Description

The MAT2A Inhibitor Screening Assay Kit is designed to measure MAT2A (Methionine Adenosyltransferase 2A) activity for screening and profiling applications. The MAT2A assay kit comes in a convenient 384-well format, with purified recombinant MAT2A enzyme, L-Methionine, ATP, MAT2A assay buffer, and colorimetric detection reagent for 400 enzyme reactions.

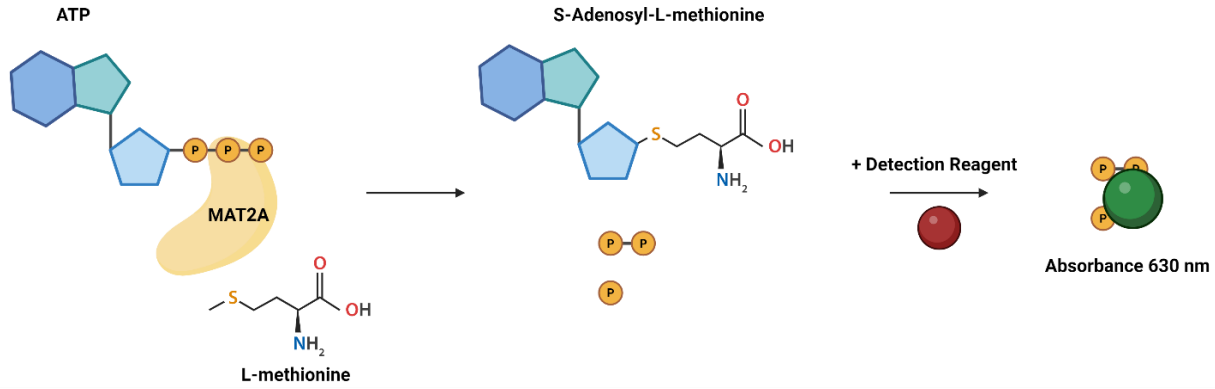

Figure 1: Illustration of the mechanism behind the MAT2A Inhibitor Screening Assay Kit.

Background

MAT2A (methionine adenosyltransferase 2A), also known as AdoMet Synthase 2, is the rate limiting enzyme in the methionine cycle, where it catalyzes the formation of S-adenosylmethionine (SAM) from methionine and ATP. It is the catalytic subunit of the S-adenosylmethionine synthetase complex. The levels of SAM are crucial for many cellular processes, including the modification of DNA, RNA and histones. In liver, MAT2a expression is associated with growth, dedifferentiation, and cancer. In CRPC (castration-resistant prostate cancer) it plays a role in maintaining the cancer stem cell stemness and tumorigenesis, potentially by promoting H3K4me2 of pro-tumorigenic non-canonical AR (androgen receptor) targets. The use of MAT2A inhibitors showed promise in CRPC models, when combined with AR and EZH2 (enhancer of zeste homolog 2) inhibitors. It is a synthetic lethal target in MTAP (methylthioadenosine phosphorylate)-deficient cancers, making it a promising oncotherapy target.

Applications

Study enzyme kinetics and screen small molecule inhibitors of MAT2A for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
71401	MAT2A, His-Tag*	250 µg	-80°C
82188	ATP (750 µM)	1 ml	-20°C
82189	L-Methionine (750 µM)	1 ml	-20°C
78869	5x MAT2A Assay Buffer	2 x 3 ml	-20°C
74001	Colorimetric Detection Reagent**	2 x 10 ml	+4°C
79962	Transparent, 384-well plate	1	Room Temp.

**The concentration of the protein is lot-specific and will be indicated on the tube.*
***Colorimetric Detection Reagent is used to measure the free phosphate from the MAT2A reaction. Any source of inorganic phosphate can interfere with the assay.*

Materials Required but Not Supplied

- UV/Vis spectrophotometer microplate reader capable of reading absorbance at 630 nm
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform (optional)
- Aluminum foil

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

- The final concentration of DMSO in the assay should not exceed 1%.
- Some compounds may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound with the assay results.
- Colorimetric Detection Reagent is used to measure the free phosphate from the MAT2A reaction. Any source of inorganic phosphate can interfere with the assay.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Positive Control” and “Test Inhibitor” conditions.
- 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 FIDAS-5 (Millipore #504173) 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.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

1. Thaw 5x MAT2A Assay Buffer.

2. Prepare 1x MAT2A Assay Buffer by diluting 5x MAT2A Assay Buffer 5-fold with water.

   Note: Dilute only enough buffer required for the assay. Store remaining 5x MAT2A Assay Buffer at -20°C in single-use aliquots.

