



6330 Nancy Ridge Drive, Suite 103  
San Diego, CA 92121  
Tel: (858) 450-0048

## Homocysteine Enzymatic Assay Kit

Catalog Number: BQ 002-EACD

### Intended Use

Enzymatic homocysteine assay is intended for the *in vitro* quantitative determination of total L-homocysteine in serum or plasma. The assay can assist in diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria.

\*\* Patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azuridine triacetate may have higher levels of Hcy due to metabolic interference with Hcy metabolism. \*\*

### Clinical Significance

Homocysteine (Hcy) is a thiol-containing amino acid produced by the intracellular demethylation of methionine. Total homocysteine (tHcy) represents the sum of all forms of Hcy including forms of oxidized, protein bound and free.

Elevated level of tHcy has emerged as an important risk factor in the assessment of cardiovascular disease<sup>1-3</sup>. Excess Hcy in the blood stream may cause injures to arterial vessels due to its irritant nature, and result in inflammation and plaque formation, which may eventually cause blockage of blood flow to the heart. Elevated tHcy levels are resulted from four major causes including:

- genetic deficiencies in enzymes involved in Hcy metabolisms such as cystathionine beta-synthase (CBS), methionine synthase (MS), and methylenetetrahydrofolate reductase (MTHFR);
- nutritional deficiency in B vitamins such as B<sub>6</sub>, B<sub>12</sub> and folate;
- renal failure for effective amino acid clearance;
- drug interactions such as nitric oxide, methotrexate and phenytoin that interfere with Hcy metabolisms.

Elevated levels of tHcy are also linked with Alzheimer's disease<sup>4</sup> and Osteoporosis<sup>5</sup>. Guidelines for tHcy determination in clinical laboratories have recently been established.<sup>6</sup>

### Assay Principle

The Bio-Quant Homocysteine Enzymatic assay is based on a novel assay principle that assesses the co-substrate conversion product (a molecule that is not a substrate of the Hcy conversion enzyme, and does not contain any element from sample Hcy) instead of assessing co-substrate or Hcy conversion products of Hcy as described in the literature. In this assay, oxidized Hcy is first reduced to free Hcy which then reacts with a co-substrate, S-adenosylmethionine (SAM) catalyzed by a Hcy S-methyltransferase to form methionine (the Hcy conversion product of Hcy) and S-adenosylhomocysteine (SAH, the co-substrate conversion product). SAH is assessed by coupled enzyme reactions including SAH hydrolase, adenosine (Ado) deaminase and glutamate dehydrogenase, wherein SAH is hydrolyzed into adenosine (Ado) and Hcy by SAH hydrolase. The formed Hcy that is originated from the co-substrate SAM is cycled into the Hcy conversion reaction by Hcy S-methyltransferase. This forms a co-substrate conversion product based enzyme cycling reaction system with significant amplification of detection signals. The formed Ado is immediately hydrolyzed into Inosine and ammonia which reacts with glutamate dehydrogenase with concomitant conversions of NADH to NAD<sup>+</sup>. The concentration of Hcy in the sample is indirectly proportional to the amount of NADH converted to NAD<sup>+</sup> ( $\Delta A_{340nm}$ ).

### Materials provided

The reagents provided in the Bio-Quant Homocysteine kit is:

| Catalog No. | Kit size   |
|-------------|--|
| BQ 002-EACD | Reagent 1: 1 x 27 mL<br>Reagent 2: 1 x 5.5 mL<br>Reagent 3: 1 x 4 mL |

Note: Calibrator sold separately

### Materials required but not provided

An analyzer capable of dispensing 3 reagents and of measuring absorbance at 340 nm with temperature control (37°C).

