ab228559 – Homocysteine Assay Kit (Fluorometric)

For quantification of total homocysteine in biological fluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab228559

Storage and Stability

Store kit at -20°C and protect from light.

Materials Supplied

Item	Quantity	Storage Condition
Developer Solution XI	5 ml	-20°C
Reducing Agent II	300 µl	-20°C
Fluorogenic Probe Solution	5 ml	-20°C
Homocysteine Assay Buffer	25 ml	-20°C
Homocysteine Disulfide Standard	1 vial	-20°C
Homocysteine Enzyme Mix	1 vial	-20°C

PLEASE NOTE: Developer Solution XI was previously labelled as Developer Solution, and Reducing Agent II as Disulfide Reducing Agent (DTT). 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:

- Multiwell fluorescence microplate reader
- Precision multi-channel pipette and reagent reservoir
- White 96-well plates with flat bottom

Reagent Preparation

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

Reducing Agent II: Provided as a 100 mM stock solution. Aliquot and store at -20°C, avoid repeated freeze/thaw cycles.

Homocysteine Enzyme Mix: Reconstitute in 330 µl of Homocysteine Assay Buffer to generate a 10X stock solution. Divide into
aliquots and store at -20°C, protected from light. Avoid repeated freeze/thaw cycles.

Fluorogenic Probe Solution: Warm to RT prior to use. Store at 4°C, protected from light.

Developer Solution XI: Warm to RT prior to use. Store at 4°C, protected from light.

Homocysteine Disulfide Standard: Reconstitute in 220 µl of dH2O for a 1 mM solution. Store at -20°C, stable for 5 freeze/thaw cycles.

Sample Preparation

1. Aliquot enough Homocysteine Assay Buffer for the number of reactions to be performed.
2. Add Reducing Agent II to Homocysteine Assay Buffer at a 1:100 ratio (10 µl of 100 mM DTT stock per 1 ml of Homocysteine Assay Buffer) immediately prior to use.
3. Collect plasma or serum samples by standard methods (keep on ice for immediate use or store at -80°C for future experiments).
4. Add 10 µl of undiluted serum/plasma to desired well(s) in a white, flat bottom 96-well plate. For each test sample, prepare a parallel sample well to serve as the sample background control.
5. Add 160 µl of Homocysteine Assay Buffer (with DTT) to all sample reaction wells (bringing the volume to 170 µl/well).

   ∆ **Notes:**

   a) Always prepare fresh Homocysteine Assay Buffer with DTT. Once prepared, keep buffer with DTT on ice and use within 4 hrs.

   b) The normal physiological range for homocysteine in human plasma is 6-12 µM; however levels can be dramatically higher in certain cases. For unknown samples, we suggest doing a pilot experiment to ensure readings are within the range of the standard curve (50-500 pmol/well or 5-50 µM). Samples with higher levels of homocysteine may be diluted with Homocysteine Assay Buffer or PBS.

   c) Plasma or serum samples exhibiting lipemia or excessive turbidity should be clarified by centrifugation prior to use. Hemolytic samples should not be used, due to release of homocysteine from lysed red blood cells.

   d) To ensure accurate determination of homocysteine in test samples or for samples with a low concentration of homocysteine, we recommend spiking samples with a known amount of Homocysteine Disulfide Standard (50 pmol).

Standard Curve Preparation

1. Prepare a 25 µM solution of Homocysteine Disulfide Standard by adding 10 µl of the 1 mM Homocysteine Disulfide stock to 390 µl of Homocysteine Assay Buffer.
2. Add 0, 1, 2, 4, 6, 8, and 10 µl of the 25 µM Standard into a series of wells, generating 0, 50, 100, 200, 300, 400 and 500 pmol of free homocysteine/well upon disulfide reduction (each mole of Homocysteine Disulfide Standard generates 2 moles of free L-homocysteine upon reduction).
3. Adjust the volume to 170 µl/well with Homocysteine Assay Buffer (with DTT).

Homocysteine Enzyme Mix Reaction:

1. Incubate the plate at 37°C for 30 mins with gentle shaking to liberate free homocysteine in sample and standard curve wells. Remove the plate from the incubator and allow it to cool to room temperature for 5 mins.
2. Prepare a 1X solution Homocysteine Enzyme Mix by diluting the reconstituted 10X stock with Homocysteine Assay Buffer (without DTT) at a 1:10 ratio. For test sample and standard curve reactions, prepare 30 µl of 1X Homocysteine Enzyme Mix (containing 3 µl reconstituted 10X stock and 27 µl Homocysteine Assay Buffer) per well. For sample background control wells, prepare 30 µl of Homocysteine Assay Buffer (without DTT).
3. Add 30 µl of 1X Homocysteine Enzyme Mix to each test sample and standard curve well and 30 µl of Homocysteine Assay Buffer (without DTT) to each sample background well. Mix well and incubate the plate at room temperature for 5 mins, protected from light.
4. During the 5 min incubation period, prepare enough Fluorogenic Developer Mix for the number of reactions being performed. For each reaction well, mix 30 µl Fluorogenic Probe Solution and 20 µl Developer Solution XI. Add 50 µl Fluorogenic Developer Mix to all sample, background control and standard curve wells and mix well (bringing the final volume to 250 µl/well).

   ∆ **Notes:**

   a) The 5 min enzymatic reaction incubation time must be consistent for both the standard curve and sample wells. We recommend using a multi-channel pipette and reagent reservoir for addition of Homocysteine Enzyme Mix and Fluorogenic Developer Mix.

b) Once prepared, the Fluorogenic Developer Mix should be used immediately. Do not store Fluorogenic Developer Mix

Measurement

Following addition of Fluorogenic Developer Mix, incubate plate for 15 min at RT with continuous shaking (to ensure adequate mixing). Measure the fluorescence of all sample, background and standard curve wells at Ex/Em = 658/708 nm in endpoint mode.

Calculation

- For the Homocysteine Standard Curve, subtract the zero standard (0 pmol/well) reading from all of the standard readings, plot the background-subtracted values and calculate the slope of the standard curve.
- For test samples, calculate the corrected sample fluorescence (F_S) by subtracting the background control RFU reading from the sample reading: $F_S = RFU_S - RFU_{BC}$.
- For unspiked samples, apply the F_S values to the standard curve to get B pmol of homocysteine in the well.

$$\boldsymbol{Sample\ Total\ Homocysteine\ Concentration} = \frac{\mathrm{B}}{\mathrm{V}} \times D\ = pmol/\mu ul\ = \mu M$$

Where: **B** = Amount of homocysteine, calculated from the standard curve (in pmol)
V = Volume of sample added to the well (10 µl)
D = Sample dilution factor (if applicable, D=1 for undiluted samples)

Δ **Note:** For spiked samples, calculate B by subtracting the corrected sample reading (Fs) from the corrected spiked sample reading ($F_{S+spike}$). Each mole of spiked Homocysteine Disulfide Standard is equivalent to 2 moles of free Homocysteine upon reduction**.**

$$\boldsymbol{Amount\ of\ Homocysteine\ in\ Spiked\ Sample\ Well\ (B)} = \left(\frac{F_s}{(F_{s+spike}) - F_s}\right) \times (2 \times Homocysteine\ Disulfide\ Spike\ (pmol))$$

Technical Support

For all technical or commercial enquiries please go to:
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