Homocysteine (Hcy) Assay Kit

Catalog No.: abx294100

Size: 100 tests

Detection Range: 0 µmol/L – 50 µmol/L

Storage: Store all components at 4°C. Store Reagent 1 and Reagent 2 in the dark.

Application: For detection and quantification of Homocysteine concentration in serum.

Introduction

Homocysteine is an amino acid that is biosynthesized in the body. High levels of Homocysteine in serum (hyperhomocysteinemia) is a marker of cardiovascular disease, and a risk factor for coronary artery disease. High levels of Homocysteine is also found to be correlated with the occurrence of blood clots, heart attacks, and strokes.

Abbexa's Homocysteine (Hcy) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Homocysteine concentration. Oxidised Homocysteine (Hcy) is reduced by tri-ethyl phosphine (TCEP) to form free Homocysteine, which then reacts with a substrate to produce adenosine. The adenosine is then dehydrogenated immediately into inosine and ammonia. The ammonia then reacts with NADH, catalysed by glutamate dehydrogenase, to produce NAD+. The decrease in NADH causes a decrease in absorbance at 340 nm which is proportional to the concentration of Homocysteine in the sample.

Kit components

1. Reagent 1: 2 × 37 ml
2. Reagent 2: 2 × 10 ml
3. Blank Standard (0 µmol/L): 1 ml
4. Homocysteine Standard (28 µmol/L): 1 ml

Materials required but not provided

1. Biochemical analyzer (340 nm)
   or Spectrophotometer (340 nm)
2. Double-distilled water
3. Normal saline (0.9 % NaCl)
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Vortex mixer
8. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 1000 × g for 20 mins. If precipitate appears, centrifuge again. Do not use samples containing sodium fluoride. Avoid samples with hemolysis, turbidity, or severe blood lipid levels. Assay immediately or aliquot and store at 4°C for up to 1 week or -20°C for long-term storage.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Normal saline (0.9 % NaCl), then carry out the assay procedure.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- A high protein diet is not recommended before taking samples due possible elevated Hcy levels.

2. Reagents

- Equilibrate all reagents to room temperature before use.

Note:

- When using Reagent 2, which contains sodium azide, use appropriate PPE to avoid contact with skin and clothing. If Reagent 2 comes into contact with skin, wash immediately with a large amount of water and seek medical treatment if necessary.

B. Assay Procedure

Pre-heat the incubator to 37°C and ensure it has reached a stable temperature before use.

Biochemical analyzer procedure:

Set up the Biochemical analyzer using the following parameters:

Temperature	37°C
Reaction direction	Down
Calibration method	Linear
Sample volume	13 µl
Reagent 1	240 µl
Reagent 2	65 µl

Method	Rate method
Delay time	120 s
Detection time	120 s
Dominant wavelength	340 nm
Auxilary wavelength	405 nm

Spectrophotometer procedure:

1. Mark microcentrifuge tubes for each standard, sample, and blank. Each sample requires a corresponding control. *It is strongly recommended to prepare all the tubes in duplicate*.
2. Add 39 µl of sample to the sample tube.
3. Add 39 µl of Blank Standard (0 µmol/L) to the blank tube.
4. Add 39 µl of Hymocysteine Standard (28 µmol/L) to the standard tube.
5. Add 720 µl of Reagent 1 to each tube.
6. Mix thoroughly, then incubate all tubes at 37°C for 4 minutes.
7. Add 195 µl of Reagent 2 to each tube.
8. Mix fully, then incubate all tubes at 37°C for 2 minutes.
9. Set the spectrophotometer to zero with double-distilled water. Measure the OD value at 340 nm with a 1 cm optical path cuvette. Record this as: $A_{1\ Sample}$, $A_{1\ Standard}$, and $A_{1\ Blank}$, respectively.
10. Leave to stand for 2 minutes. Set the spectrophotometer to zero with double-distilled water. Measure the OD value at 340 nm with a 1 cm optical path cuvette. Record this as $A_{2\ Sample}$, $A_{2\ Standard}$, and $A_{2\ Blank}$, respectively.

C. Calculation of Results

1. Serum samples:

$$\text{Homocysteine concentration (µmol/L)} = \mathbf{F} \times \mathbf{C} \times \frac{(\Delta A/\text{min}_{\ \text{Sample}} - \Delta A/\text{min}_{\ \text{Blank}})}{(\Delta A/\text{min}_{\ \text{Standard}} - \Delta A/\text{min}_{\ \text{Blank}})}$$

where:

$\Delta A/min_{\ Sample}$	($A_{1\ Sample} - A_{2\ Sample}$) / 2 minutes
$\Delta A/min_{\ Standard}$	($A_{1\ Standard} - A_{2\ Standard}$) / 2 minutes
$\Delta A/min_{\ Blank}$	($A_{1\ Blank} - A_{2\ Blank}$) / 2 minutes
C	Concentration of Hymocysteine Standard (28 µmol/L)
F	The dilution factor of sample before assaying.

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.