

Introduction

1. AutoPure Homocysteine is a reagent kit for quantitative determination of L - homocysteine in human serum and plasma on automated clinical chemistry analyzers.
2. AutoPure Homocysteine is a ready-to-use, two liquid reagent system.
3. With AutoPure Homocysteine, the assay is linear up to 50 µmol/l.

Principle

AutoPure Homocysteine assay is based on the measurement of co - substrate conversion product.

Oxidized Homocysteine from the sample is reduced to free Homocysteine which then reacts with SAM to form methionine and SAH. SAH is assessed by coupled enzyme reactions where in adenosine is formed. The adenosine formed is hydrolyzed into inosine and ammonia which reacts with glutamate dehydrogenase with concomitant conversions of NADH to NAD⁺. The concentration of Homocysteine is proportional to the amount of NADH converted to NAD⁺ and is measured as change in absorbance at 340 nm.

Reagent Storage, Stability & Handling

AutoPure Homocysteine is a ready-to-use, two liquid reagent system.

Shelf life

Stable till expiry date indicated on the label when stored at 2^o–8^oC.

On – Board Reagent Stability

R1 : 60 days at 2^o–8^oC after opening.

R2 : 60 days at 2^o–8^oC after opening.

Protect the reagent from light and contamination.

Do not freeze the reagent.

Components & Concentration of Reagent

Component	Concentration
R1	
• S-adenosylmethionine (SAM)	0.1mM
• NADH	> 0.2 mM
• TCEP	> 0.5 mM
• 2-oxoglutarate	5 mM
R2	
Glutamate dehydrogenase	10 KU/l
SAH hydrolase	3.0 KU/l
Adenosine deaminase	5.0 KU/l
Hcy methyltransferase	5.0 KU/l

Specimen Collection & Preservation

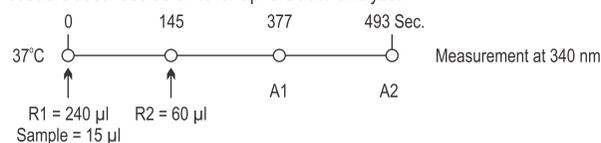
Collect sample using standard sampling tube. Fresh serum and EDTA/heparinised plasma are the specimens of choice.

Haemolysed, turbid or severely lipemic specimens are not recommended for this assay.

After separation of plasma from cells, Homocysteine is stable for at least 4 days at room temperature and for 2 weeks at 2^o–8^oC.

Procedure

AutoPure Homocysteine can be used on various automated analyzers. The procedure described below is for Sphera auto-analyzer.



Calculations

Fully automated system automatically calculates the homocysteine concentration of each sample

Application Sheet

Refer to application sheet for details. For additional system applications, contact our local Accurex representative.

Calibration

For calibration, it is recommended to use AutoPure Homocysteine calibrator from Accurex Biomedical Pvt. Ltd. Other commercially available homocysteine calibrators have not been tested with this assay and cannot be supported by Accurex. Refer to the AutoPure Homocysteine calibrator kit package insert for a description of assignment procedures and instructions. Calibration materials have concentrations around medical decision level.

Calibration frequency

The calibration curve is stable for 11 days.

Re - calibration is recommended

- Whenever the reagent lot is changed.
- As per the requirement of quality control procedures.

Quality Control

Each batch of AutoPure Homocysteine is assayed with multiple quality control sera prior to release.

To ensure adequate quality control, it is recommended that the laboratory should use a normal and abnormal commercial reference control serum. It should be realized that the use of quality control material checks both the reagent and instrument functions together.

If the control values fall outside the specified limits, each of the below criteria should be cross - checked and corrected:

- Proper instrument function – wavelength setting, light source and temperature control.
- Cleanliness of probes and cuvettes.
- Bacterial contamination of wash water used by the instrument.
- Expiry date of the reagent kit.

Expected Values

Serum/Plasma Homocysteine : < 15 µmol/l

Note:

Expected range varies from population to population. It is therefore recommended that each laboratory should establish its own normal range. For diagnostic purposes, the homocysteine results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Performance Characteristics

Linearity

With AutoPure Homocysteine, the assay is linear up to 50 µmol/l. Determine samples with higher concentrations via the rerun function. On analyzers without rerun function, manually dilute the sample with higher concentrations using distilled / deionized water (e.g. 1 + 4). Multiply the result by the appropriate dilution factor (e.g. 5).

