ab234041 – Methionine Assay Kit (Fluorometric)

For the measurement of Methionine in plasma, cell lysates and serum.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab234041

Storage and Stability

All components in this kit are shipped on blue ice and are suitable for storage at -80°C, unless reconstituted. Upon receipt, immediately store kit at -80°C in the dark. Individual components may be stored at alternative temperatures as show in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.
Aliquot components in working volumes before storing at the recommended temperature.
Avoid repeated freeze-thaws of reagents.

Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer 45	25 mL	-80°C or -20°C	-20°C
Buffer Supplement I	1 vial	-80°C or -20°C	-20°C
Developer Mix C	200 µL	-80°C or -20°C	-20°C
Met Enzyme Mix I	200 µL	-80°C	-80°C
Developer Mix A	1 vial	-80°C or -20°C	-20°C
OxiRed™ Probe	0.2 mL	-80°C or -20°C	-20°C
Methionine Standard	100 µL	-80°C or -20°C	-20°C
Sample Clean-up Mix	1 vial	-80°C or -20°C	-20°C

PLEASE NOTE: Assay Buffer 45 was previously labelled as Assay Buffer XLV and Methionine Assay Buffer, and Buffer Supplement I as Methionine Buffer Supplement, and Developer Mix C as Development Enzyme Mix III and Methionine Developer, and Met Enzyme Mix I as Methionine Enzyme Mix I, and Developer Mix A as Development Enzyme Mix I and Methionine Enzyme Mix II, and OxiRed™ Probe as Methionine Probe. The composition has not changed.

Materials Required, Not Supplied

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

- Plate Reader capable of 37°C temperature setting and fluorescence readings
- 96-well plate (preferably opaque black)
- 10k Spin columns for sample preparation

Reagent Preparation

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

Assay Buffer 45: Store at -20 °C. Warm to room temperature (RT) before use. Stable for two months.

Buffer Supplement I: Add 220 µl of Assay Buffer 45 to the vial. Pipet up and down to mix well. Store at -20 °C. Stable for two months.

Met Enzyme Mix I and Developer Mix C: Ready to use. Divide into aliquots and store at -80 °C. Stable for two months.

Developer Mix A and Sample Clean-Up Mix: Add 220 µl of Assay Buffer 45 to each vial of Developer Mix A and Sample Clean-Up Mix. Pipet up and down to mix well. Store at -20 °C. Stable for two months.

OxiRed™ Probe and Methionine Standard (10 mM): Ready to use. Warm to RT before use. Store at -20 °C. Stable for two months.

Assay Protocol

Sample Preparation

- For blood plasma and serum, pre-treat 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) and retain the ultrafiltrate. Add 2-20 µl of ultrafiltrate per well and bring up the volume to 50 µl with Assay Buffer 45. For each sample, prepare two parallel wells, one for determination of methionine and one as the sample background control.

Standard Curve Preparation:

- Prepare 100 µM Methionine Standard as follows:
  a) Generate the 100 µM Met Stock by adding 10 µl 10 mM Met Solution to 990 µl Assay Buffer 45. 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 Assay Buffer 45.

Reaction Mix:

1. 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:

Item	Reaction Mix	Background Control Mix (per well)
Assay Buffer 45	41.6 µl	43.6 µl
OxiRed™ Probe	0.4 µl	0.4 µl
Buffer Supplement I	2 µl	2 µl
Met Enzyme Mix I	2 µl	---
Developer Mix A	2 µl	2 µl
Developer Mix C	2 µl	2 µl

2. 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 OxiRed™ Probe should be diluted 1:5 in Assay Buffer 45 immediately prior to running the experiment. If using diluted OxiRed™ Probe, use 40 µl Buffer and 2 µl diluted OxiRed™ Probe per well in the Reaction Mix, and 42 µl Buffer with 2 µl diluted OxiRed™ Probe per well in the Background Control Mix.

Measurement

Incubate plate at 37 °C for 30 min and read fluorescence in end point mode (Ex/Em= 535/587 nm).

Calculation

- Subtract the 0 Methionine Standard reading from all Standard readings, and plot the background subtracted Methionine Standard to generate the Methionine 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:

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

Technical Support

For all technical or commercial enquiries please go to:
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www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)