Vitamins and Others

Analysis of Vitamins

Introduction

Vitamin is a group of low-molecular weight compounds which are required in small amounts for normal health and metabolism. With few exceptions, humans cannot synthesize most vitamins and therefore need to obtain them from food and supplements. Insufficient levels of vitamins result in deficiency diseases, e.g., scurvy and pellagra, which are due to the lack of vitamin C and niacin, respectively. Analysis of vitamin levels of biological samples is helpful for determining human nutritional requirements, and assisting diagnosis of related diseases. In addition, analysis of vitamin in pharmaceutical preparations, food and food supplement products also plays an important role for the health and food sciences, and is one essential part of the compendium of evidence which defines nutritional status, and thus guides public health decisions about nutrient requirements, and the dietary or medical interventions in individuals. We have developed a suite of antibodies and antigens dedicated to vitamin immunoassays, and moving forward, we will roll out an expanded portfolio of products that act as core raw materials for accurate vitamin immunoassays across human fluid specimens and food/beverage matrices.

Products

Vitamins	Conjugate	Antibody
5MTHF	√	√
α-CEHC	√	√
Vitamin-B7 (Biotin)	√	√
25OH-Vitamin-D	√	√
Vitamin-A	√	
Vitamin-B12	√	
Folic Acid	√	
Vitamin-B6	√	
Vitamin-B1	√	
Vitamin-B2	√	
Vitamin-K1	√	
Vitamin-K2	√	

Vitamins

5-Methyltetrahydrofolate (5MTHF)

Folate is a group of water-soluble vitamin B9 vitamers, which plays an essential role for nucleic acid and protein synthesis by donating one-carbon units. Folate deficiency for women at childbearing age will increase the risk of offspring with neural-tube defects. For the general population, low folate levels can contribute to depression, cancers, Alzheimer's disease, dementia, and raised homocysteine level which is identified as a risk factor of cardiovascular disease and a lot of other disorders. Accurate quantification of the blood folate level is important for the evaluation of the clinical folate status. However, the microbiological assay and competitive protein-binding assay, two of the main methods for current clinic folate determination, can provide only an estimation of ''total folate'' which include both the active and inactive forms. Although various chromatographic and mass techniques have been utilized for specific determination of individual folate forms, their application for clinic screening of folate level isn't widely accepted due to the inconvenience for getting a clinically significant result. 5-Methyltetrahydrofolate (5MTHF) is the predominant active circulating folate coenzyme in plasma, its quantitative determination provides a more straightforward and valuable parameter for the diagnosis of folate deficiency. On the light of this need, we generated anti-5-MTHF McAb for developing sensitive immunoassay to evaluate the 5-MTHF blood level.

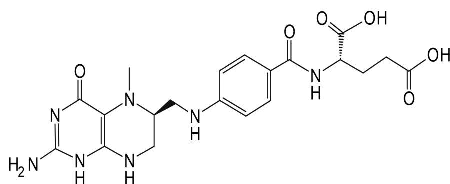

Fig. 1. The chemical structure of 5MTHF

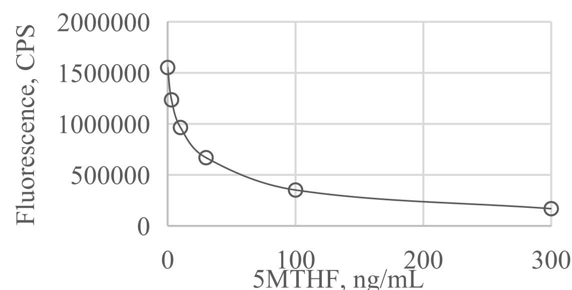

Fig. 2. The typical calibration curve of 5MTHF-DELFIA using McAb-37

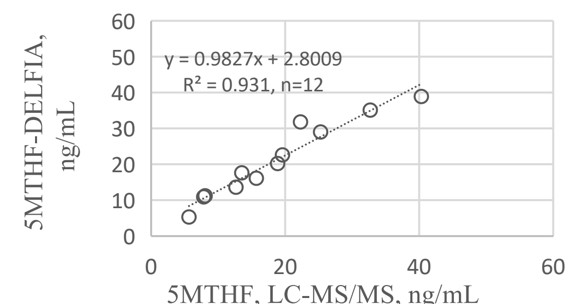

Fig. 3. The agreement between 5MTHF concentration obtained by LC-MS/MS and the DELFIA using McAb-37

Product Type	Catalog #	Description
Mouse monoclonal antibody	• Anti-5MTHF McAb-37	LOD < 5ng/mL by DELFIA using McAb-37. No cross-reaction was observed with 1000ng/mL of folic acid and methotrexate; The cross-reaction for folinic acid was ~0.6%. The key performances of the 5MTHF-DELFIA using McAb37 are presented in Fig. 2 and 3.
Conjugate	• 5MTHF-PEG-Biotin	Paired with anti-5MTHF McAb-37 for 5MTHF determination.

