Key Publications and Patents


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

Vitamin B<sub>6</sub> dependence of one—carbon metabolism pathway

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**A/C Vitamin B<sub>6</sub> Kit**

**Application:**
- Determination of B<sub>6</sub> status (B<sub>6</sub> deficiency or overdose)
- Low B<sub>6</sub> indicates hyperhomocysteinaemia, inflammation, anemia, and depression

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**ENZYMATIC VITAMIN B<sub>6</sub> ASSAY**

**Application:**
- Determination of B<sub>6</sub> status (B<sub>6</sub> deficiency or overdose)
- Low B<sub>6</sub> indicates hyperhomocysteinaemia, inflammation, anemia, and depression

**www.anticancer.com**
2013
Pyridoxal 5’-phosphate (PLP), the biologically active form of vitamin B₆, 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. The dependence upon exogenous sources to maintain adequate levels of Vitamin B₆ 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 an 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

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 B₆). 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 H₂S by the enzymatic reaction. H₂S combines with DBPDA, the combination of which forms chromophore. The absorbance of this compound is read at 675 nm (660~680nm).

Assay Protocol of A/C B₆ Assay

### Step 1
1. Add 100 µl of Working Assay Buffer
2. Add 100 µl of Working Binding Buffer
3. Shake and Incubate at 37°C for 40 minutes

### Step 2
1. Add 25 µl of Chromogen RI and 15 µl of Chromogen RII
2. Shake and Incubate at 37°C for 20 minutes

### Step 3
1. Add 25 µl of Chromogen RI and 15 µl of Chromogen RII
2. Shake and Incubate at 37°C for 10 minutes

Read at between 660-680nm wavelength.