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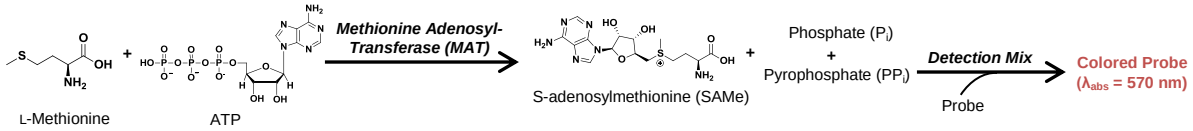
Methionine Adenosyltransferase Activity Assay Kit (Colorimetric)

02/20

(Catalog # K2033-100; 100 Reactions; Store at -20°C)

I. Introduction:

Methionine Adenosyltransferases (MATs, EC 2.5.1.6), also known as Adenosylmethionine Synthetases are a family of enzymes that synthesize *S*-adenosyl-L-methionine (SAMe) from L-methionine and ATP. SAMe is a primary biochemical alkylation agent (one of only two possible methyl group donors) and is also a vital metabolic precursor of the trans-sulfuration and polyamine synthesis (aminopropylation) pathways. All organisms express at least one MAT enzyme. In mammals, three isozymes of MAT have been identified. MAT1 and MAT3 isozymes are predominantly expressed in the liver, whereas MAT2A is expressed in most tissues. Upregulation of the MAT2A isozyme has been linked to several human diseases. MAT2A has become a popular drug target for novel cancer therapeutics as well as for hepatic fibrosis and non-alcoholic fatty liver disease. **BioVision's Methionine Adenosyltransferase Activity Assay Kit** enables the rapid measurement of MAT activity in complex biological matrices. The assay is based on the detection of pyrophosphate, which is generated stoichiometrically during the generation of SAMe. Pyrophosphate is enzymatically metabolized to an intermediate product, which reacts with the probe to form a stable chromophore that is detected by absorbance at 570 nm. The assay is homogeneous, simple to perform and does not require complicated sample processing. The assay is high-throughput adaptable and has a limit of quantification of 2 mU MAT activity per well.



II. Application:

- Rapid assessment of MAT activity in biological samples or recombinant MAT preparations

III. Sample Type:

- Human or animal soft tissue (*i.e.* brain, liver, lung, etc.) homogenates
- Cultured cell lysates (adherent or suspension cells)
- Heterologously expressed recombinant MAT preparations

IV. Kit Contents:

Components	K2033-100	Cap Code	Part Number
MAT Assay Buffer	25 ml	WM	K2033-100-1
MAT Probe	200 µl	Red	K2033-100-2
MAT Substrate Mix	1 vial	White	K2033-100-3
Detection Enzyme Mix	200 µl	Blue	K2033-100-4
Detection Cofactor Mix	1 vial	Amber	K2033-100-5
Developer Mix	1 vial	Green	K2033-100-6
MAT Positive Control	200 µl	Purple	K2033-100-7
Pyrophosphate Standard	200 µl	Orange	K2033-100-8

V. User Supplied Reagents and Equipment:

- Multiwell microplate spectrophotometer (capable of reading absorbance at 570 nm)
- Precision multi-channel pipette and reagent reservoir
- Clear 96-well plate with flat bottom

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Allow the MAT Assay Buffer to warm to room temperature (RT) prior to use. Read entire protocol before performing the assay procedure.

- **MAT Probe:** Provided as a solution in DMSO. Divide into aliquots and store at -20°C, protected from light. Prior to use, warm solution to RT. After use, promptly retighten the cap to minimize adsorption of airborne moisture.
- **MAT Substrate Mix** and **Detection Cofactor Mix:** Reconstitute with 220 µl of dH_2O, aliquot as desired and store at -20°C. Avoid repeated freeze/thaw cycles.
- **Detection Enzyme Mix** and **MAT Positive Control:** Store at -20°C, thaw and keep on ice while in use.
- **Developer Mix:** Reconstitute with 220 µl of MAT Assay Buffer. Divide into aliquots and store at -20°C, protected from light. Avoid repeated freeze/thaw cycles.
- **Pyrophosphate Standard (1 mM):** Provided as 1 mM stock solution. Store at -20°C, stable for at least 3 freeze/thaw cycles.

