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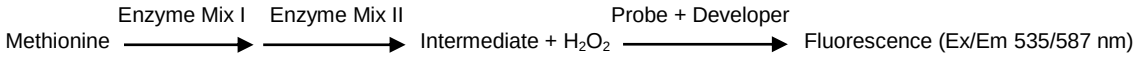
Methionine Assay Kit (Fluorometric)

rev 06/19

(Catalog # K442-100; 100 assays; Store at -20°C)

I. Introduction:

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*-adenosylmethionine, another product of Met metabolism, is an important co-factor 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 amino-sulfonic 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. BioVision's 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 Ex/Em= 535/587 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.



II. Applications:

- Determination of methionine concentration in biological samples.

III. Sample Type:

- Biological Samples: Serum, Plasma, Cell/Tissue Lysates

IV. Kit Contents:

Components	K442-100	Cap Code	Part Number
Met Assay Buffer	25 ml	WM	K442-100-1
Met Buffer Supplement	1 vial	Orange	K442-100-2
Met Enzyme Mix I	200 µl	Purple	K442-100-3
Met Enzyme Mix II	1 vial	Green	K442-100-4
Met Developer	200 µl	Blue	K442-100-5
Met Probe	200 µl	Red	K442-100-6
Sample Clean-Up Mix	1 vial	Clear	K442-100-7
Met Standard (10 mM)	100 µl	Yellow	K442-100-8

V. User Supplied Reagents & Equipment:

- Plate Reader capable of 37°C temperature setting and fluorescence readings
- 96-well plate (preferably opaque black)
- 10k Spin columns for sample preparation (BV Cat. No. 1997)

VI. Storage and Reagents Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **Met Assay Buffer:** Store at -20°C. Warm to room temperature (RT) before use. Stable for two months.
- **Met Buffer Supplement:** Add 220 µl of Met Assay Buffer to the vial. Pipet up and down to mix well. Store at -20°C. Stable for two months.
- **Met Enzyme Mix II and Sample Clean-Up Mix:** Add 220 µl of Met Assay Buffer to each vial of Met Enzyme Mix II and Sample Clean-Up Mix. Pipet up and down to mix well. Store at -20°C. Stable for two months.
- **Met Developer and Met Enzyme Mix I:** Ready to use. Store at -20°C. Stable for two months.
- **Met Probe and Met Standard (10 mM):** Ready to use. Warm to RT before use. Store at -20°C. Stable for two months.

