



**Crystal Chem**

# **Rat Homocysteine Kit Instructions**

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

**Catalog #80450  
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  $\mu\text{mol/L}$ . 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  $\text{Fe}^{+3}$  to form a chromophore, which can be measured using a fluorescence reader.

**D. Kit Storage**

1. Upon receipt of the Rat Homocysteine kit, store it at  $-20^{\circ}\text{C}$  and avoid light exposure (do not freeze the kit or hold it at temperatures above  $25^{\circ}\text{C}$ ).
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  $\mu\text{L}$  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)  
 Fluorescence reader (capable of EX 660 / EM 710)

#### **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 (5  $\mu$ L), pipetting should be done as carefully as possible. A high quality 10  $\mu$ L 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**

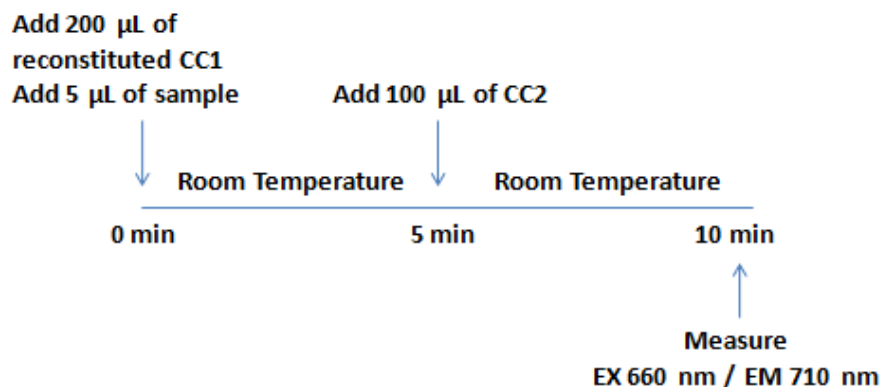
1. Reagent CC1 Working Solution  
For 20 tests, dissolve one vial of Reagent CC1 with 4.2 mL of Buffer. The Reagent CC1 working solution should be prepared freshly before use, which is stable for 24 hours at 2-8° C.
2. Buffer  
Provided as ready to use.
3. Standards 1-3  
Provided as ready to use.
4. 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  $\mu$ L of reconstituted Reagent CC1 and 5  $\mu$ L 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  $\mu$ L 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 end-point using a fluorescence reader (measure EX 660 nm / EM 710 nm).

**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 end-point 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 end-point values for each sample. The homocysteine concentration is expressed as  $\mu$ mol/L.  
**Note:** *Samples with a high rat homocysteine concentration (40  $\mu$ mol/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  $\mu$ mol/L.

### J.2. Precision

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

***Warranty***

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