Description

The METTL3/METTL114 Complex Chemiluminescent Assay Kit is designed to measure the activity of METTL3 (methyltransferase 3, N6-adenosine-methyltransferase complex catalytic subunit)/ METTL14 (methyltransferase 14, N6-adenosine) complex activity for screening and profiling applications. The METTL3/METTL14 assay kit comes in a convenient 96-well format, with enough recombinant purified METTL3/METTL14 complex, primary and secondary antibodies, S-adenosylmethionine (SAM), pre-coated plate with RNA substrate, blocking buffer and detection reagents for 100 enzyme reactions.

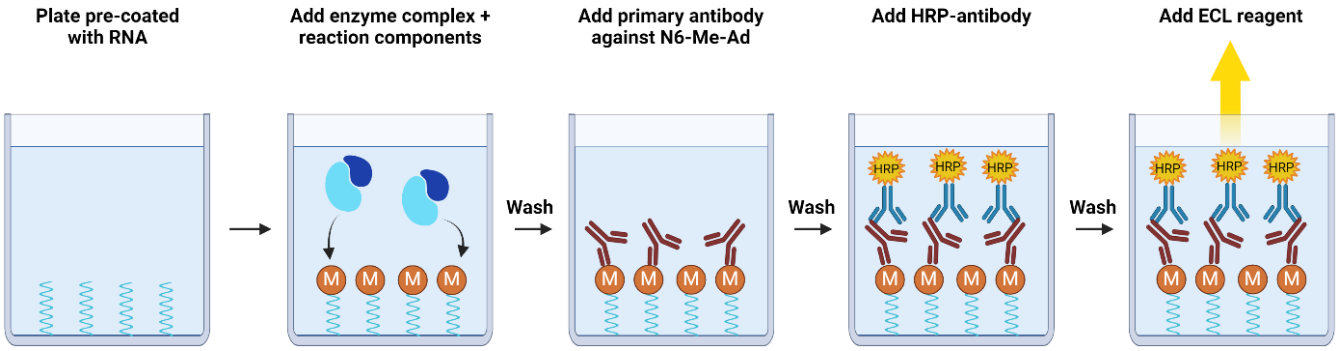

Figure 1: Illustration of the METTL3/METTL114 Complex Chemiluminescent Assay Kit assay principle.

Background

METTL3 (methyltransferase 3, N6-adenosine-methyltransferase complex catalytic subunit)/METTL4 (methyltransferase 4, N6-adenosine) and METTL14 (methyltransferase 14, N6-adenosine) form the core of a complex involved in N6-methyladenosine addition to RNA, one of the most common mRNA modifications, and belong to the group known as “writer” proteins. METTL3 expression is altered in cancer via different processes. For instance, in pancreatic cancer cigarette smoke condensate influences METTL3 promoter hypomethylation and increases its expression. This complex can act as a tumor suppressor or activator and promote chemoresistance. It has been linked to acute myeloid leukemia (AML), liver, lung, bladder cancer and others. Interestingly METTL3 can activate certain oncogenes by simply recruiting eIF3 (eukaryotic translation initiation factor 3), a process that does not require its catalytic activity. Its role in cancer has made it an attractive target in oncology. STM2457, a highly selective potent METT3/METTL14 inhibitor, resulted in AML cells undergoing differentiation and apoptosis. A deeper understanding of the role of this complex in cancer and development of new therapeutics around it will benefit the cancer therapy field.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

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Supplied Materials

Catalog #	Name	Amount	Storage
100105	METTL3/METTL14 Protein Complex, FLAG-Tag, His-Tag*	20 µg	-80°C
52120	20 µM S-Adenosylmethionine	250 µl	-80°C
52140Z4	Primary Antibody 29	100 µl	-80°C
78368	4x METTL Assay Buffer	3 ml	-20°C
	Blocking Buffer 5	50 ml	+4°C
	0.5 M DTT	200 µl	-20°C
52131H	Secondary HRP-Labeled Antibody 2	10 µl	-80°C
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
	White microplate precoated with RNA Substrate	1	Room Temp

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- 1x PBS (phosphate buffer saline) Buffer
- PBST Buffer (1x PBS, containing 0.05% Tween-20)
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

This kit is compatible with up to 1% final DMSO concentration.
Use RNAse-free conditions.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “No-Substrate Control”, “Positive Control”, and “Test Inhibitor” conditions.

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- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using STM2457 as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

Step 1:

1. Rehydrate each pre-coated well with 150 µl of PBST Buffer.

2. Incubate 15 minutes at Room Temperature (RT).

3. Tap the plate onto clean paper towel to remove the liquid.

4. Thaw 20 µM S-adenosylmethionine on ice. Briefly spin the tube containing the enzyme to recover its full content.

   Note: Store remaining S-adenosylmethionine in single use aliquots (5 µl minimum volume per aliquot) at -80°C immediately.

5. Add 30 µl of 0.5 M DTT to 4x METTL Assay Buffer. This makes 4x Assay Buffer.

6. Prepare a Master Mix (25 µl/well, except "No-Substrate Control"): N wells x (7.5 µl of 4x Assay Buffer + 2.5 µl of 20 µM S-adenosylmethionine + 15 µl of distilled water).

7. Add 25 µl of Master Mix to all wells, except the "No-Substrate Control" wells.

8. Prepare a Deficient Master Mix (25 µl/"Substrate Control" well): N wells x (7.5 µl of 4x Assay Buffer + 17.5 µl of distilled water).