Note: Fresh serum is recommended as specimen for testing 5MTHF. EDTA-plasma should be avoided because of the rapid oxidative degradation of 5MTHF in this matrix. The use of antioxidative agents such as β-mercaptoethanol and ascorbic acid is helpful for stabilizing 5MTHF in samples and calibrators.

Vitamins

α-Carboxyethyl Hydroxychroman (α-CEHC)

Molecules with vitamin-E (VE) antioxidant activity include tocopherol and tocotrienol in their -α, β, δ, and γ types, among which, α-tocopherol is the major lipid soluble antioxidant in vivo for protecting against peroxidation. As the major metabolite of α-tocopherol, α-CEHC increases in the urine with the increased plasma α-tocopherol concentrations. A significant association between α-tocopherol intake and urinary α-CEHC was observed in some strictly controlled investigations, indicating that urinary α-CEHC level reflects recent α-tocopherol intake, and could be used as a measure of intake of VE during the previous period of time. Our high-affinity and specific anti-α-CEHC McAb can be used to establish immunoassay for sensitive quantification of low concentrations of a-CEHC, which may facilitate the evaluation of effectiveness of VE supplementation, understanding VE metabolism and drug-nutrient interactions.

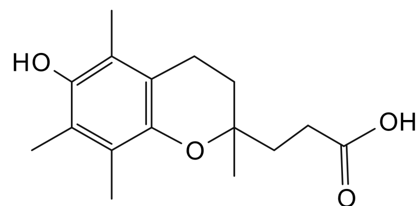

Fig. 1. The chemical structure of α-CEHC

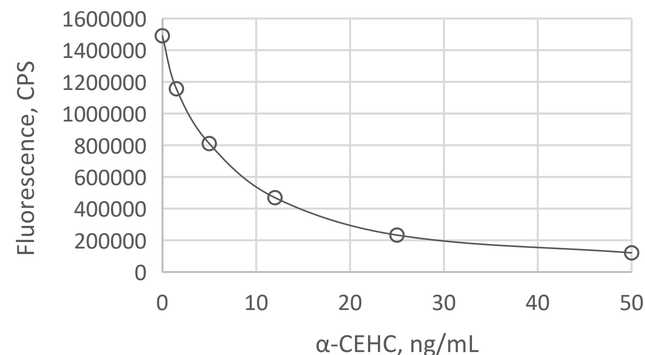

Fig. 2. The typical calibration curve of α-CEHC-DELFIA using McAb-1

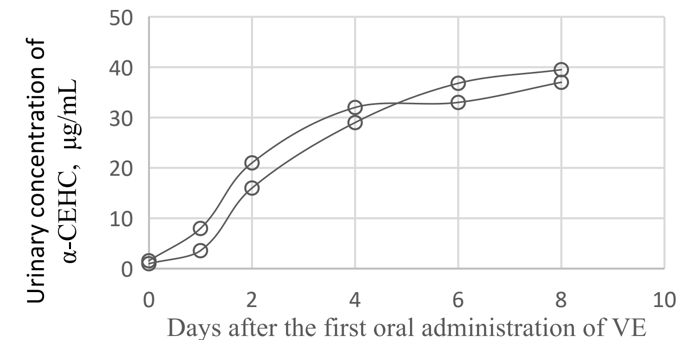

Fig. 3. The α-CEHC concentration in urine after oral VE administration at a daily dose of 800IU for two male volunteers

Product Type	Catalog #	Description
Mouse monoclonal antibody	• Anti-α-CEHC-McAb-1	LOD < 0.5ng/mL by DELFIA using McAb-1. No cross-reactivity was observed with γ-CEHC at 100μg/ml. Fig. 3 shows the time-dependent concentration profile of α-CEHC in urine following oral VE administration at a daily dose of 800IU for two male volunteers, determined by the McAb-1 based α-CEHC-DELFIA.
Conjugate	• α-CEHC-PEG-NH2	Paired with anti-α-CEHC McAb for α-CEHC determination.