### Reagent Composition

| Active Ingredients         | Concentration |
|----------------------------|---------------|
| S-adenosylmethionine (SAM) | 0.1 mM        |
| NADH                       | 0.2 mM        |
| TCEP                       | 0.5 mM        |
| 2-oxoglutarate             | 5.0 mM        |
| Glutamate dehydrogenase    | 10 KU/L       |
| SAH hydrolase              | 3.0 KU/L      |
| Adenosine deaminase        | 5.0KU/L       |
| Hcy methyltransferase      | 5.0KU/L       |

### Precautions

This reagent is for *in vitro* diagnostic use only. DO NOT INGEST. Avoid contact with skin and eyes. Contains sodium azide which may react with lead or copper plumbing to form explosive compounds. Flush drains with copious amounts of water when disposing of this reagent. Calibrators and controls are human serum based. Specimens containing human sourced materials should be handled as if potentially infectious, using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395). Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product.

### Reagent Stability and Storage

The Bio-Quant Homocysteine Enzymatic assay reagents, calibrators, and controls should be stored at 2 – 8 °C. DO NOT FREEZE. The reagents, calibrators, and controls are stable when stored as instructed until the expiration date on the label. Do not mix reagents of different lots.

### Reagent Preparation

R1, R2 and R3 are ready-to-use liquid stable reagents. Calibrators and controls are ready-to-use stable liquids.

### Specimen Collection and Handling

Fresh serum, heparin plasma, or EDTA plasmas can be used in the Hcy assay. It is important to centrifuge blood samples immediately after collection to separate the plasma from the blood cells. If immediate centrifugation is not possible, collected blood specimens should be kept on ice and centrifuged within an hour. Hemolysed or turbid specimens or severely lipemic specimens are not recommended for Hcy assay. After separation of plasma from cells, Hcy is stable for at least 4 days at room temperature, stable for several weeks at 0 – 8 °C, and stable for several months or years at -20°C.<sup>7</sup>

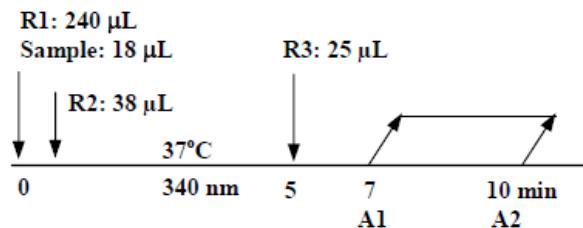
### Calibration

For Cobas Mira application: use the Cobas Mira blank solution and Calibrator 2 as standard. For other analyzers, calibrators are available. Calibrators are sold separately.

The calibration curve is stable for at least five days.

### Assay Procedure

Before sampling, gently swirl the calibrator and control vial several times to ensure homogeneity. After each use, promptly replace the cap and return to 2 – 8 °C storage.



### Results

Results are printed out in µmol/L. Note: Samples with values greater than 50 µmol/L should be diluted 1:1 with water and rerun. Multiply results by 2.

### Quality Control

We recommend that each laboratory use Hcy controls to validate the performance of Hcy reagents. A set of normal and abnormal ranges of Hcy controls is available from Bio-Quant Laboratories (Cat. No. BQ 002-EACN). The range of acceptable control limits should be established by individual laboratories.

### Reference Range

In most of the U.S. clinical laboratories, 15 µmol/L is used as the cut-off value for normal level of Hcy for adults.<sup>8-9</sup> In Europe, 12 µmol/L is used as the cut-off value. However, each laboratory is recommended to establish a range of normal values for the population in their region.

### Limitations

- The reagent should be clear. It should be discarded if it becomes turbid or the initial absorbance is less than 0.5 at 340nm (light path 0.6cm).
- The measuring range of the assay is from 3-50 µmol/L.
- Addition of 3-deazaadenosine to inhibit Hcy production in red cells has been suggested. However, the Bio-Quant Hcy assay can not use samples containing 3-deazaadenosine since it inhibits one of the key enzymes used in the assay.
- S-adenosylhomocysteine (SAH) will cause a significant positive interference. However, SAH is either not detectable or at sub nmole/L concentrations in normal plasma, and should not cause concern.
- Patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azuridine triacetate may have higher levels of Hcy due to metabolic interferences with Hcy metabolism.

### Performance Characteristics

#### Limits of Detection

The sensitivity of the assay was found to be <1.5 µmol/L.