VII. Methionine Assay Protocol:

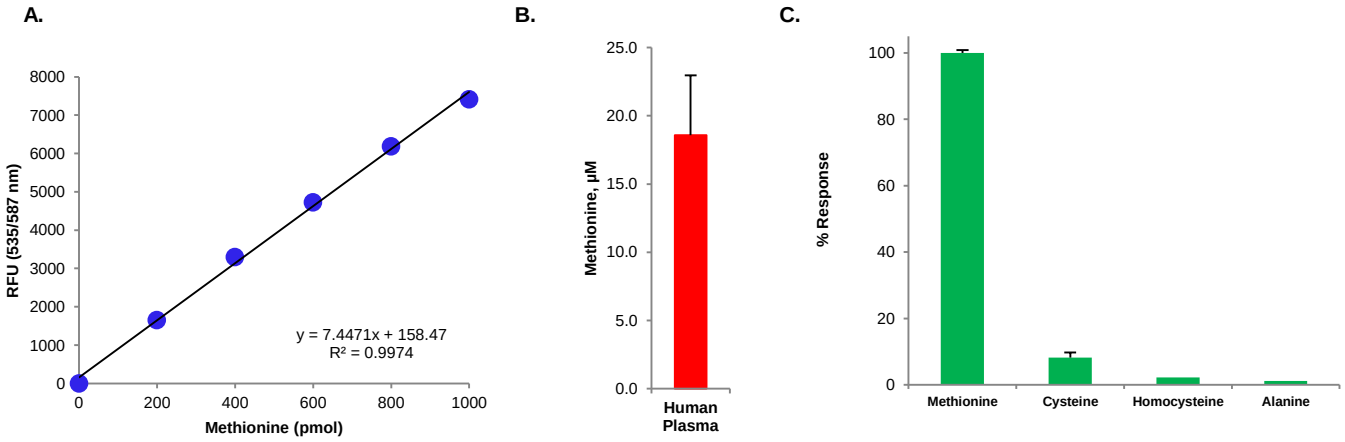
1. **Sample Preparation:** For blood plasma and serum, pretreat samples by adding 2 µl Sample Clean-Up Mix to 100 µl sample and incubate at 37°C for 30 min. Following incubation, filter samples by spinning through a 10 kDa spin column (10000 x *g*, 4 °C, 10 min; BV #1997) and retain the ultrafiltrate. Add 2-20 µl of ultrafiltrate per well and bring up the volume to 50 µl with Met Assay Buffer. For each sample, prepare two parallel wells, one for determination of methionine and one as the sample background control.
2. **Standard Curve Preparation:** Prepare 100 µM Met Standard as follows:
   a. Generate the 100 µM Met Stock by adding 10 µl 10 mM Met Solution to 990 µl Met Assay Buffer. Mix well.
   b. Add 0, 2, 4, 6, 8, and 10 µl of the 100 µM Met Stock to each well individually to generate standards of 0, 200, 400, 600, 800, and 1000 pmol Met/well. Adjust the volume of each well to 50 µl with Met Assay Buffer.
3. **Reaction Mix**: Mix enough reagent for the number of samples and standards to be performed: For each well (samples and standards), prepare 50 µl Reaction Mix. For sample background wells, prepare 50 µl Background Control Mix:

	Reaction Mix (per well)	Background Control Mix (per well)
Met Assay Buffer	41.6 µl	43.6 µl
Met 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

Add 50 µl Reaction Mix and 50 µl Background Control Mix to the respective parallel sample wells.
Note: If only several experiments are to be run, the Met Probe should be diluted 1:5 in Met Assay Buffer immediately prior to running the experiment. If using diluted Met Probe, use 40 µl Buffer and 2 µl diluted Probe per well in the Reaction Mix, and 42 µl Buffer with 2 µl diluted Probe per well in the Background Control Mix.

4. **Measurement:** Incubate plate at 37°C for 30 min and read fluorescence in end point mode (Ex/Em= 535/587 nm).
5. **Calculations:** Subtract the 0 Met standard reading from all standard readings, and plot the background subtracted Met standards to generate the Met standard curve (from 0-1000 pmol Met). For sample readings, subtract the reading obtained from the parallel reaction containing Background Control Mix. Apply the background-subtracted values to the standard curve to calculate the Met concentration:

$$\textbf{Methionine Concentration} = \left(\frac{\text{Met amount from standard curve (pmol)}}{\text{Vol. of sample(µl)}}\right) x\ Dilution\ Factor \left(\frac{pmol}{\mu l}\ or\ \mu M\right)$$



Figures. (A) Met Standard Curve. (B) Methionine in human plasma: The assay was run on various volumes of treated plasma and the results averaged. Met concentration was determined to be 18.6 µM in human plasma (2-12 µl; undiluted). **(C) Specificity of the Assay:** The assay was run in the presence of 1 nmole (1000 pmole) each of the amino acids Methionine, Cysteine, Homocysteine, and Alanine. Numbers are relative to 100% response to methionine.

VIII. RELATED PRODUCTS:

Glycine Assay Kit (K589)
Glutamine Assay Kit (K556)
Cysteine Assay Kit (K558)
Glutamate Assay Kit (K629)
Alanine Assay Kit (K652)
Tyrosine Assay Kit (K573)
Aspartate Assay Kit (K552)
Phenylalanine Assay Kit (K572)

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