Technical Bulletin

Methionine Adenosyltransferase Activity Assay Kit (Colorimetric)

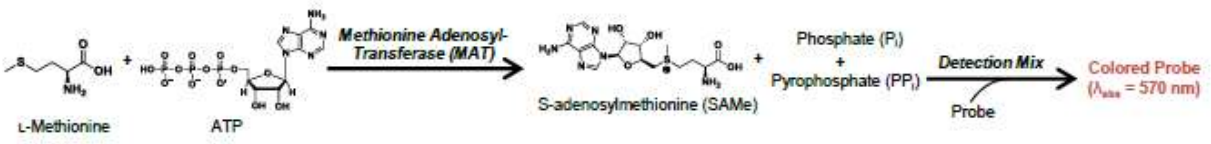
Catalog Number MAK431

Product Description

Methionine Adenosyltransferases (MATs), 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 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.

The 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 suitable for high-throughput applications and has a limit of quantification of 2 mU MAT activity per well.

The kit is suitable for the rapid assessment of MAT activity in human or animal soft tissue (i.e., brain, liver, lung, etc.), homogenates, cultured cell lysates (adherent or suspension cells), and heterologously expressed recombinant MAT preparations.



Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- MAT Assay Buffer 25 mL
  Catalog Number MAK431A
- MAT Probe 200 µL
  Catalog Number MAK431B
- MAT Substrate Mix 1 vial
  Catalog Number MAK431C
- Detection Enzyme Mix 200 µL
  Catalog Number MAK431D
- Detection Cofactor Mix 1 vial
  Catalog Number MAK431E
- Developer Mix 1 vial
  Catalog Number MAK431F
- MAT Positive Control 200 µL
  Catalog Number MAK431G
- Pyrophosphate Standard 200 µL
  Catalog Number MAK431H

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96 well clear flat-bottom plate. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Bradford Reagent
  (Catalog Number B6916 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store kit at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

MAT Assay Buffer: Ready to use. Allow the MAT Assay Buffer to warm to room temperature prior to use.

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 room temperature. After use, promptly retighten the cap to minimize adsorption of airborne moisture.

MAT Substrate Mix and Detection Cofactor Mix: Reconstitute each vial with 220 µL of purified water, 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 vial 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 three freeze/thaw cycles.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

1. Homogenize mammalian soft tissues (~50 mg) or pelleted, pre-washed cells (~5 × 10^6 cells) in 500 µL of ice-cold MAT Assay Buffer.
2. Incubate the homogenate on ice for 5 minutes and centrifuge at 10,000 × g and 4 °C for 15 minutes.
3. Collect the supernatant and keep on ice until use. Tissue homogenates and cell lysates can also be aliquoted and stored at -80 °C for future experiments.
4. Measure sample protein concentration using Bradford reagent.
5. 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. The Sample volume and/or dilution factor required can vary based upon the nature of the sample. For unknown samples, perform a pilot experiment by testing several amounts to ensure the readings are within the range of the Standard Curve.
6. Adjust the volume of all Sample (S) and Sample Background Control (BC) wells to 50 µL per well with MAT Assay Buffer.

Positive Control

Dilute the MAT Positive Control 1:5 with MAT Assay Buffer immediately before use (e.g., mix 20 µL MAT Positive Control with 80 µL MAT Assay Buffer). Once diluted, the MAT Positive Control should be kept on ice and used within 2 hours. **Do not freeze** diluted MAT Positive Control. 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.

Standard Curve Preparation

Using Pyrophosphate Standard (1 mM) stock solution, prepare Pyrophosphate Standards according to Table 1. Mix well.

Table 1.
Preparation of Pyrophosphate Standards

Well	1 mM Pyro-phosphate Standard	MAT Assay Buffer	Pyro-phosphate (nmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	2
3	4 µL	46 µL	4
4	6 µL	44 µL	6
5	8 µL	42 µL	8
6	10 µL	40 µL	10

Reaction Mix

1. Preincubate the plate for 10 minutes at 37°C to allow for temperature equilibration.
2. During the preincubation, prepare Reaction Mix and Sample Background Mix according to Table 2. Mix enough reagents for the number of assays to be performed. For each well containing Standard or Sample (S), prepare 50 µL of Reaction Mix. For each well containing Sample Background Control (BC), prepare 50 µL of Sample Background Mix. Mix well.