#### Accuracy

Correlation studies were performed by testing 66 serum and plasma samples in comparison with an existing commercial Hcy assay method. The linear regression gives a correlation of  $r^2$  value of 0.976, slope of 0.98 and y intercept of 0.87.

#### Precision

Precision studies were conducted according to the NCCLS EP-5 protocol. Within precisions (CV%) for three levels of Hcy controls are 2.2% for 7 µM Hcy, 3.0% for 12 µM Hcy and 1.8% for 29.5 µM Hcy. Total imprecision for three levels of Hcy controls are 4.1% for 7 µM Hcy, 5.9% for 12 µM Hcy and 4.0% for 29.5 µM Hcy.

#### Linearity

The assay is linear up to 50 µmol/L HCY.

### Interference

An interference study was performed by testing a serum sample spiked with varied concentrations of endogenous substances. The following substances normally present in the serum produced less than 10% deviation when tested with the following stated concentrations: 500 µM NH<sub>4</sub>Cl, 1 mM NaPi, 1 mM NaF, 2500 mg/dL Triglycerides, 20 mg/dL Bilirubin, 1200 m/dL Hemoglobin, 0.5 mM\* Glutathione, 10 mM Ascorbic Acid, 1 mM L-Cysteine, 20 µM S-Adenosylmethionine (SAM), 100 µM\*\* Adenosine, 100 µM\* Cystathionine.

\* Glutathione was originally tested at 1 mM level, the interference was +13.5%. When retested at 0.5 mM level, the interference was less than 10%.

\*\* The concentrations tested are about 5-10 times higher than the normal range of serum levels.

### References

1. Eikelboom JW, et. al. *Ann Intern Med* 131:363-75, (1999)
2. Scott J, Weir D. *Q J Med* 89: 561– 3 (1996)
3. Nygard O, *N Engl J Med.* 337(4):230-6 (1997)
4. Seshadri S. *et al. N. Engl. J. Med.* 346:477-483 (2002)
5. McLean R. *et al. N. Engl. J. Med.* 350: 2042-2049 (2004)
6. Refsum H. *Clinical Laboratory News* May 2002, pp 2-14
7. Guttormsen AB *et al. J Nutr.* 124(10):1934-41 (1994)
8. Vilaseca et al. *Clin. Chem.* 43: 690-692 (1997)
9. Faure-Delanef et al. *Am. J. Hum. Genet.* 60: 999-1001 (1997)

### Cobas Miras Application Parameter Settings

Temperature 37 °C

Use Cobas Mira blank solution for blank and calibrator 2 as standard.

|                    |                    |                             |
|--------------------|--------------------|-----------------------------|
| <b>GENERAL</b>     |                    | Test Range Low: NO          |
| Measurement Mode   | : Absorb           | High: NO                    |
| Reaction Mode      | : R-S-SR1-SR2      | Normal Range Low: NO        |
| Calibration Mode   | : Slope Avg        | High: NO                    |
| Reagent Blank      | : Reag/Sol         |                             |
| Cleaner            | : NO               | Number of Steps: 1          |
| Wavelength         | :340 nm            | Calc Step A: ENDPOINT       |
| Decimal Position   | : 1                | Readings First: 16 Last: 24 |
| Unit               | : µmol/L           |                             |
| <b>ANALYSIS</b>    |                    | <b>CALIBRATION</b>          |
| Post DIL. Factor   | : NO               | Calib. Interval: EACH RUN   |
| Conc.Factor        | : NO               | Blank Sol-Pos: *            |
| Sample Cycle       | : 1                | Reag. Range low: NO         |
| Volume             | : 15.0 µl          | High: NO                    |
| Dilution Name      | : H <sub>2</sub> O | Blank Range Low: NO         |
| Volume             | : 10.0 µl          | High: NO                    |
| Reagent Cycle      | : 1                |                             |
| Volume             | : 240 µl           | <b>STANDARD POS: *</b>      |
| Start R1 Cycle     | : 2                | STD 1: *µmol/l              |
| Volume             | : 38.0 µl          | STD 2: NO                   |
| Dilution Name      | : H <sub>2</sub> O | STD 3: NO                   |
| Volume             | : 5.0 µl           |                             |
| Start R2 Cycle     | : 11               | Replicate: SINGLE           |
| Volume             | : 24.0 µL          | <b>CONTROL</b>              |
| Dilution Name      | : H <sub>2</sub> O |                             |
| Volume             | : 5.0 µl           | CS1 Pos: NO                 |
| <b>CALCULATION</b> |                    | CS2 Pos: NO                 |
| Sample Limit       | : NO               | CS3 Pos: NO                 |
| Reaction Direction | : Decrease         |                             |
| Check              | : OFF              |                             |
| Conversion Factor  | : 1.0000           |                             |
| Offset             | : 0.0000           |                             |

