. Read type: endpoint. Sensitivity may be adjusted based on luminescence of a control without enzyme (typically we set this value as 100 when using Synergy 2 plate reader).

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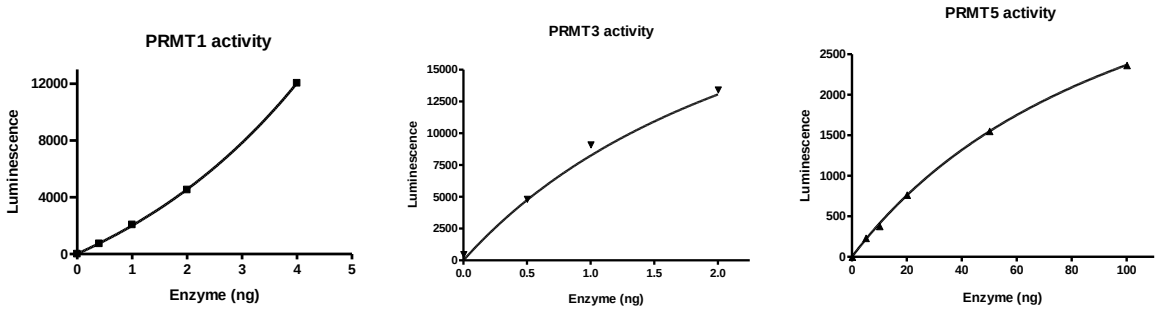
Example of Assay Results:



PRMT1, PRMT3, and PRMT5 enzyme activity, measured using the Histone H4(R3) Methyltransferase Assay Kit, BPS Bioscience #52074. 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

PRMT1 recombinant protein (E. coli)	#51040	50 µg
PRMT1 recombinant protein (Sf9)	#51041	20 µg
PRMT3 recombinant protein	#51043	50 µg
PRMT4 (CARM 1) recombinant protein	#51047	20 µg
PRMT5 recombinant protein (HEK293)	#51045	20 µg
PRMT5/MEP50 recombinant protein (Sf9)	#51048	20 µg
PRMT6 recombinant protein	#51046	20 µg
PRMT1 Chemiluminescent Assay Kit	#52004L	100 reactions
PRMT3 Chemiluminescent Assay Kit	#52005L	100 reactions
PRMT5 Chemiluminescent Assay Kit	#52002L	100 reactions
PRMT1 Homogeneous Assay Kit	#52054	384 reactions
PRMT3 Homogeneous Assay Kit	#52055	384 reactions
PRMT5 Homogeneous Assay Kit	#52052	384 reactions

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	Methyltransferase enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzymes (PRMT1, BPS Bioscience #51040; PRMT3, #51043; PRMT5, #51045). Store enzymes 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.
Chemiluminescence signal is erratic or varies widely	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/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 (PRMT1, BPS Bioscience #51040; PRMT3, #51043; PRMT5, #51045) to create a standard curve.