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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Storage and Stability

Store kit at -80°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if 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 XLV/Methionine Assay Buffer	25 mL	-80°C	-20°C
Buffer Supplement I/Methionine Buffer Supplement	1 vial	-80°C	-20°C
Development Enzyme Mix III/Methionine Developer	200 µL	-80°C	-80°C
Met Enzyme Mix I/Methionine Enzyme Mix I	200 µL	-80°C	-80°C
Development Enzyme Mix I/Methionine Enzyme Mix II	1 vial	-80°C	-20°C
OxiRed Probe/Methionine Probe	200 µL	-80°C	-20°C
Methionine Standard/Methionine Standard (10 mM)	100 µL	-80°C	-20°C
Sample Clean-up Mix	1 vial	-80°C	-20°C

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

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

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

Development Enzyme Mix I/Met Enzyme Mix II and Sample Clean-Up Mix: Add 220 µl of Assay Buffer XLV/Met Assay Buffer to each vial of Development Enzyme Mix I/Met Enzyme Mix II and Sample Clean-Up Mix. Pipet up and down to mix well. Store at -20 °C. Stable for two months.

OxiRed Probe/Met Probe and Methionine Standard/Met 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 XLV/Met Assay Buffer. 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/Met Standard as follows:
  a) Generate the 100 µM Met Stock by adding 10 µl 10 mM Met Solution to 990 µl Assay Buffer XLV/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 Assay Buffer XLV/Met Assay Buffer.

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 XLV/Met Assay Buffer	41.6 µl	43.6 µl
OxiRed Probe/Met Probe	0.4 µl	0.4 µl
Buffer Supplement I/Met Buffer Supplement	2 µl	2 µl
Met Enzyme Mix I	2 µl	---
Development Enzyme Mix I/Met Enzyme Mix II	2 µl	2 µl
Development Enzyme Mix III/Met Developer	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/Met Probe should be diluted 1:5 in Assay Buffer XLV/Met Assay Buffer immediately prior to running the experiment. If using diluted OxiRed Probe/Met Probe, use 40 µl Buffer and 2 µl diluted OxiRed Probe/Probe per well in the Reaction Mix, and 42 µl Buffer with 2 µl diluted OxiRed Probe/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/Met Standard reading from all Standard readings, and plot the background subtracted Methionine Standard/Met Standards to generate the Methionine Standard/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:

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

Technical Support

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