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Product Information

Methionine Assay Kit

Catalog Number **MAK347**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Methionine (Met) is one of the twenty proteogenic amino acids encoded by the genetic code, and one of nine essential amino acids, meaning it cannot be synthesized *in vivo* and must be obtained from dietary sources. Met is one of two sulfur-containing amino acids (the other being cysteine) and can serve as a precursor in cysteine biosynthesis. S-Adenosyl-methionine, another product of Met metabolism, is an important cofactor and methyl donor in a number of biological reactions such as methylation and polyamine synthesis. Met is also an intermediate species in the synthesis of phospholipids such as lecithin, and other small precursor molecules including taurine, an aminosulfonic acid. Oxidation of Met generates methionine sulfoxide, a species whose concentration tends to increase with age and is known to cause protein misfolding and loss-of-function of numerous enzymes. This version of the amino acid can be restored back to methionine by another enzyme, methionine sulfoxide reductase. Biologically, Met levels can affect important events such as angiogenesis, protein synthesis, and cartilage production.

The Methionine Assay Kit utilizes an enzymatic mechanism by which metabolism of methionine is coupled with the stoichiometric generation of hydrogen peroxide. A probe generates fluorometric signal that can be quantified at λ_{ex} = 535 nm/λ_{em} = 587 nm. The assay shows greater than 10-fold specificity for methionine over cysteine and greater than 40-fold selectivity over other standard and non-standard amino acids. After utilization of a proprietary Sample Clean-Up Mix, the method is suitable for use in blood samples, and can detect concentrations as low as 0.5 μM, or 25 pmole methionine.

The kit is suitable for the determination of methionine concentration in serum, plasma, cell cultures, and tissue lysates.

Components

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

Met Assay Buffer Catalog Number MAK347A	25 mL
Met Buffer Supplement Catalog Number MAK347B	1 vial
Met Enzyme Mix I Catalog Number MAK347C	200 μL
Met Enzyme Mix II Catalog Number MAK347D	1 vial
Met Developer Catalog Number MAK347E	200 μL
Met Probe Catalog Number MAK347F	200 μL
Sample Clean-Up Mix Catalog Number MAK347G	1 vial
Met Standard (10 mM) Catalog Number MAK347H	100 μL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Black flatbottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Fluorescence multiwell plate reader, capable of 37 °C temperature setting
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)

Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The kit is shipped on wet ice. Store components at –20 °C, protected from light upon receiving. Briefly centrifuge small vials prior to opening.

Preparation Instructions.

Reagent Preparation

Met Assay Buffer, Met Probe, and Met Standard (10 mM) – Warm to room temperature prior to use.

Met Buffer Supplement – Add 220 µL of Met Assay Buffer to the vial. Pipet up and down to mix well. Store at –20 °C. Solution is stable for two months once reconstituted.

Sample Clean-Up Mix and Met Enzyme Mix II – Reconstitute each vial with 220 µL of Met Assay Buffer and pipette up and down to mix well. Solutions are stable for two months once reconstituted.

Procedure
Sample Preparation
1. For blood plasma and serum, pretreat samples by adding 2 µL of Sample Clean-Up Mix to 100 µL of sample.
2. Incubate at 37 °C for 30 minutes.
3. Following incubation, filter samples by spinning through a Corning Spin-X UF concentrator at 10,000 × g and 4 °C for 10 minutes.
4. Retain ultrafiltrate.
5. Add 2–20 µL of ultrafiltrate per well and bring up the volume to 50 µL with Met Assay Buffer.
6. For each sample, prepare two parallel wells, one for determination of methionine and one to serve as the sample background control.

Standard Curve Preparation
Prepare a 100 µM Met Standard solution by adding 10 µL of Met Standard (10 mM) to 990 µL of Met Assay Buffer and mix well. Prepare Met Standards in a series of wells in a black, flat-bottom 96-well plate as described in Table 1.

Table 1.
Preparation of Met Standards

Well	100 µM Premix	Met Assay Buffer	Met (pmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	200
3	4 µL	46 µL	400
4	6 µL	44 µL	600
5	8 µL	42 µL	800
6	10 µL	40 µL	1,000

Reaction Mix
Mix enough reagent for the number of samples and standards to be performed: For each well (samples and standards), prepare 50 µL of Reaction Mix according to Table 2. For sample background wells, prepare 50 µL of Background Control Mix according to Table 2.