Vitamins

Biotin (Vitamin-B7)

Biotin is a water-soluble vitamin belonging to the B-complex, which is found in small quantities in all living cells. It exists in eight isomer forms, but only D-biotin is biologically active. In humans, biotin functions as the cofactor of four carboxylases, which catalyze essential steps in mammalian intermediary metabolism. Biotin deficiency may cause a series of symptoms including periorificial dermatitis, conjunctivitis, developmental delay in infants and children, alopecia, ataxia, hypotonia, seizures, skin infection, thinning hair, skin rashes, and impaired immune function. Oral administration of pharmacological doses of biotin is usually effective and can reverse most of the symptoms, including the neurological ones. Determination of biotin in biological fluids can be used to evaluate the effectiveness of biotin supplementation and assist the diagnosis of biotin deficiency. Our anti-biotin McAb-26 can be applied to establish sensitive and specific immunoassay for determination of biotin levels in human fluids, pharmaceutical preparations, food and food supplement products.

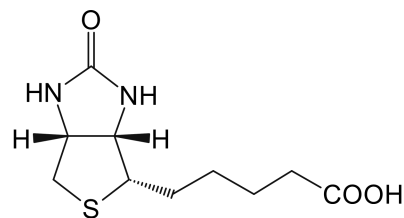

Fig. 1. The chemical structure of D-biotin

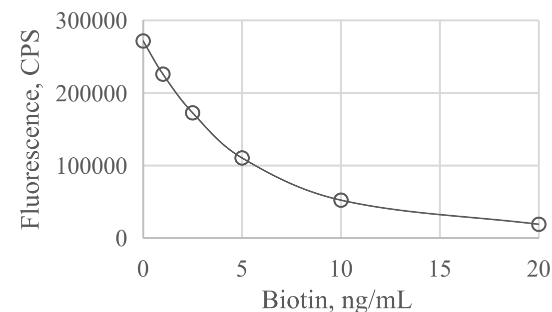

Fig. 2. The typical calibration curve of biotin-DELFIA using McAb-26

Product Type	Catalog #	Description
Mouse monoclonal antibody	• Anti-biotin-McAb-26	LOD < 0.5ng/mL by biotin-DELFIA. The cross-reactivities are < 5.9% for bisnorbiotin and < 1.5% for biotin sulfoxide.
Conjugate	• Biotin-PEG-Biotin	Paired with anti-biotin McAb-26 for specific detection of biotin.

Vitamins

25OH-VD Dissociating Agent

When immunoassays are used to detect the total concentration of small-molecule analytes in blood, it is often necessary to fully dissociate the small molecules from their binding proteins. This allows the analyte to be available for binding to antibodies, enabling accurate measurement of the total concentration of the small-molecules. Therefore, effective dissociation of protein-bound small molecules in specimens is a critical step for the accurate immunoanalysis of many small molecules, such as T3, T4, E2, P, T, FA, VB12, 25-OH-VD, FK506, RAPA, CSA, voriconazole, posaconazole, and others. There are three primary methods commonly used to dissociate protein-bound small molecules in specimens.

1) Organic solvent extraction. Organic solvents can denature proteins in the specimen, abolishing their ability to bind small molecules. This is a standard sample pretreatment for detecting small molecules via chromatography and mass spectrometry, and is also sometimes used in small-molecule immunoassays. This method achieves complete dissociation of small molecules from proteins while simultaneously eliminating major interfering substances in specimens, such as heterophilic antibodies and RF. However, it requires a centrifugation step to obtain the supernatant as sample, making the sample processing relatively cumbersome.

2) **Displacement and blocking method.** To simplify the sample pretreatment, small-molecule blockers can be directly added to the assay system to release the target analytes. This method is easy to implement but requires careful optimization of the type and concentration of the blocker to ensure efficient analyte dissociation without significantly impairing antibody-antigen binding. Although it does not eliminate interfering factors in specimens as mentioned in the first method, its simplicity and good dissociation efficiency make it one of the most commonly used approaches for small-molecule immunoassays.

3) **Limited denaturation of binding proteins.** Organic solvent extraction causes severe denaturation of blood proteins, leading to protein aggregation that requires centrifugation to obtain the supernatant. This centrifugation step not only increases sample processing complexity but also creates insurmountable barriers to full automation of the immunoassay. Fortunately, for small-molecule immunoassays where effective blockers are unavailable, limited denaturation of binding proteins can achieve efficient small-molecule dissociation without inducing protein precipitation. This method avoids the complexity of organic solvent extraction, is compatible with fully automated instruments, and delivers satisfactory detection accuracy.

Following extensive screening researches, we have developed a single-component 25OH-VD dissociating agent, which can effectively improve the accuracy of competitive or double-antibody sandwich immunoassays for 25OH-VD determination, and features excellent storage stability and ease of use.