\* Value Input by operator

### Hitachi 917 Parameter Settings

Temperature: 37 °C

Use the following parameters with calibrator sets for calibration.

|                              |   |
|------------------------------|---|
| ** ANALYZE **                |   |
| TEST NAME                    | : [HCY]                                 |
| ASSAY/POINT                  | : [2 point end] [10] [24] [34] [0] [0]  |
| WAVE (SUB/MAIN)              | : [700] [340]                           |
| S. VOL. (NORMAL)             | : [18.0] [0.0] [0]                      |
| S. VOL. (DECREASE)           | : [18.0] [0.0] [0]                      |
| S. VOL. (INCREASE)           | : [18.0] [0.0] [0]                      |
| DILUENT                      | : [WATER] [0]                           |
| REAGENT VOL (R1)             | : [240] [0] [xxxxx] [ 0 ]               |
| REAGENT VOL (R2)             | : [38] [0] [xxxxx] [ 0 ]                |
| REAGENT VOL (R3)             | : [25] [0] [xxxxx] [ 0 ]                |
| REAGENT VOL (R4)             | : [0] [0] [xxxxx] [ 0 ]                 |
| ABS LIMIT                    | : [32000] [DECREASE] TWIN TESTS: [ ]    |
| PROZONE LIMIT                | : [-32000] [0] [ LOWER]                 |
| CELL DETERGENT               | : [Detergent 1]                         |
| ** CALIBRATION **            |   |
| CALIB TYPE                   | : [Linear] [ ]                          |
| POINT                        | : [2] SPAN POINT [2]                    |
| WEIGHT                       | : [0]                                   |
| AUTO CALIBRATION             |   |
| TIME OUT                     | CHG. OVER                               |
| BLANK                        | : [0] CHANGE LOT: [ ]                   |
| SPAN                         | : [0] CHANGE BOTTLE: [ ]                |
| 2POINT                       | : [0]                                   |
| FULL                         | : [0]                                   |
| SD LIMIT                     | : [100]                                 |
| DUPLICATE LIMIT              | : [200]                                 |
| SENSITIVITY LIMIT            | : [0]                                   |
| S1ABS RANGE                  | : [-32000] [32000]                      |
| ** RANGE **                  |   |
| TEST CODE                    | : [xxx]                                 |
| UNIT                         | : [μmol/l] DATA MODE [on board]         |
| CONTROL INTERVAL             | : [0]                                   |
| INST. FACTOR (Y=aX + b)      | : a = [1.0] b = [0.0]                   |
| TECHNICAL LIMIT [0.5] [50]   |   |
| EXPECTED VALUE               |   |
| MALE [ ]                     |   |
| FEMALE [ ]                   |   |
| ** STANDARD CONCENTRATION ** |   |
| STANDARD SOLUTION            |   |
| CONC                         | : [*] [*] [0] [0] [0] [0]               |
| POSITION                     | : [*] [*] [0] [0] [0] [0]               |
| SAMPLE                       | : [18.0] [18.0] [0.0] [0.0] [0.0] [0.0] |
| PRE-DILUENT                  |   |
| VOLUME                       | : [0.0] [0.0] [0.0] [0.0] [0.0] [0.0]   |
| DILUENT                      | : [0] [0] [0] [0] [0] [0]               |
| CALIB CODE                   | : [0] [0] [0] [0] [0] [0]               |

