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Data Sheet

PRDM9 Chemiluminescent Assay Kit

Catalog #:79625
Size: 96 reactions

DESCRIPTION: The *PRDM9 Chemiluminescent Assay Kit* is designed to measure PRDM9 activity for screening and profiling applications, using purified PRDM9. The *PRDM9 Chemiluminescent Assay Kit* comes in a convenient format, with 8-well strips pre-coated with histone H3 peptide substrate, an antibody against methylated lysine on Histone H3, a secondary HRP-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, and enough purified PRDM9 enzyme for 100 enzyme reactions. The key to the *PRDM9 Chemiluminescent Assay Kit* is a highly specific antibody that recognizes methylated K36 residue of Histone H3. 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. Next, primary antibody is added. Finally, the plates are treated with an HRP-labeled secondary antibody followed by the addition of the HRP substrate to produce chemiluminescence that can be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100078	PRDM9	>1 µg	-80°C	***Avoid freeze/ thaw cycles!***
52120	20 µM S-adenosylmethionine	250 µl	-80°C	
52140L	Primary antibody 12	12.5 µl	-80°C	
52160	4x HMT assay buffer 1 (*add DTT)	3 ml	-20°C	
52131H	Secondary HRP-labeled antibody 2	10 µl	-80°C	
79556	Blocking buffer	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	

MATERIALS REQUIRED BUT NOT SUPPLIED:
TBST buffer (1 x TBS, pH 8.0, containing 0.05% Tween-20)
0.5 M DTT (dithiothreitol) in anhydrous DMSO
Luminometer or microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips
Rotating or rocker platform

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

STABILITY: Up to one year from date of receipt when stored as directed.

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REFERENCE(S): Powers NR, *et al. PLoS Genet.* 2016 Jun 30;12**(6):**e1006146.

ASSAY PROTOCOL:

Step 1:

1) Rehydrate the microwells by adding 200 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 strip 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 contents of the tube. Aliquot **S-adenosylmethionine** into single use aliquots and store at -80˚C. *Note: **S-adenosylmethionine** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

3) Add 125 µl of 0.5 M DTT to **4x HMT assay buffer 1**. Prepare the master mixture: N wells × (7.5 µl **4x HMT assay buffer 1** + 2.5 µl **20 µM S-adenosylmethionine** + 15 µl distilled water). Add 25 µl of master mixture to all wells labeled "Positive Control", "Test Sample" and "Blank". For wells labeled "Substrate control", add 7.5 µl **4x HMT assay buffer 1** + 17.5 µl water.

	Blank	Substrate Control	Positive Control	Test Sample
4x HMT assay buffer 1	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
Distilled water	15 µl	17.5 µl	15 µl	15 µl
Test Inhibitor	–	–	–	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	
1x HMT assay buffer 1	20 µl	–	–	–
Diluted PRDM9 (0.1 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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

5) Thaw **PRDM9 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **PRDM9 enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **PRDM9 enzyme** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

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6) Prepare **1x HMT assay buffer 1** by diluting **4x HMT assay buffer 1** with 3 parts distilled water. Prepare only enough **1x HMT assay buffer 1** as required for the assay. Store remaining **4x HMT assay buffer 1** in aliquots at -20°C.

7) Dilute PRDM9 enzyme in **1x HMT assay buffer 1** to 0.1 ng/µl (2 ng/20 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use. *Note: Diluted enzyme may not be stable. Dilute the enzyme immediately before use.*

8) Add 20 µl of **1x HMT assay buffer 1** to the wells designated "Blank".

9) Initiate reaction by adding 20 µl of diluted **PRDM9 enzyme** to the wells designated "Positive Control", "Substrate Control", and "Test Sample ". Incubate at room temperature for one hour.

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

11) Add 100 µl of **Blocking buffer** to every well. Shake on a rotating platform for 10 minutes. Remove supernatant as described above.

Step 2:

1) Dilute "**Primary antibody 12**" 800-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 1-10 and 1-11.

Step 3:

1) Dilute "**Secondary HRP-labeled antibody 2**" 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 with TBST buffer and incubate in **Blocking buffer** 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**. 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 other values.

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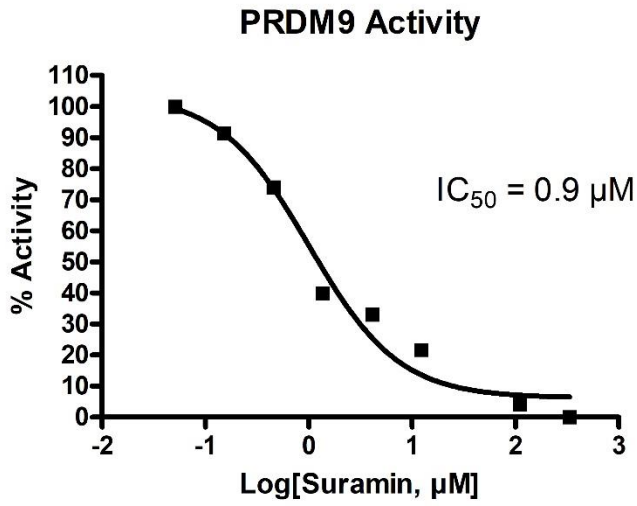
Reading Chemiluminescence:

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).

Example of Assay Results:



PRDM9 enzyme activity, measured using the *PRDM9 Chemiluminescent Assay Kit*, BPS Bioscience #79625. 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 support@bpsbioscience.com*

RELATED PRODUCTS

Product Name	Catalog #	Size
PRDM9	100078	50 µg
PRDM12	50320	50 µg
PRDM14	50321	50 µg
SETD2 (PRDM2)	53019	50 µg
SETD2 Chemiluminescent Assay Kit	52060	96 rxns.

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	PRDM9 enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (PRDM9, BPS Bioscience #100078). Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for 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 (PRDM9, BPS Bioscience #100078) to create a standard curve.