Catalog #80454

V.2

Crystal Chem

Rat Homocysteine Kit Instructions

For the quantitative determination of total homocysteine
in rat serum and plasma

Catalog #80454
96 Assays

For research use only. Not for use in diagnostic procedures.

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A. Intended Use

The Rat Homocysteine kit is for the quantitative determination of total L-homocysteine in rat serum and plasma. The homocysteine concentration is expressed as µmol/L. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Homocysteine is a non-protein amino acid that is biosynthesized from methionine. Elevated levels of total homocysteine (which represents the sum of all forms of homocysteine including forms of oxidized, protein bound and free) has emerged as an important risk factor in the assessment of cardiovascular disease. Elevated levels of total homocysteine have also been linked with Alzheimer's disease and osteoporosis. As a result, the accurate measurement of homocysteine in experimental animals is becoming increasingly important.

C. Principle of the Assay

The Rat Homocysteine kit is based on a proprietary assay principle. Total homocysteine is degraded by a recombinant homocysteinase producing hydrogen sulfide. Hydrogen sulfide combines with N,N-dibutyl phenylene diamine in the presence of Fe^{+3} to form a chromophore, which can be measured using an absorbance reader.

D. Kit Storage

1. Upon receipt of the Rat Homocysteine kit, store it at -20°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
CC1	Reagent CC1 (Lyophilized)	5 vials
CC2	Reagent CC2 (Liquid)	1 x 15 mL
BUF	Buffer (Liquid)	1 x 50 mL
CAL1	Calibrator 1 (Liquid)	1 x 0.25 mL
CAL2	Calibrator 2 (Liquid)	1 x 0.25 mL
CAL3	Calibrator 3 (Liquid)	1 x 0.25 mL
CON1	Control 1 (Liquid)	1 x 0.25 mL
CON2	Control 2 (Liquid)	1 x 0.25 mL

E.2. Materials required but not provided

Microplates (350 µL well volume or greater)
Micropipettes and disposable tips
Clean glass tubes and test tube racks
Volumetric flasks
1X PBS (phosphate buffered saline, pH 7.2-7.5)
Microplate reader (capable of A_{660})

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.

G. Maximizing Kit Performance

1. Given the small sample volumes required (20 µL), pipetting should be done as carefully as possible. A high quality 50 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each calibrator and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Fresh rat serum, heparin plasma, or EDTA plasma can be used in the assay. It is important to centrifuge blood samples immediately after collection to separate the plasma from the blood cells, as synthesis of total homocysteine will take place in red blood cells after sampling. If immediate centrifugation is not possible, collected blood specimens should be kept on ice and centrifuged within an hour. After separation of plasma from cells, samples are stable for four weeks at 2-8° C, and stable for several months or years at –20° C.

Food consumption can affect circulating total homocysteine levels. Protein or fat-rich meals give higher total homocysteine values and should be avoided late in the day before sampling. Overnight fasting is recommended before blood is drawn. Standardized sampling procedures are crucial due to the above-mentioned influencing factors.

Note: *Hemolysed or turbid specimens or severely lipemic specimens are not recommended.*

I. Assay Procedure

I.1. Preparation of reagents

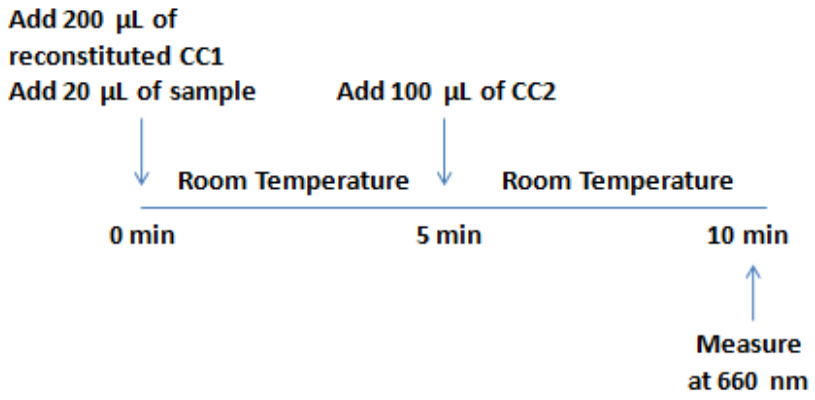
1. Reagent CC1 Working Solution
   For 20 tests, dissolve one vial of Reagent CC1 with 4.2 mL of Buffer. The Reagent CC1 working solution should be prepared freshly before use, which is stable for 24 hours at 2-8° C.
2. Buffer
   Provided as ready to use.
3. Standards 1-3
   Provided as ready to use.
4. Controls 1-2
   Provided as ready to use.

I.2. Assay procedure

All reagents should be brought to room temperature for at least 30 minutes prior to use. Reagents should be stored at -20°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. Add 200 µL of reconstituted Reagent CC1 and 20 µL of sample, calibrator, or control into each well (as needed) of a microplate and mix well by repeated pipetting avoiding bubbles.
2. Allow microplate to incubate at room temperature over 5 minutes.
3. Add 100 µL of Reagent CC2 into each well of the microplate and mix well by repeated pipetting avoiding bubbles.
4. Allow microplate to incubate at room temperature for another 5 minutes.
5. Measure absorbance using a microplate reader (measure A_{660}).

Figure 1. Summary of assay procedure



I.3. Determining the rat homocysteine concentration

1. Using linear graph paper, construct the homocysteine calibration curve by plotting the absorbance reading for each calibrator on the Y axis versus the corresponding homocysteine concentration on the X axis.
   Note: *Calibrator values vary per lot and should be obtained from the calibrator labels. The calibrators should be run every time the assay is performed.*
2. Rat homocysteine concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. The homocysteine concentration is expressed as µmol/L.
   Note: *Samples with a high rat homocysteine concentration (40 µmol/L or higher) should be diluted with 1X PBS Solution and rerun.*

J. Performance characteristics

J.1. Assay range

The Rat Homocysteine assay has a linear range from 4-40 µmol/L.

J.2. Precision

The assay has a within-run and total precision of CV < 10%.

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