\* Value Input by operator

### Hitachi 911 Parameter Settings

Temperature: 37 °C

Use the following parameters with calibrator sets for calibration.

|  |                           |        |        |       |       |       |
|--|---------------------------|--------|--------|-------|-------|-------|
| ** ANALYZE **                                    |                           |        |        |       |       |       |
| TEST NAME  | : [HCY]                   |        |        |       |       |       |
| ASSAY CODE                                       | : [2 point end] [10] [ ]  |        |        |       |       |       |
| ASSAY/POINT                                      | : [22]-[31][0]-[0] SERUM  |        |        |       |       |       |
| S. VOL. (NORMAL)                                 | : [18.0] [0.0] [0]        |        |        |       |       |       |
| S. VOL. (DECREASE)                               | : [18.0] [0.0] [0]        |        |        |       |       |       |
| S. VOL. (INCREASE)                               | : [18.0] [0.0] [0]        |        |        |       |       |       |
| ABS LIMIT  | : [0]                     |        |        |       |       |       |
| PROZONE LIMIT                                    | : [-32000]                |        |        |       |       |       |
| REAGENT VOL (T1)                                 | : [240] [0] [xxxxx] [ 0 ] |        |        |       |       |       |
| REAGENT VOL (T2)                                 | : [38] [0] [xxxxx] [ 0 ]  |        |        |       |       |       |
| REAGENT VOL (T3)                                 | : [25] [0] [xxxxx] [ 0 ]  |        |        |       |       |       |
| REAGENT VOL (T 4)                                | : [0] [0] [xxxxx] [ 0 ]   |        |        |       |       |       |
| WAVELENGTH(2 <sup>ND</sup> /PRIMARY) [700]/[340] |                           |        |        |       |       |       |
| DILUENT/RGT. STABILITY [00000]/[0000]            |                           |        |        |       |       |       |
| URINE  |                           |        |        |       |       |       |
| [0] [0] [0]                                      |                           |        |        |       |       |       |
| [0] [0] [0]                                      |                           |        |        |       |       |       |
| [0] [0] [0]                                      |                           |        |        |       |       |       |
| [32000] [DECREASE]                               |                           |        |        |       |       |       |
| [32000] [LOWER]                                  |                           |        |        |       |       |       |
| ** CALIBRATION **                                |                           |        |        |       |       |       |
| CALIB TYPE                                       | : [Linear] [2] [2] [0]    |        |        |       |       |       |
| AUTO TIMEOUT                                     | : BLANK [0]               |        |        |       |       |       |
|  | : SPAN [0]                |        |        |       |       |       |
|  | : 2 POINT [0]             |        |        |       |       |       |
|  | : FULL [168]              |        |        |       |       |       |
| AUTO CHANGE                                      | : LOT [CANCEL]            |        |        |       |       |       |
|  | : BOTTLE [CANCEL]         |        |        |       |       |       |
| SELECT TEST VIA KEYBOARD                         | : ENTER                   |        |        |       |       |       |
| SD LIMIT   | : [100]                   |        |        |       |       |       |
| DUPLICATE LIMIT                                  | : [32000]                 |        |        |       |       |       |
| SENSITIVITY LIMIT                                | : [0]                     |        |        |       |       |       |
| S1ABS LIMIT                                      | : [-32000] [32000]        |        |        |       |       |       |
| COMPENSATED LIMIT                                | : [ ]                     |        |        |       |       |       |
| TEST CODE  | : [HCY] [xxx]             |        |        |       |       |       |
| DATA MODE  | : [on board]              |        |        |       |       |       |
| CONTROL INTERVAL                                 | : [0]                     |        |        |       |       |       |
| INST. FACTOR (Y=aX + b)                          | : a = [1.0] b = [0.0]     |        |        |       |       |       |
| TECHNICAL LIMIT [0.5] [50]                       |                           |        |        |       |       |       |
| EXPECTED VALUE                                   |                           |        |        |       |       |       |
| TECHNICAL LIMIT [2] [50]                         |                           |        |        |       |       |       |
| TEST NAME [HCY]                                  | UNIT [μmol/l]             |        |        |       |       |       |
| REPORT NAME                                      | [USER DEFINED]            |        |        |       |       |       |
| STD  | CONC.                     | POS    | SAMPLE | PRE.  | DIL   | CALIB |
| (1)  | [*]                       | [*]    | [0]    | [0]   | [0]   | [0]   |
| (2)  | [*]                       | [*]    | [0]    | [0]   | [0]   | [0]   |
| (3)  | [18.0]                    | [18.0] | [0.0]  | [0.0] | [0.0] | [0.0] |
| (4)  | [0.0]                     | [0.0]  | [0.0]  | [0.0] | [0.0] | [0.0] |
| (5)  | [0]                       | [0]    | [0]    | [0]   | [0]   | [0]   |
| (6)  | [0]                       | [0]    | [0]    | [0]   | [0]   | [0]   |

