Methionine Assay kit

ARG83377 Methionine Assay kit is an assay kit for Methionine in Serum, plasma, urine, Cell culture supernatants, cell lysate and tissue lysates.

Catalog number: ARG83377

Package: 100 assay

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INTRODUCTION

L-methionine is the L-enantiomer of methionine. It has a role as a nutraceutical, a micronutrient, an antidote to paracetamol poisoning, a human metabolite and a mouse metabolite. It is an aspartate family amino acid, a proteinogenic amino acid, a methionine and a L-alpha-amino acid. It is a conjugate base of a L-methioninium. It is a conjugate acid of a L-methioninate. It is an enantiomer of a D-methionine. It is a tautomer of a L-methionine zwitterion.

PRINCIPLE OF THE ASSAY

ARG83377 Methionine Assay Kit measures Methionine content in biological samples. Methionine and ATP are converted by s-adenosylmethionine synthetase to s-adenosylmethionine, inorganic phosphate, and pyrophosphate. Phosphoenolpyruvate and pyrophosphate are converted by phosphate pyruvate dikinase to pyruvate. Pyruvate is converted by pyruvate oxidase in the presence of phosphate and oxygen into acetyl phosphate, carbon dioxide, and hydrogen peroxide. Samples are compared to a known concentration of Methionine standard. The intensity of the color is measured at a wavelength of 540 nm. The concentration of Methionine in the sample is then determined by comparing the O.D. of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store 10X Assay Buffer at RT, Store AdoMetS and PPDK at -80°C

Store other component at ≤ -20°C. Use the kit before expiration date.

Component	Quantity	Storage information
Standard	50 µL	-20°C
10X Assay Buffer	25 mL	RT
s-adenosylmethionine synthetase (AdoMetS)	150 µL	-80°C
Phosphate pyruvate dikinase (PPDK)	200 µL	-80°C
Adenosine triphosphate (ATP)	50 µL	-20°C
Adenosine monophosphate (AMP)	50 µL	-20°C
Phosphoenolpyruvate (PEP)	50 µL	-20°C
Sodium phosphate dibasic (Na2HPO4)	50 µL	-20°C
Probe	50 µL	-20°C
HRP-Streptavidin Solution	10 µL	-20°C
Flavin Adenine Dinucleotide (FAD)	50 µL	-20°C
Thiamine Pyrophosphate (TPP)	50 µL	-20°C
Pyruvate Oxidase	300 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 nm
- Flat bottomed 96-well black microplate and tube.
- 1X PBS and deionized water
- Pipettes and pipette tips
- 10 kDa molecular weight cutoff spin filter

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection.
- Upon received, store 10X Assay Buffer at RT, Store AdoMetS and PPDK at -80°C Store other component at ≤ -20°C. Use the kit before expiration date and avoid freeze / thaws.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to 4°C / room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Urine - Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Cell Lysates: Wash cells 3 times with cold PBS prior to lysis. Lyse cells with sonication or homogenation in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at -80°C. Perform dilutions in PBS.

Tissue Lysates: Sonicate or homogenize tissue sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at -80°C. Perform dilutions in PBS.

REAGENT PREPARATION

- **1X Assay Buffer:** Dilute the 10X Assay Buffer with deionized water to yield 1X Assay Buffer. Store at RT.

- **Reaction Mix and Control Mix:**

  Prepare two separate mixtures according to the table below.

Component	Reaction Mix (20 assays)	Negative Control Mix (20 assays)
AdoMetS	30 µL	-
PPDK	40 µL	40 µL
ATP	10 µL	10 µL
AMP	10 µL	10 µL
PEP	10 µL	10 µL
HRP	2 µL	2 µL
Pyruvate Oxidase	60 µL	60 µL
FAD	10 µL	10 µL
TPP	10 µL	10 µL
Na2HPO4	10 µL	10 µL
Fluorometric Probe	10 µL	10 µL
1X Assay Buffer	798 µL	828 µL
Total	**1000 µL**	**1000 µL**

- **Standards**: Add 5 µl of 10.0 mM stock standard into 495 µl 1X Assay Buffer to generate a standard with 100 µM of Tyrosine. Dilute the standards with 1X Assay Buffer serves as zero standard (blank standard, 0 µM). The example of the standards dilution table is as below:

Standard	Methionine (µM)	Volume of 1X Assay Buffer (µL)	Volume of Methionine (µL)
S1	100	495	5 (10 mM stock)
S2	50	250	250(S1)
S3	25	250	250(S2)
S4	12.5	250	250(S3)
S5	6.25	250	250(S4)
S6	3.13	250	250(S5)
S7	1.56	250	250(S6)
S8	0	250	0

ASSAY PROCEDURE

Each samples should be assayed in at least duplicates, one to be treated with AdoMetS and one without, to measure endogenous background.

1. Add **50 µl** of **standard** and **sample** to each wells.
2. Add **50 µL** of **Reaction Mix** to **standard** and half of sample wells.
3. Add **50 µL** of **Negative Control Mix** to other half of sample wells.
4. Mix well and Incubate for 30 min at 37°C.

   Note: This assay is continuous (not terminated), therefore may be measured at multiple time points to follow the reaction kinetics.
5. Read O.D. with a microplate reader at **590 nm** immediately.

CALCULATION OF RESULTS

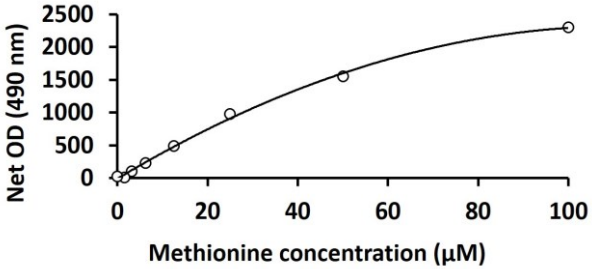
1. Calculate the average absorbance value for each set of standards and samples.
2. Subtract the average value of Standard 0 from all standard value.
3. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
4. Subtract the sample well values without AdoMetS from the sample well values containing AdoMetS (Control Mix) to obtain the difference. The absorbance difference is due to the L-Methionine Dehydrogenase activity:

   $\Delta A = A_{\text{Reaction Mix}} - A_{\text{Negative Control Mix}}$

5. Compare the change in absorbance Δ A of each sample to the standard curve to determine and extrapolate the quantity of bile acid present in the sample. Only use values within the range of the standard curve.

EXAMPLE OF TYPICAL STANDARD CURVE

The following table shows the OD readings of a run of this assay kit with serial diluted standards



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Methionine ranged from 1.56-100 µM. The mean MDD was 0.8 µM