

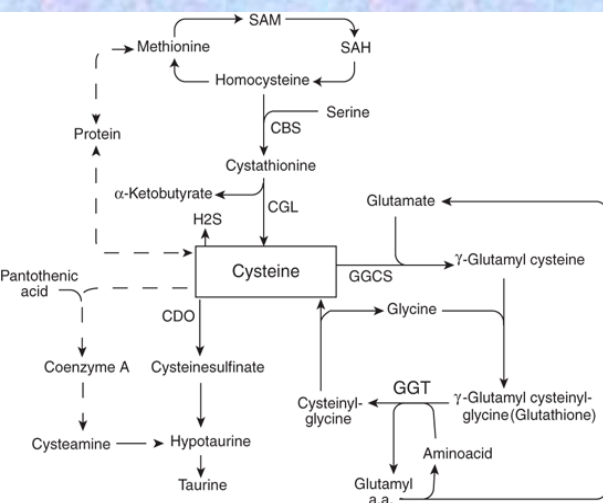
Key Publications and Patents

1. Han, Q, et al. High expression, purification, and properties of recombinant homocysteine α , γ -lyase. *Protein Expr Purif* **14**, 267-274 (1998).
2. Han, Q, et al. Homogeneous Enzymatic Colorimetric Assay for Total Cysteine. *Clinical Chemistry* 2004; v. 50, p.1229-1231.
3. Han, Q., and Hoffman, R.M. Enzymatic assay for total plasma Cys. *Nature Protocols* 3, 1778-1781, 2008. Han, Q. and Hoffman, RM.
4. Tan, Y., Tang, L., Sun, X., Zhang, N., Han, Q., Xu, M., Baranov, E., Tan, X-Z., Tan, X-Y., Rashidi, B., An, Z., Perry, A.W., and Hoffman, R.M. Total-homocysteine enzymatic assay. *Clinical Chemistry* 46, 1686-1688, 2000.
5. Elshorbagy, AK., Smith, AD., Kozich, V., and Refsum, H. Cysteine and Obesity. *Obesity* (2012) 20, 473-481.

Patents:

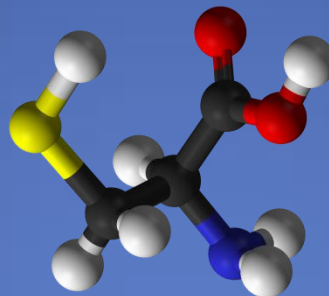
- China ZL02823051.5
- Europe 1,456,399
- Australia 2002346474
- Japan 4319545
- Canada 2,466,503
- So. Korea 10-976967

Cysteine – metabolic pathway



A/C DIAGNOSTICS

A/C L-Cysteine Kit



AntiCancer Inc.

7917 Ostrow St., San Diego, CA 92111

TEL: 858-654-2555, Fax: 858-268-4175

Email: all@anticancer.com

Locations also in Beijing, Nanjing,

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A/C DIAGNOSTICS

ENZYMATIC L-CYSTEINE ASSAY

FOR RESEARCH USE ONLY



Application:

- **High plasma cysteine linked to obesity (Plasma concentrations of cysteine, but not of other sulfur amino acids, correlate strongly with fat mass and BMI in men and women).**
- **Also high plasma cysteine is related to cardiovascular disease, metabolic syndrome and cancer.**

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Background:

Recent studies have demonstrated that plasma total cysteine (tCYS) levels are a risk factor of vascular disease in the coronary, cerebral, and peripheral vessels.

People with high levels of the amino acid cysteine carry 6 -10 kilograms more fat than other people. Professor Refsum's research indicate that cysteine plays a key role in how the body metabolises energy, stores fat, and breaks down fat .

Measurement of plasma total cysteine levels is very impotent to diagnoses risk for these diseases, and which could help to prevent and treat life-threatening obesity.

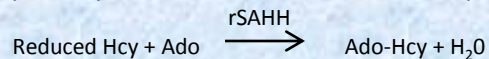
A/C Enzymatic total Cysteine Assay

The A/C Enzymatic total Cysteine assay is based on a three reagents protocol with two recombinant enzymes are used and a following DBPDA color reaction quantified in a absorbance reader. The basic version is run in 96-microtiter plates.

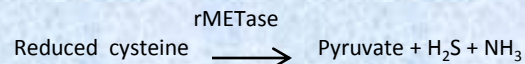
- 10 or 20 µl of sample
- Endpoint measurement at 675 nm (660-680nm)
- Measurement Range of 16.9 - 600 µmol/L
- Easy to adapt on liquid handing robots.
- High-throughput
- Three steps in 40 minutes for 40 test samples

Principle of the A/C tCYS Assay

Step 1, samples are reduced by DTT to generate free reduced L-Cys and HCY. Simultaneous use of rSAHH with excess adenosine converts the reduced HCY to S-adenosylhomocysteine, and eliminates interference by HCY.



Step 2, rMETase is added to generate H₂S from L-cysteine.



Step 3, H₂S combines with DBPDA to form an absorbent/fluorescent compound. For this assay, the absorbance is read at 675 nm .

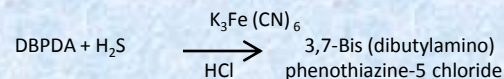


Fig. 1. tCYS Assay Dilution Linearity

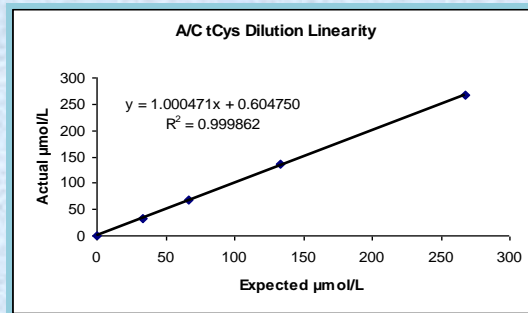
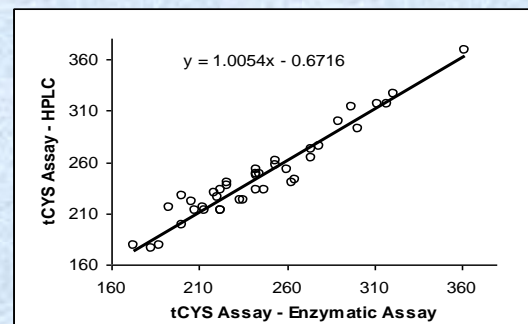


Fig. 2, Correlation of A/C Enzymatic tCYS Assay with HPLC



Assay Protocol of A/C tCYS Assay

Step 1

10 or 20 µl of sample



Add 200 µl of working R1



Shake and Incubate at 37°C for 20 minutes

Step 2



add 40 µl of working R2



Shake and Incubate at 37°C for 10 minutes

Step 3 Add 25 µl of Chromogen RII and 15 µl of Chromogen RIII.



Shake and Incubate at 37°C for 10 minutes



Read at between 660- 680nm wavelength.