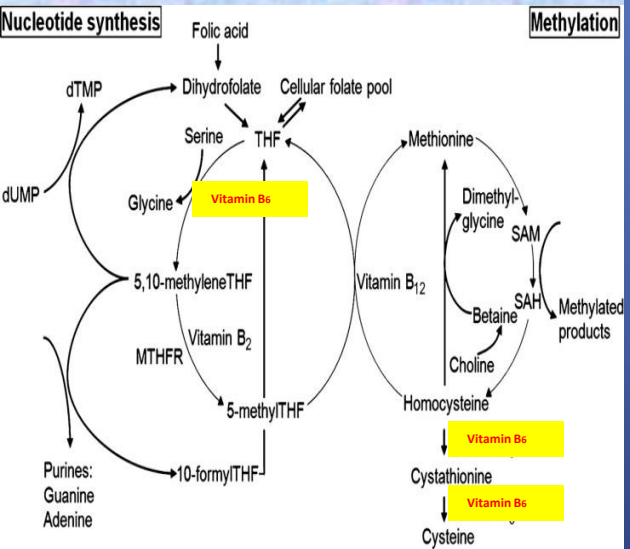
Key Publications and Patents

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, Xu M, Tang L, Tan X-Z, Tan X-Y, Tan Y, Hoffman RM. Homogeneous nonradioactive enzymatic assay for plasma pyridoxal 5-phosphate. *Clin Chem* **48**, 1560-1564 (2002).
3. Han, Q. and Hoffman, RM. Nonradioactive enzymatic assay for plasma and serum vitamin B_6. *Nature Protocols V. 3, No. 12 1815—19 (2008).*
4. Morris MS, Picciano MF, Jacques PF, Selhub J. Plasma pyridoxal 5'-phosphate in the US population: the National Health and Nutrition Examination Survey, 2003-2004. *Am J Clin Nutr 87, 1446-1454, (2008).*

Patents:

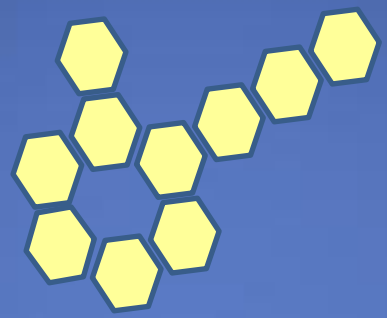
- US 6,426,194
- Australia 780,804
- Canada 2,361,077
- China ZL00803327.7
- Japan 4,098,475

Vitamin B6 dependence of one – carbon metabolism pathway



A/C DIAGNOSTICS

A/C Vitamin B6 Kit



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Locations also in Beijing, Nanjing, Seoul and Osaka

A/C DIAGNOSTICS

ENZYMATIC VITAMIN B6 ASSAY

FOR IN VITRO DIAGNOSTIC USE ONLY

FDA 510(K) K111260

Application:

- Determination of B6 status (B6 deficiency or overdose)
- Low B6 indicates hyperhomocysteinaemia, inflammation, anemia, and depression

www.anticancer.com

2013

Background:

Pyridoxal 5'-phosphate (PLP), the biologically active form of vitamin B_6, is an essential cofactor for multiple enzymes and involved in the synthesis and catabolism of neurotransmitter, homocysteine (HCY) transsulfuration, and the metabolism of other amino acids, fats, and glycogen. Large surveys in the US (NHANES) showed that B6 deficiency usually occurs in association with *neurological abnormalities including depression and cognitive dysfunction, anemia, impaired nutrient metabolism, steroid hormone function, immune function. T*he dependence upon exogenous sources to maintain adequate levels of Vitamin B_6 in the body makes it clinically desirable to measure pyridoxal 5'-phosphate.

A/C Enzymatic B6 Assay

The A/C Enzymatic B6 assay is based on a four reagent protocol with a single PLP dependent apo-enzyme and a following DBPDA color reaction quantified in a absorbance reader. The basic version is run in 96-microtiter plates.

- 10 µl of sample
- Endpoint measurement at 675 nm (660-680nm)
- Measurement Range of 15.6 – 250 nmol/L
- Easy to adapt on liquid handing robots.
- High-throughput
- The A/C Enzymatic B6 Assay successfully measured vitamin B6 levels in large US population for CDC-NHANES (2003-2004).

Principle of the A/C B6 Assay

The assay is based on a PLP-dependent recombinant homocysteine-α,γ-lyase (rHCYase), which is prepared in the apo-enzyme form by stripping off the cofactor PLP (vitamin B_6). The restoration of enzymatic activity by reconstitution of the holoenzyme depends on the amount of PLP in the plasma bound to apo-enzyme and production of H_2S by the enzymatic reaction. H_2S combines with DBPDA, the combination of which forms chromophore. The absorbance of this compound is read at 675 $_{nm}$ (660- -680nm).

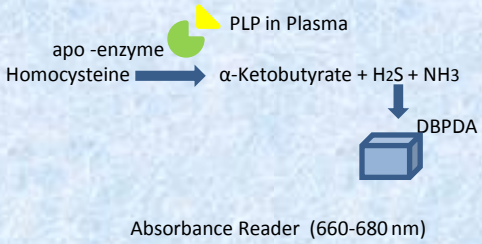


Fig. 1. Calibration Curve of A/C Enzymatic B6 Assay

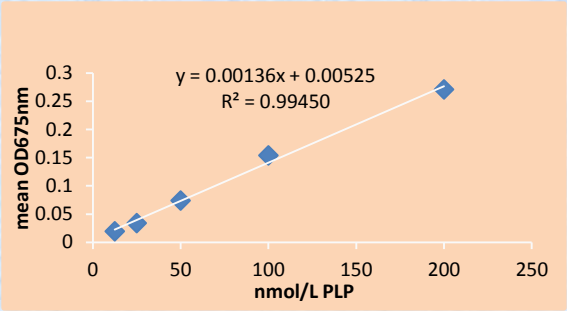
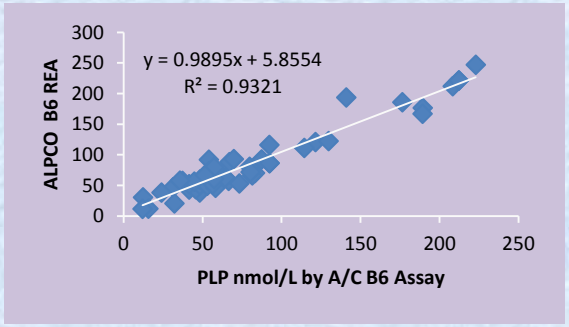


Fig. 2, Correlation of A/C Enzymatic B6 Assay with ALPCO B6 REA



Assay Protocol of A/C B6 Assay

Step 1

10 µl of sample

Add 150 µl of Working Binding Buffer

Shake and Incubate at 37°C for 60 minutes

Step 2

Add 100 µl of Working Assay Buffer

Shake and Incubate at 37°C for 20 minutes

Step 3

Add 25 µl of Chromogen RI and 15 µl of Chromogen RII

Shake and Incubate at 37°C for 10 minutes

Read at between 660- 680nm wavelength.