\* Value Input by operator

\*\* The above reagent parameter **has not been fully validated** for this analyzer. The parameters are based on Bio-Quant's knowledge of the analyzer and reagents, and should perform adequately. However, you should use these parameters as guidelines in conjunction with your Quality Control Program for validation.

**Bechman Synchron CX Parameter Settings**

Temperature 37 °C

Use the following parameters with calibrator sets for calibration.

|                               |              |                       |         |
|-------------------------------|--------------|-----------------------|---------|
| Test Name:                    | HCY          | User Defined No.:     | *       |
| Chemistry Name:               | Homocysteine | No. of Calibrators:   | [5]     |
| Reaction Type:                | [RATE 1]     | Calibrator 1:         | *       |
| Units:                        | [µmol/l]     | 2:                    | *       |
| Decimal Precision:            | [X.XX]       | 3:                    | *       |
|                               |              | 4:                    | *       |
| Reaction Direction:           | [Negative]   | 5:                    | *       |
| Calculation Factor:           | 0            | 6:                    | 0.00    |
| Math Model:                   | [9]          | Cal Time Limit:       | 336 hrs |
| Primary Wavelength:           | [340]        | Secondary Wavelength: | [380]   |
| Primary Inject RGT:           |              | Secondary Inject RGT: |         |
| A: 240                        |              | C:25                  |         |
| B: 38                         |              | Add Time: 560 SEC     |         |
| Sample Volume:                | 15           |                       |         |
| <u>Reagent Blank:</u>         |              | <u>Reaction</u>       |         |
| Start Read:                   | 520 sec      | Start Read:           | 357 sec |
| End Read:                     | 536 sec      | End Read:             | 480 sec |
| <u>Usable Range:</u>          |              |                       |         |
| Lower Limit:                  | 1.5          |                       |         |
| Upper Limit:                  | 50           |                       |         |
| <u>Error Detection Limits</u> |              | <u>Reaction</u>       |         |
| Reagent Blank                 |              | Low ABS Limit:        | 1.5     |
| Low ABS Limit:                | -1.5         | High ABS Limit:       | 1.5     |
| High ABS Limit:               | 1.5          |                       |         |
| <u>Substrate Depletion</u>    |              |                       |         |
| Initial Rate:                 | -99.99       |                       |         |
| Delta Abs                     | 1.5          |                       |         |
| <u>Multipoint Span</u>        |              |                       |         |
| 1-2: -0.001                   | 2-3: -0.001  |                       |         |
| 3-4: -0.001                   | 4-5: -0.001  |                       |         |
| 5-1: -0.001                   |              |                       |         |

\* Value Input by operator

**Dimension AR Parameters**

Temperature 37 °C

Use the following parameters with calibrator sets for calibration.

