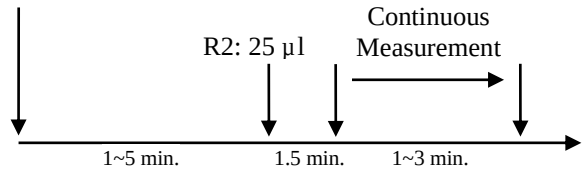
Pointe Chemistry Reagent Series
Homocysteine Test Kit

Detection of Homocysteine in Human Serum or Plasma on Chemistry Analyzers

Cat. No. R033K11 ***Hcy Reagent Kit***

SUMMARY OF TEST PROCEDURE

Figure 1: Procedure Diagram



*Refer to Figure 1 and the package insert for detail

Table 1: Instrument Parameters*

Calibration method	2-pointe linear	Slope of reaction	Decrease
Testing wavelength	Dλ : 340 nm Sλ : 405 nm	Sample volume	16.5 µl
Test method	Rate Method	R1 volume	250 µl
Reaction temperature	37℃	R2 volume	25 µl

INTENDED USE

Pointe Chemistry Reagent Series **Hcy Reagent Kit** (the Kit) is an assay intended for *in vitro* quantitative detection of Homocysteine in human serum or plasma on automated clinical chemistry analyzers.

SUMMARY AND EXPLANATION

Homocysteine is a sulphur-containing amino acid which is believed in causing arterial and venous atherothrombotic disease. It cannot be obtained from diet but only biosynthesized from methionine. In 1969 an American doctor indicated this relationship on patients with extremely elevated plasma Hcy concentration. A lot of studies also showed that it is associated with an increased risk of myocardial infarction and stroke because the elevated Hcy may reflects the increase in occurrence of blood clots.

TEST PRINCIPLES

The Kit utilizes enzymatic and kinetic reactions to measure the amount of homocysteine (µmol/L) in human serum or plasma.

$$\text{Homoctsteine + Serine} \xrightarrow{\text{CBS}} \text{L-cystathionine}$$

$$\text{L-cystathionine} \xrightarrow{\text{CBL}} \text{Homoctsteine + Pyruvate + } NH_3$$

$$\text{Pyruvate + } NADH^{+} \xrightarrow{\text{LDH}} \text{Lactate + NAD}$$

Oxidized homocysteine is reduced to free homocysteine which reacts with serine by the catalysis of cystathionine β-synthase to produce L-cystathionine. L- cystathionine converts back to homocysteine in the presence of cystathionine β-lyase and pyruvate and ammonia are also formed in the same reaction. Pyruvate is then oxidazed to lactate catalyzed by lactate dehydrogenase to tranfer hydrogen from $NADH^{+}$ to NAD. The process is quantified by measuring the absorabances at 340 nm in a kinetic fashion.

The rate of increase in absorbance at 340 nm is directly proportional to the amount of Hcy in the sample.

MATERIALS PROVIDED

Reagents:

R1	Lactate dehydrogenase Serine NADH	>35 KU/L 0.76 mmol/L 0.47 mmol/L
R2	Cystathionine β-synthase Cystathionine β-lyase EDTA	>20% KU/L >10 KU/L >0.1 g/L

MATERIALS NEEDED BUT NOT PROVIDED

1. Automated chemistry analyzer

INSTRUMENT

The Kit is applicable on most automated chemistry analyzers. Refer to specific instrument application for suggested settings.

STORAGE AND STABILITY

Store the reagents at 2-8℃. Avoid direct sunlight. The Kit is stable through the expiration date when stored properly. The reagent is stable for 1 month at 2-8℃ after opening.

PRECAUTIONS

1. The Kit is for *in vitro* diagnostic use only. Not for use in humans or animals.
2. The instructions must be followed to obtain accurate results.
3. Do not use the reagents beyond the expiration date.
4. Treat all specimens as infectious. Proper handling and disposal procedures of specimens and test materials should be strictly followed.

SPECIMEN COLLECTION AND HANDLING

Follow standard laboratory procedures to collect serum preventing hemolysis.

It is recommended to perform test immediately after sample collection. If the test cannot be done immediately, specimens can be stored at 2~8℃ for 48 hours.

TEST PROCEDURE (see Figure 1)

Reagent 1 and 2 are liquid stable ready-to-use, no preparation needed.

Calibration: Recommend using Pointe calibrator set for optimal results.

Test procedure: see Figure 1 and Table 1 for instrument parameter setup. Refer to specific instrument application for suggested setting.

1. Add 16.5 µl of sample and 250µl of R1; mix well and incubate at 37℃ for 1~5 minutes.
2. Add 25µl of R2; mix well and incubate at 37℃ for 1.5 minutes.
3. Take continuous optical density measurement for 1~3 minutes.
4. Calculate average Δ A /min

RESULT

The homocysteine concentration in µmol/L can be obtained by the following calculation:

$$\frac{\text{Abs. sample/min}}{\text{Abs. standard/min}} \times \text{Standard Conc. (μmol/L)} = \text{Hcy Conc. (μmol /L)}$$

Please refer to instrument application if testing under different conditions.

EXPECTED VALUES

4.0~15.4 μmol/L
It is recommended for each laboratory to establish its own expected values

QUALITY CONTROL

Using Pointe controls with known concentration is recommended before each batch of tests to ensure the test is properly performed and all reagents and the instrument are functional as specified.

LIMITATIONS

1. The Kit is for *in vitro* use on automated chemistry analyzers only.
2. The test result from the Kit should not be used as the only basis for definite diagnosis.
3. Samples with homocysteine exceeding the maximum measurement range should be diluted with saline and retested.

PERFORMANCE CHARACTERISTICS

Linearity: 0~50 μmol/L (R≥0.990)

Accuracy: Bias proportion 90%~110%

Precision: Within Run: CV≤8%;
Run-to-Run: CV≤10%

Reagent Blank Absorbance: at 340nm wavelength and 10 mm optical diameter, O.D. ≥1.0

REFERENCES

1. Bogdan N. Manolescu *et al.*, Acta Biochimica Polonica 57(4):467-477 (2010)
2. Bruno Zappacosta *et al.*, Clinical Biochemistry 39(1):62-66 (2006)
3. McCully K. S., Am J pathol. 56:111-128 (1969)

Not Intended for Sale in the United States.

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