VII. Methionine Adenosyltransferase Activity Assay Protocol:

1. Sample Preparation:

a. Homogenize mammalian soft tissues (~50 mg) or pelleted, pre-washed cells (~5 x 10^6 cells) in 500 µl ice-cold MAT Assay Buffer. Incubate the homogenate on ice for 5 min and centrifuge at 10,000 x *g* and 4°C for 15 min. Collect the supernatant and keep on ice until use. Note: Tissue homogenates and cell lysates can also be aliquoted and stored at -80°C for future experiments.

b. Add 2-20 µl of the test sample(s) to desired wells in a clear, flat-bottom 96-well plate. For each Test Sample, prepare two parallel Sample wells, with one well serving as a Sample Background Control. Adjust the volume of all Sample and Sample Background Control wells to 50 µl per well with MAT Assay Buffer.

c. For Positive Control, dilute the MAT Positive Control at 1:5 ratio with MAT Assay Buffer immediately before use (for example, mix 20 µl MAT Positive Control with 80 µl MAT Assay Buffer). Add 10-20 µl of the diluted MAT Positive Control to desired well(s) and adjust the volume to 50 µl per well with MAT Assay Buffer.

Notes:

- The Sample volume and/or dilution factor required can vary based upon the nature of the Sample. For Unknown Samples, we suggest doing a pilot experiment by testing several amounts to ensure the readings are within the range of the Standard Curve.
- Once diluted, the MAT Positive Control should be kept on ice and used within 2 hr. Do not freeze diluted MAT Positive Control.
- We recommend measuring sample protein concentration using the Bradford reagent (BioVision Cat. #K810) or a comparable protein assay.

2. **Standard Curve Preparation:** Use Pyrophosphate Standard (1 mM) stock solution. Add 0, 2, 4, 6, 8 and 10 µl of Pyrophosphate Standard into a series of wells and adjust the volume to 50 µl per well with MAT Assay Buffer yielding 0, 2, 4, 6, 8 and 10 nmole/well Pyrophosphate Standard.

3. **Reaction Mix Preparation:**

a. Preincubate the plate for 10 min at 37°C to allow for temperature equilibration. During the preincubation, prepare Reaction Mix and Sample Background Mix according to the table below. Make a sufficient amount of each type of mix to add 50 µl to all assay wells of that type. *Remember to account for the Standard Curve wells when calculating the amount of Reaction Mix to prepare.*

	Reaction Mix	Sample Background Mix
Detection Enzyme Mix	2 µl	2 µl
Detection Cofactor Mix	2 µl	2 µl
MAT Substrate Mix	2 µl	—
Developer Mix	2 µl	2 µl
MAT Probe	2 µl	2 µl
MAT Assay Buffer	40 µl	42 µl

b. Add 50 µl of the Reaction Mix to all Sample, Positive Control (if applicable) and Standard Curve wells. Add 50 µl of the Sample Background Mix to all Sample Background Control well(s).