Channel: X  
Name: XXX  
Sample

Time: 0.0 sec  
Volume: 15 µL  
Chase: 10 µL  
Mix: Moderate

**First Reagent**

Time: -60.0 sec  
Component 1: (A) 240 µL  
Component 2: (B) 38 µL  
Component 3: ( ) 0 µL  
Chase: 25 µL  
Mix: Moderate

**Second Reagent**

Time: 257.3 sec  
Component 1: (C) 25 µL  
Component 2: ( ) 0 µL  
Component 3: ( ) 0 µL  
Chase: 25 µL  
Mix: Moderate

**Third Reagent**

Time: \*\*\* sec  
Component 1: ( ) 0 µL  
Component 2: ( ) 0 µL  
Component 3: ( ) 0 µL  
Chase: 0 µL  
Mix: None

**Photometry**

P1 Time: 360.0  
P2 Time: 600.0  
P3 Time: \*\*\*  
P4 Time: \*\*\*

**Reagent Cartridge**

|             |     |     |     |     |     |     |
|-------------|-----|-----|-----|-----|-----|-----|
| Well:       | 1   | 2   | 3   | 4   | 5   | 6   |
| Component:  | (A) | (A) | (A) | (A) | (B) | (C) |
| Aliquots:   | 15  | 15  | 15  | 15  | 60  | 60  |
| Life [hrs]: | 720 | 720 | 720 | 720 | 720 | 720 |

Reagent Cartridge life: 720 HRS;  
Calibration Interval: 168 HRS  
Standard Curve: Linear  
Measurement Mode: Absorbance

MAU Calculation:

```
{
C=RATE (P1, P2, 340NM, 383NM);
RETURN C;
}
```

**Modular P Parameter Settings**

Temperature 37 °C

Use the following parameters with calibrator sets for calibration.

No. Test Type \*\*\* Materials Needed but Not Provided:  
 \* HCY Ser/Pl Open Channel Barsheet Open Channel Reagent Barcodes

**Analyze**

Assay/Time/Point [2 Point End] [10] [24] [34] [0] [0]  
 Wave (2<sup>nd</sup> Primary) [700] / [340]  
 S. Vol (Normal) [13.0] [0.0] [0]  
 S. Vol (Decrease) [13.0] [0.0] [0]  
 S. Vol (Increase) [13.0] [0.0] [0]  
 Diluent [Water] [0] Timing  
 Reagent R1 [168] [20] [317] [ 0] bottle size L  
 Reagent R2 [27] [20] [317] [ 0] T2 bottle size M  
 Reagent R3 [20] [20] [317] [ 0] T3 bottle size M  
 Reagent R4 [00] [0] [317] [ 0]  
 Abs.  
 Limit [32000] [Decrease] 2 Tests [ ]  
 Prozone Limit [-32000] [ 0] [Lower]  
 Cell Detergent [Detergent 1]  
 Twin Test Cancel

**Calib**

Calibration Type [Logit-Log 5P] [0]  
 Point [5] Span [ 5 ]  
 Weight [0]  
 Auto Calibration Time Out Change Over  
 Blank [0] Lot [ ]  
 Span [0] Bottle [ ]  
 2 Point [0]  
 Full [168]  
 SD Limit [100]  
 Duplicate Limit [10%] [32000 Abs]  
 Sensitivity Limit [-99999] [99999]  
 S1Abs Limit [-32000] [32000]

**Range**

Application Code \* [ ] Unit [µmol/L]  
 Report Name\* [User Defined]  
 Data Mode [Active]  
 Control Interval\* [ ]  
 Instrument Factor (Y=aX+b) a=[1.0] b=[0.0]  
 Technical Limit [2.0] [50]  
 Repeat Limit \* [ ] [ ]  
 Expected Value \* Qualitative  
 [100]  
 [Y] -99999 99999 [Cancel]  
 (Male) [100]  
 [Y] -99999 99999 (1) [0] -  
 -99999 99999 (2) [0] -  
 [100]  
 [Y] -99999 99999 (3) [0] +-  
 (Female) [100]  
 [Y] -99999 99999 (4) [0] ++  
 -99999 99999 (5) [0] +++  
 (Default) [Male] Range 3 (6) [0] ++++