Table 2.
Preparation of Reaction Mixes

Reagent	Reaction Mix (per well)	Background Control Mix (per well)
Met Assay Buffer	41.6 µL	43.6 µL
Met Probe	0.4 µL	0.4 µL
Met Buffer Supplement	2 µL	2 µL
Met Enzyme Mix I	2 µL	–
Met Enzyme Mix II	2 µL	2 µL
Met Developer	2 µL	2 µL

Note: If only a couple of experiments (1 or 2 wells) are to be run, the Met Probe should be diluted 5-fold in Met Assay Buffer immediately prior to running the experiment. If using diluted Met Probe, use 40 µL of Buffer and 2 µL of diluted Probe per well in the Reaction Mix, and 42 µL of Buffer with 2 µL of diluted Probe per well in the Background Control Mix.

Assay Reaction
1. Add 50 µL of Reaction Mix and 50 µL of Background Control Mix to the respective parallel sample wells.
2. Incubate plate at 37 °C for 30 minutes.
3. Read fluorescence in end point mode (λ_{ex} = 535 nm/λ_{em} = 587 nm).

Results

Subtract the 0 Met standard reading from all standard readings, then plot the background-subtracted Met standards to generate the standard curve (from 0-1000 pmol Met). For sample readings, subtract the reading obtained from the parallel reaction containing Background Control Mix. Compare the background-subtracted values to the standard curve to calculate Met concentration:

Methionine Conc. (pmol/μL or μM) =

$$\frac{\text{Met from standard curve (pmol)}}{\text{Volume of sample (μL)}} \times \text{Dilution Factor}$$

Figure 1.
Typical Met Standard Curve

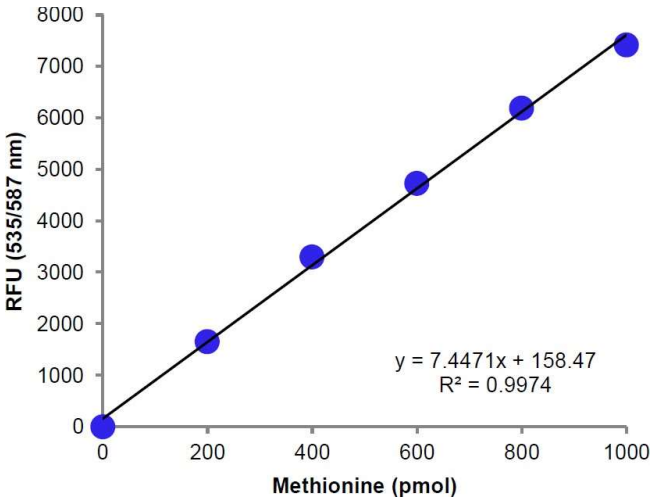
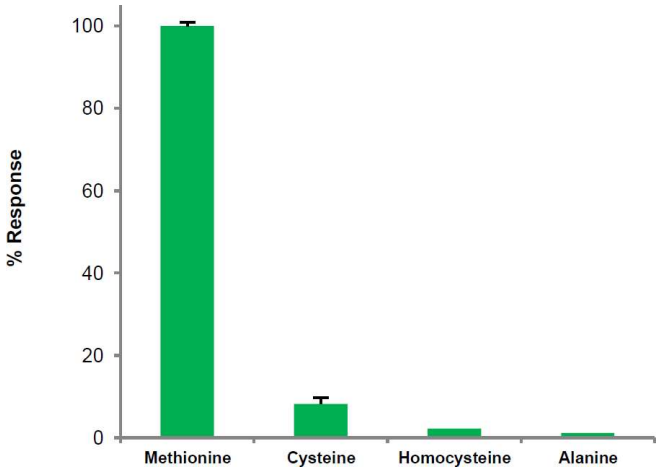


Figure 2.
Assay Specificity



The assay was run in the presence of 1 nmole (1,000 pmole) each of the amino acids Methionine, Cysteine, Homocysteine, and Alanine. Numbers are relative to 100% response to methionine.

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