Product Type	Catalog #	Description
Dissociating Agent	• VD Release-1	• The dissociating agent only needs to be mixed with specimen at a predetermined ratio to achieve rapid and efficient release of 25OH-VD from its binding proteins, thereby enhancing the accuracy of both competitive or double-antibody sandwich 25OH-VD immunoassays. • This product is compatible with whole blood, serum and plasma samples.

Others

Angiotensin I (AI)

Renin is an enzyme (EC 3.4.99.19) secreted from the juxtaglomerular cells of the kidney, it acts on the angiotensinogen to release angiotensin I (AI), a physiologically inactive decapeptide. This in turn is converted to angiotensin II, a potent vasoconstrictor that stimulates release of aldosterone. The overall effect is an increase in blood pressure. Renin is the limiting enzyme in the formation of angiotensin II and in the biological activity of the renin-angiotensin-aldosterone system. Plasma renin activity (PRA) can be assessed by incubating samples under appropriate conditions and measuring the concentration of AI liberated from the endogenous substrate angiotensinogen. Determination of PRA under different conditions of suppression and stimulation is useful for differentiating primary from secondary hyperaldosteronism, for subdividing hypertensive patients into different treatment protocols, and for screening patients to identify those with unilateral hypertension. Accurate quantification of AI is a prerequisite for accurate assessment of PRA. Our anti-AI McAb can be applied to establish sensitive immunoassay for determination of the AI levels in plasma.

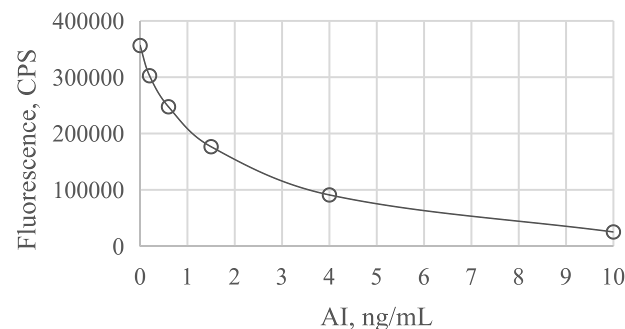

Fig. 2. The typical calibration curve of AI-DELFIA using McAb-12

Product Type	Catalog #	Description
Mouse monoclonal antibody	• Anti-AI McAb-12	LOD < 0.03ng/mL by DELFIA using McAb-12. The cross-reaction is < 1.6% with angiotensin II (AII).
Conjugate	• AI-PEG-Biotin	Paired with anti-AI antibodies for AI determination.

Others

Angiotensin II (AII)

Angiotensin II (AII) is an octapeptide (Asp1-Arg2-Val3-Tyr4-Ile5-His6-Pro7-Phe8) with potent vasoconstrictor properties. AII is formed by cleavage of a dipeptide from the carboxy-terminus of angiotensin I (AI). As the active component of the renin-angiotensin system, AII causes vasoconstriction and increases arterial pressure by binding to angiotensin II type 1 receptors. Moreover, angiotensin II stimulates the secretion of aldosterone, which causes an increase in sodium levels and fluid retention. All of these events can cause hypertension, left ventricular hypertrophy, and heart failure. Accordingly, accurate monitoring of the levels of angiotensin II in plasma is important for the management of hypertension and heart diseases. Our anti-AII McAb can be used to establish sensitive immunoassay for determination of the AII levels in plasma.

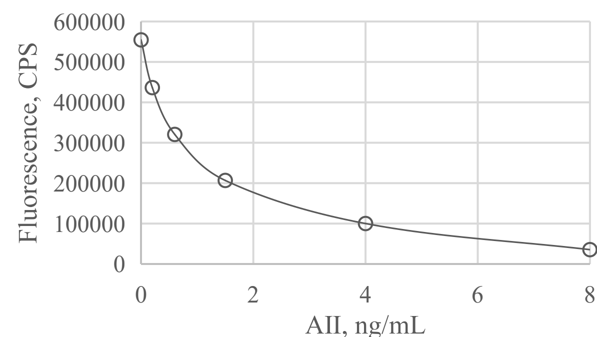

Fig. 2. The typical calibration curve of AII-DELFIA using McAb-12

Product Type	Catalog #	Description
Mouse monoclonal antibody	• Anti-AII McAb-9	LOD < 0.02ng/mL by DELFIA using McAb-9. The cross-reaction is < 3.2% with angiotensin I (AI).
Conjugate	• AII-PEG-Biotin	Paired with anti-AII antibodies for AII determination.