**Others**

|                  |         |         |         |         |         |       |
|------------------|---------|---------|---------|---------|---------|-------|
| <Standard>       | (1)     | (2)     | (3)     | (4)     | (5)     | (6)   |
| Calib Code*      | [431]   | [432]   | [433]   | [434]   | [435]   | [ ]   |
| Concentration ** | [*****] | [*****] | [*****] | [*****] | [*****] | [ 0 ] |
| Rack             |         |         |         |         |         |       |
| Position *       | [ ]     | [ ]     | [ ]     | [ ]     | [ ]     | [ ]   |
| Sample Volume    | [13.0]  | [13.0]  | [13.0]  | [13.0]  | [13.0]  | [0.0] |
| Diluted S. Vol   | [0.0]   | [0.0]   | [0.0]   | [0.0]   | [0.0]   | [0.0] |
| Diluted Volume   | [0.0]   | [0.0]   | [0.0]   | [0.0]   | [0.0]   | [0.0] |

\* User Defined

\*\*\*\*\* Input concentration of HCY calibrators

. \*\* The above reagent parameter **has not been fully validated** for this analyzer. The parameters are based on Bio-Quant’s knowledge of the analyzer and reagents, and should perform adequately. However, you should use these parameters as guidelines in conjunction with your Quality Control Program for validation.

## Bechman Synchron LX Parameter Settings

Temperature 37 °C

Use the following parameters with calibrator sets for calibration.

### PROCEDURE:

1. Program a user-defined channel with the parameters listed blow. For more detailed instructions refer to the SYNCHRON LX Clinical Systems Operating Instructions.
2. Load cartridge as described in Synchron LX Operating Instructions.
3. Calibrate using the two Homocysteine calibrators enclosed in this kit.

### USER DEFINED PARAMETERS

|                         |               |                       |              |
|-------------------------|---------------|-----------------------|--------------|
| Test Name:              | HCY           | No. of Calibrators    | 2            |
| Reaction Type:          | [RATE 1]      | Calibrator #1:        | ***          |
| Units:                  | [µmol/l]      | #2:                   | ***          |
| Decimal Precision:      | [X.XX]        | #3:                   | 0.00         |
|                         |               | #4:                   | 0.00         |
| Reaction Direction:     | [Negative]    | #5:                   | 0.00         |
| Calculation Factor:     | 1             | #6:                   | 0.00         |
| Math Model:             | [Linear]      | Cal Time Limit:       | 336 hr       |
| Primary Wavelength:     | [340] nm      | Secondary Wavelength: | [380] nm     |
| <br>                    |               |                       |              |
| REAGENTS                | First Inject  | Second Inject         | Third Inject |
| Component:              | [A]           | [B]                   | [C]          |
| Dispense Volume:        | 180 µL        | 28 µL                 | 18 µL        |
| Inject Time:            |               | -180 sec              | 272 sec      |
| Sample Volume:          | 13 µL         |                       |              |
| REAGENT BLANK:          |               | REACTION 1:           | REACTION 2:  |
| Start Read:             | -50 sec       | Start Read:           | 360 Sec      |
| End Read:               | -10 sec       | End Read:             | 600 Sec      |
| USABLE RANGE:           |               |                       |              |
| Lower Limits:           | 1.00          |                       | Upper Limit: |
| ERROR DETECTION LIMITS: | BLANK 1:      | REACTION 1:           | REACTION 2:  |
| Low ABS Limit:          | -1.500        | Low ABS Limit:        | -1.500       |
| High ABS Limit:         | 2.200         | High ABS Limit:       | 2.200        |
| Low Rate Limit:         | 2.200         | Low Rate Limit:       | 2.200        |
| High Rate Limit:        | -1.500        | High Rate Limit:      | -1.500       |
| Mean Deviation:         | 2.200         | Mean Deviation:       | 2.200        |
| SUBSTRATE DEPLETION:    |               |                       |              |
| Initial Rate:           | -99.999 *     |                       |              |
| Delta Abs               | 2.10          |                       |              |
| MULTIPOINT SPAN:        | 1-2: -0.001 * |                       |              |

\*\*\* Input the HCY calibrator concentration

The above reagent parameter **has not been fully validated** for this analyzer. The parameters are based on Bio-Quant's knowledge of the analyzer and reagents, and should perform adequately. However, you should use these parameters as guidelines in conjunction with your Quality Control Program for validation.