9. Add 25 µl of Deficient Master Mix to the "No-Substrate Control" wells.

10. Thaw **METTL3/METTL14** complex on ice. Briefly spin the tube containing the enzyme to recover its full content.

11. Dilute 4x Assay Buffer 4-fold with distilled water. This makes 1x Assay Buffer.

12. Dilute METTL3/METTL14 complex to **10 ng/µl** with 1x Assay Buffer (20 µl/well).

13. Prepare the Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

    13.1 If the Test Inhibitor is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration.

    For the positive and negative controls, use distilled water (Diluent Solution).

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OR

13.2 If the Test Inhibitor is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 10-fold with distilled water (at this step the compound concentration is 10-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 10%.

Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in distilled water to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in distilled water (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

14. Add 5 µl of Test Inhibitor to each well labeled as "Test Inhibitor".

15. Add 5 µl of Diluent Solution to the "Positive Control", "No-Substrate Control" and "Blank" wells.

16. Initiate the reaction by adding 20 µl of diluted METTL3/METTL14 complex to the wells designated "Positive Control", "No-Substrate Control", and "Test Inhibitor."

17. Add 20 µl of 1x Assay Buffer to the "Blank" wells.

18. Incubate overnight at RT.

	Blank	**No-Substrate Control**	**Positive Control**	**Test Inhibitor**
Master Mix	25 µl	-	25 µl	25 µl
Deficient Master Mix	-	25 µl	-	-
Test Inhibitor	-	-	-	5 µl
Diluent Solution	5 µl	5 µl	5 µl	-
1x Assay Buffer	20 µl	-	-	-
Diluted METTL3/METTL14 Complex (10 ng/µl)	-	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

19. Wash plate three times with 200 µl of PBST Buffer per well.

20. Tap the plate onto clean paper towel to remove the liquid.

21. Add 100 µl of Blocking Buffer 5 to each well.

22. Incubate for 10 minutes at RT with gentle agitation.

23. Tap the plate onto clean paper towel to remove the liquid.

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Step 2:

1. Dilute 100-fold the Primary Antibody 29 with Blocking Buffer 5 (100 µl/ well).
2. Add 100 µl of diluted Primary Antibody 29 to each well.
3. Incubate for 1 hour at RT with slow agitation.
4. Wash three times with 200 µl of PBST Buffer per well and tap the plate onto clean paper towel.
5. Add 100 µl of Blocking Buffer 5 to each well.
6. Incubate for 10 minutes at RT with gentle agitation.
7. Tap the plate onto clean paper towel to remove the liquid.

Step 3:

1. Dilute 1000-fold the Secondary HRP-Labeled Antibody 2 with Blocking Buffer 5 (100 µl/well).
2. Add 100 µl of diluted Secondary HRP-Labeled Antibody 2 to each well.
3. Incubate for 30 minutes at RT with slow agitation.
4. Wash three times with 200 µl of PBST Buffer per well and tap the plate onto clean paper towel.
5. Add 100 µl of Blocking Buffer 5 to each well.
6. Incubate for 10 minutes at RT with gentle agitation.
7. Tap the plate onto clean paper towel to remove the liquid.
8. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).
9. Add 100 µl of mix per well.
10. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.
11. The “Blank” value should be 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 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 Results

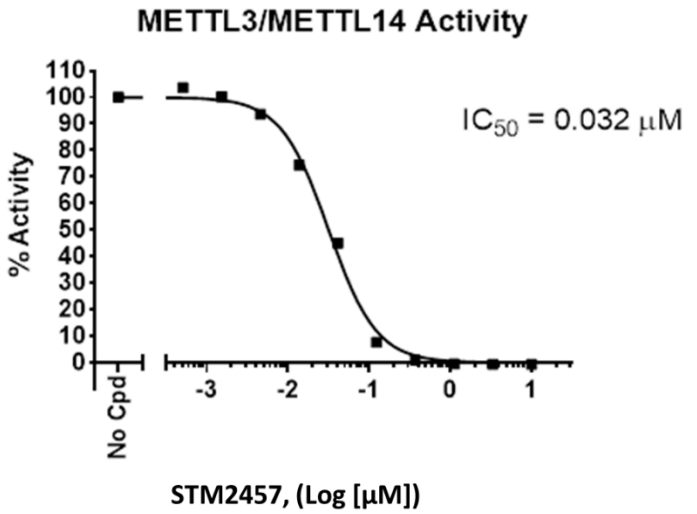

Figure 2: Inhibition of METTL3/METTL14 activity by STM2457.
METTL3/METTL14 complex was incubated with increasing concentrations of STM2457. Luminescence was measured using a Bio-Tek microplate reader.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Yankova E., *et al.*, 2021 *Nature* 593: 597-601.
Zeng C., *et al.*, 2020 *J Hematol Oncol* 13:117.
Sun Y., *et al.*, 2023 *Journal of Experimental & Clinical Cancer Research* 42:65.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
METTL3, FLAG-Tag Recombinant	100055	10 µg
MLL3/WDR5/Ash2L/RbBP5/DPY30 Complex Recombinant	100323	50 µg
MLL3 Complex Chemiluminescent Assay Kit	79758	96 reactions
MLL1 Complex Chemiluminescent Assay Kit	53008	96 reactions
MLL1/WDR5/Ash2L/RbBP5/DPY30 (MLL1/WARD Complex), FLAG, His-Tag Recombinant	51021	50 µg

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