ab284535 – Methionine Adenosyltransferase Activity Assay Kit (Colorimetric)

For the rapid assessment of MAT activity in biological samples or recombinant MAT preparations.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284535

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, store the kit components as per the respective temperatures mentioned below. Use kit within 1 year.

Materials Supplied

Item	Quantity	Storage Condition
MAT Assay Buffer	25 mL	-20°C
MAT Probe	200 µL	-20°C
MAT Substrate Mix	1 vial	-20°C
Detection Enzyme Mix	200 µL	-20°C
Detection Cofactor Mix	1 vial	-20°C
Developer Mix	1 vial	-20°C
MAT Positive Control	200 µL	-20°C
Pyrophosphate Standard	200 µL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

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 Assay Buffer: Prior to use, warm solution to RT.

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.

Methionine Adenosyltransferase Activity Assay Protocol

Sample Preparation:

1. 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.

Δ Note: *Tissue homogenates and cell lysates can also be aliquoted and stored at -80°C for future experiments.*

2. 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.
3. 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.

Δ Note: *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.*
Δ Note: *Once diluted, the MAT Positive Control should be kept on ice and used within 2 hr. Do not freeze diluted MAT Positive Control.*
Δ Note: *We recommend measuring sample protein concentration using the Bradford reagent or a comparable protein assay.*

Standard Curve Preparation:

1. 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.

Reaction Mix Preparation:

1. 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

2. 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).

Measurement

1. 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.

Δ Note: *The Pyrophosphate Standard Curve wells may be read in endpoint mode (OD at 570 nm).*

Calculation:

1. For the Pyrophosphate Standard Curve, subtract the 0 nmole/well reading from all Standard readings, plot the background-subtracted values and calculate the slope.
2. For Sample reaction wells (including paired Sample Background Control wells), choose any two time points (T1 and T2) in the linear phase of the reaction progress curves. Obtain the corresponding absorbance values at those points (A1 and A2) and determine the change in absorbance over the time interval: ΔA = A2 – A1.
3. Subtract the Sample Background Control (ΔABC) from the corresponding Sample (ΔAS) to obtain the net change in absorbance: ΔANET = ΔAS – ΔABC.
4. MAT activity is obtained by applying the net values to the Standard Curve to get B nmol of substrate metabolized during the reaction time.

MAT Specific Activity = B / (ΔT x P) = nmol/min/mg = mU/mg

Where:
B = Amount of metabolite produced, calculated from the Standard Curve (nmole)
ΔT = T2 - T1 (min)
P = Sample protein amount added per well (mg)

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.

Technical Support

For all technical or commercial enquiries please go to:

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