3. Thaw MAT2A on ice. Briefly spin the tube containing the protein to recover the full content of the tube.

4. Dilute MAT2A to 60 ng/µl with 1x MAT2A Assay Buffer (10 µl/well).

5. Prepare the Test Inhibitor (5 µl/well): for a titration, prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

   5.1 If the Test Inhibitor is water-soluble, prepare 5-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations in 1x MAT2A Assay Buffer.

   For the positive control and blank, use 1x MAT2A Assay Buffer (Diluent Solution).

   OR

   5.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 20-fold in 1x MAT2A Assay Buffer to prepare the highest concentration of the 5-fold intermediate dilutions. The concentration of DMSO is now 5%.

   Using 1x MAT2A Assay Buffer containing 5% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 5-fold the desired final concentrations.

   For positive control and blank, prepare 5% DMSO in 1x MAT2A Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

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

6. Add 10 µl of diluted MAT2A to the “Positive Control” and “Test Inhibitor” wells.

7. Add 10 µl of 1x MAT2A Assay Buffer to the “Blank” wells.

8. Add 5 µl of Test Inhibitor to each well labeled “Test Inhibitor”.

9. Add 5 µl of Diluent Solution to the “Positive Control" and “Blank” wells.

10. Incubate at Room Temperature (RT) for 30 minutes.

11. Thaw ATP (750 µM) and L-Methionine (750 µM) on ice.

12. Prepare a Master Mixture (10 µl/well): N wells x (5 µl of 5x MAT2A Assay Buffer + 2.5 µl of ATP (750 µM) + 2.5 µl of L-Methionine (750 µM)).

    N*ote: Store the remaining ATP and L-Methionine at -20°C in single use aliquots (minimum of 5 µl/ aliquot).*

13. Add 10 µl of Master Mix to every well.

14. Incubate at RT for 1 hour with gentle agitation.

Component	**Blank**	**Positive Control**	**Test Inhibitor**
Diluted MAT2A (60 ng/µl)	-	10 µl	10 µl
1x MAT2A Assay Buffer	10 µl	-	-
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
30 minutes at Room Temperature			
Master Mix	10 µl	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**

15. After the reaction, add 50 µl of Colorimetric Detection Reagent into each well.

16. Cover the plate with aluminum foil and incubate the plate at RT for 15 minutes. During the 15-minute incubation, the plate can be placed on a rocker platform (optional).

17. Set the microplate reader and read absorbance at 630 nm.

18. The “Blank” value should be subtracted from all other readings.

Example Results

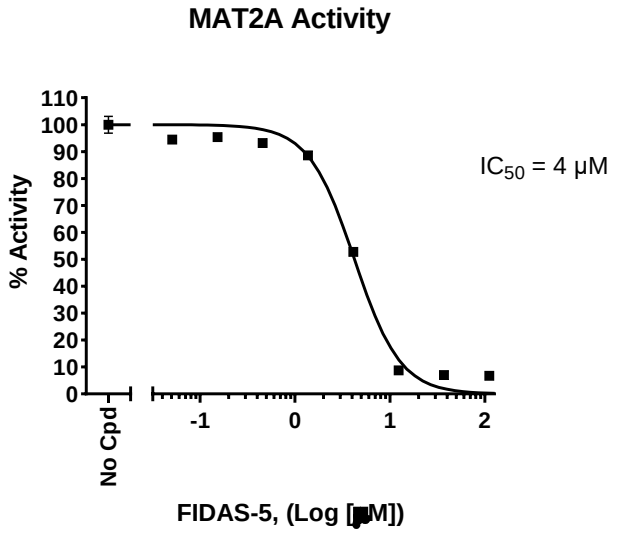

Figure 2: Inhibition of MAT2A activity by the inhibitor FIDAS-5.
MAT2A activity was measured in the presence of increasing concentrations of FIDAS-5 (Millipore #504173). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (activity in the absence of inhibitor, set at 100%).

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

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

References
Cacciatore A., *et al.*, 2024 *Nature Communications* 15 (6672).
Kalev P., *et al.*, 2021 *Cancer Cell* 39 (2):209-224.

Related Products

Products	*Catalog #*	*Size*
MAT2B, His-Tag Recombinant	79123	100 µg
MAT2A. His-Avi-Tag, Biotin-Labeled Recombinant	79308	25 µg/ 50 µg
MTAP, GST-tag Recombinant	50305	50 µg
EZH2 Chemiluminescence Assay Kit	52009L	96 reactions
EZH2-EED Binding Assay Kit	52066	384 reactions

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