Table 2.
Preparation of Reaction Mixes

Reagent	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

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

Measurement

Immediately begin measuring the absorbance at 570 nm (A_{570}) in kinetic mode for 60 minutes at 37 °C. It is strongly recommended to read 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. The Pyrophosphate Standard Curve wells may be read in endpoint mode at 570 nm.

Results

1. For the Pyrophosphate Standard Curve, subtract the 0 nmole/well reading from all Standard readings.
2. Plot the background-subtracted values and calculate the slope.
3. 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.
4. Obtain the corresponding absorbance values at those points (A_1 and A_2) and determine the change in absorbance over the time interval: $\Delta A = A_2 - A_1$.
5. Subtract the Sample Background Control (ΔA_{BC}) from the corresponding Sample (ΔA_S) to obtain the net change in absorbance: $\Delta A_{NET} = \Delta A_S - \Delta A_{BC}$.
6. MAT activity is obtained by applying the Sample net values to the Standard Curve to get B nmoles of substrate metabolized during the reaction time.

Methionine Adenosyl transferase (MAT) Specific Activity (nmol/min/mg or mU/mg) =

$$B/ (\Delta T \times P)$$

where:

B = Amount of metabolite produced, calculated from the Standard Curve (in nmole)

ΔT = Linear phase reaction time $T_2 - T_1$ (in minutes)

P = Amount of protein added to the sample well (in mg)

Figure 1.
Typical Pyrophosphate Standard Curve. One mole of Pyrophosphate Standard corresponds to the synthesis of one mole of SAMe, with release of one mole free pyrophosphate.

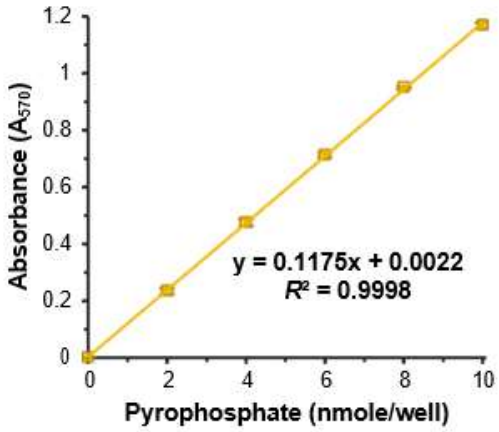


Figure 2.
Reaction kinetics of MAT activity in homogenates of rat liver (0.8 μg protein/well), rat brain (0.8 μg protein/well) and HepG2 cell lysate (10 μg protein/well).

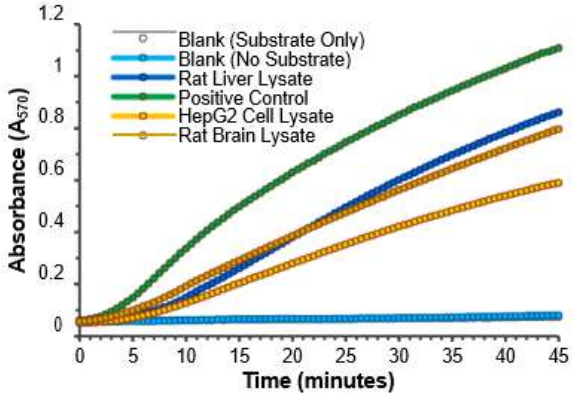
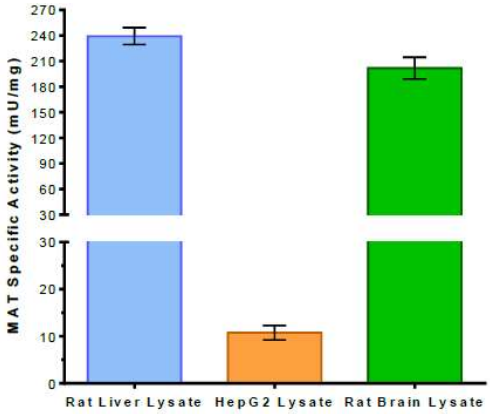


Figure 3.
Quantification of MAT activity in Samples (mean ± SEM of 4 or more independent replicates). Assays were performed according to the kit protocol.



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