Interference

There is no significant interference from samples containing up to 500 mg/dl of haemoglobin, 40 mg/dl of bilirubin, 1000 mg/dl of triglycerides, 10 mM ascorbic acid and 100 µM of cystathionine.

Precision

Reproducibility was determined using three levels of pooled human sera. The within run and between run reproducibility was determined as shown below :

Serum pool	Mean µmol/l	%CV	
		Within run (n = 20)	Between run (n = 30)
Low	7.0	4.50	5.87
Mid	15.6	3.04	5.51
High	29	2.40	2.57

Co - Relation Studies

A comparison of Homocysteine determination using AutoPure Homocysteine and Abbott AxSYM Immunochemistry System gave the following co-relation (µmol/l):

Linear Regression
y = 1.003x + 1.654
r = 0.985

References

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

Application sheet

Designations
 Code: Name: Type: Group:

1 - Pipetting
 Reagent 1 ID:
 Reagent 1 bottle:
 Reagent 2 ID:
 Reagent 2 bottle:
 Sample vol: ^{1st} ^{2nd} μl
 Reagent 1 vol: μl
 Reagent 2 vol: μl μl
 Diluent vol: μl

2 - Time
 Incubation 1: Sec
 Incubation 2: Sec
 Reading: Sec

3 - Wavelengths
 Wavelength 1: nm
 Wavelength 2: nm

4 - Washing
 Needle:
 Cuvette:

5 - Incompatibility
 1
 2
 3
 4

6 - Limits
 Blank OD min: Abs Linearity: %
 Blank OD max: Abs Subst. Depletion: Abs
 Reaction slope: Blank OD Δ max: Abs
 OD Range min: Abs Min conc: $\mu\text{mol/l}$
 OD Range max: 0.065 Abs Max conc: $\mu\text{mol/l}$

7 - Autodilution
 Rate: 5
 Max OD: 0.065 Abs

8 - Dilutions
 Serum
 1:1 1:2 1:4
 1:10 1:40 1:100
 Urine
 1:1 1:2 1:4
 1:10 1:40 1:100

9 - Pathological ranges

Minimum	Sample type	Maximum
	Serum	15

Add Remove Edit

10 - Results units
 Units 1: $\mu\text{mol/l}$
 Units 2:
 Conversion:
 Decimal digits:

* User defined ** User defined based on calibration

Designations
 Code: Name: Type: Group:

Calibration
 OK
 General
 Number of standards:
 Validity (hours): Interpolation type:

Standards		Current Calibration					
Single	Calibrator	Dilution rate	Lot	Primary concent	Final concent	OD value	Date
1	HCY C1	<input type="text" value="1"/>	1	<input type="text" value=""/>	<input type="text" value=""/>	<input type="text" value=""/>	<input type="text" value=""/>
2	HCY C2	<input type="text" value="1"/>	2	<input type="text" value=""/>	<input type="text" value=""/>	<input type="text" value=""/>	<input type="text" value=""/>
3	HCY C3	<input type="text" value="1"/>	3	<input type="text" value=""/>	<input type="text" value=""/>	<input type="text" value=""/>	<input type="text" value=""/>
4	HCY C4	<input type="text" value="1"/>	4	<input type="text" value=""/>	<input type="text" value=""/>	<input type="text" value=""/>	<input type="text" value=""/>
5	HCY C5	<input type="text" value="1"/>	5	<input type="text" value=""/>	<input type="text" value=""/>	<input type="text" value=""/>	<input type="text" value=""/>

Reagent blank Blank Correction Validity hours OD OD 0 Date

Print calibration Curve chart

Correlation
 Y = X +

AR. No.: I87A

ASHY1-2014-07-001

ISO 13485, ISO 9001 CERTIFIED COMPANY

Accurex Biomedical Pvt. Ltd.

Head Office - Mumbai. Tel.: 91(022) 67446744; Fax: 91(022) 67446755
 E-mail: accurex@vsnl.com; Website: www.accurex.org
 Plant : G-54, MIDC Tarapur, Boisar, Thane - 401 506. INDIA.
 Call us toll free on : 1800 209 8456