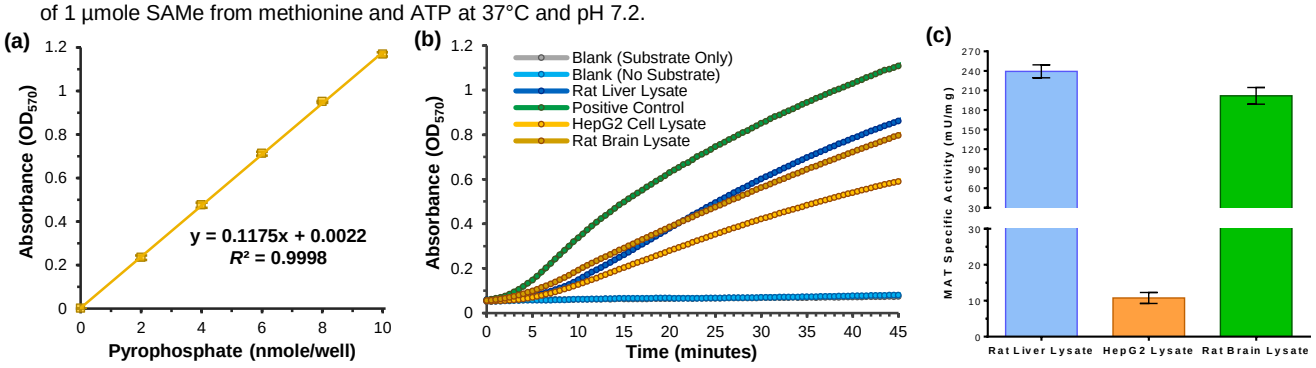
4. **Measurement:** Immediately begin measuring the absorbance at 570 nm in kinetic mode for 60 min at 37°C. We strongly recommend reading in kinetic mode in order to ensure that the measurements recorded are within the linear range of the reaction. Ideal measurement time for the linear range may vary depending upon the Sample. **Note:** The Pyrophosphate Standard Curve wells may be read in endpoint mode (OD at 570 nm).

5. **Calculations:** For the Pyrophosphate Standard Curve, subtract the 0 nmole/well reading from all Standard readings, plot the background-subtracted values and calculate the slope. For Sample reaction wells (including paired Sample Background Control wells), choose any two time points (T_1 and T_2) in the linear phase of the reaction progress curves. Obtain the corresponding absorbance values at those points (A_1 and A_2) and determine the change in absorbance over the time interval: $\Delta A = A_2 - A_1$. Subtract the Sample Background Control (ΔA_{BC}) from the corresponding Sample (ΔA_S) to obtain the net change in absorbance: $\Delta \boldsymbol{A}_{NET} = \Delta \boldsymbol{A}_S - \Delta \boldsymbol{A}_{BC}$. MATactivity is obtained by applying the net values to the Standard Curve to get *B* nmoles of substrate metabolized during the reaction time.

$$\textbf{Methionine Adenosyltransferase (MAT) Specific Activity} = \frac{\boldsymbol{B}}{\Delta \boldsymbol{T} \times \boldsymbol{P}} = \textbf{nmol/min/mg} \equiv \textbf{mU/mg}$$

Where: $\boldsymbol{B}$ is the amount of metabolite produced, calculated from the Standard Curve (in nmole)
$\Delta \boldsymbol{T}$ is the linear phase reaction time $T_2 - T_1$ (in min)
$\boldsymbol{P}$ is the amount of protein added to the Sample well (in mg)

MAT Unit Definition: One unit of MAT activity is the amount of enzyme that generates 1 µmole of pyrophosphate per min by synthesis of 1 µmole SAMe from methionine and ATP at 37°C and pH 7.2.



Figures: (a) Pyrophosphate Standard Curve. One mole of Pyrophosphate Standard corresponds to the synthesis of one mole of SAMe, with release of one mole free pyrophosphate. **(b)** Reaction kinetics of MAT activity in homogenates of rat liver (0.8 µg protein/well), rat brain (0.8 µg protein/well) and HepG2 cell lysate (10 µg protein/well). **(c)** Quantification of MAT activity in Samples (mean ± SEM of 4 or more independent replicates). Assays were performed according to the kit protocol.

VIII. Related Products:

Methionine Assay Kit (K442)	Homocysteine Assay Kit (K531)	Adenosylhomocysteinase Activity Assay Kit (K807)
Pyrophosphate Assay Kit (K586)	AdoMet Synthetase, E. coli (P1211)	S-Adenosylmethionine ELISA Kit (E4541)

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