

# Reliable vitamin determination in food and feed

**VitaFast® - microbiological microtiterplate test to quantitate, vitamin B1 (thiamine), vitamin B2 (riboflavine), vitamin B6 (pyridoxine), vitamin B3 (niacin), vitamin B5 (pantothenic acid), vitamin B12 (cyanocobalamin), folic acid and vitamin B7 (biotin)**

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## Introduction

An increasing number of food products are now being enriched with vitamins. The vitamin content is monitored by manufacturers and control authorities to check compliance with labelling regulation.

The sample preparations play an important role but they vary considerable from one laboratory to the next. For the determination of vitamin enriched foods, a hot water extraction is usually sufficient. However, to measure the total vitamin content (including the native vitamins), the sample has to be treated with enzymes. Folic acid, vitamin B12 and biotin are generally present at very low concentrations in the µg / 100 g range. Most analytical methods are not able to give precise and reproducible results at such low levels.

Chromatographic methods such as HPLC are often the preferred method despite large inconveniences in the determination of folic acid, biotin and vitamin B12. The traditional microbiological method is very tedious as micro-organisms have to be cultured and stored, assay mediums have to be controlled, etc; but this method is still the golden standard in vitamin analysis.

VitaFast® test Kits are very user friendly as the reagents are ready to use.

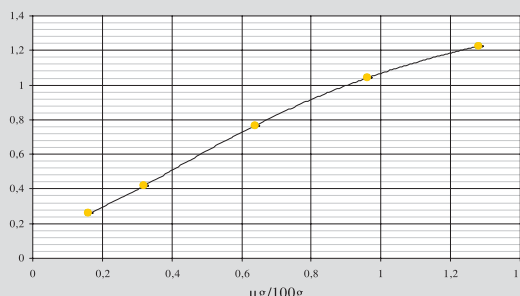
## Methods

The innovative microbiological assays for vitamins in test kit format (VitaFast®) are very user friendly. The vitamins are extracted from the sample and the extract is diluted. In the case of folic acid, e. g., the diluted extract and the folic acid assay-medium are pipetted into the wells of a microtiter plate which is coated with *Laktobacillus rhamnosus* (ATCC Nr. 7469). The growth of *Laktobacillus rhamnosus* is dependent on the supply of folic acid. Following the addition of folic acid as a standard or as a compound of the sample, the bacteria grow until the vitamin is consumed. The incubation is carried out in the dark at 37 °C (98.6 °F) for 44 - 48 h. The intensity of metabolism or growth in relation to the extracted folic acid is measured as turbidity and compared to a standard curve. The measurement is done using an ELISA reader at 610 - 630 nm (alternatively at 540 - 550 nm).

The concentration of the standard contained in the test kit is quality controlled by HPLC. The purity of the assay medium is checked by the ISO certified manufacturer ifp. The whole product line has been validated with internationally available reference materials from NIST, CRM and AACC and also with inter-laboratory studies. All these quality measures ensure a high analytical performance for the end user.



The VitaFast® test kit contains a microtiter plate coated with micro-organisms (96 wells), an additional holder, three adhesive foils, three bottles assay-medium, three standard bottles and three bottles sterilized water.



The standard curve from the quality assurance certificate for VitaFast® Folic Acid measured at 630 nm. The coefficient of variation of the standards is smaller than 5 %.

## Sensitivity and Specificity

Reference material	Target concentration in µg / 100 g	Measured concentration in µg / 100 g
NIST 1846 Infant formula	129 (101 - 157)	120 (Dilution 200)
		127 (Dilution 300)
		124 (Dilution 400)
		137 (Dilution 500)

The validation of the VitaFast® Folsäure / Folic Acid with the reference material NIST1846 Infant Formula shows excellent results. The VitaFast® product line has been validated with all kinds of available reference materials such as CRM, AACC, FAPAS, etc. with satisfying results.

Concentration	Vitamin B <sub>1</sub> µg / g	Vitamin B <sub>2</sub> µg / g	Vitamin B <sub>6</sub> µg / g
FAPAS target concentration*	81.2	88.6	90.2
VitaFast® results	86	82	95

\* The target concentration is the statistical average of all participants.

ifp has participated with the VitaFast® test kits in the FAPAS Proficiency Testing for vitamins (Report 2139, Vitamins in Liquid Supplement, April-June 2006). Most of the participants used HPLC but the microbiological method using VitaFast® test kits gave comparable results.

Concentration	VitaFast® in mg / 100 ml	Recovery after spiking with 0,4 mg / 100 ml
Multi-Vitamin-Juice	1.1 (dilution 1:10)	98%
	1.2 (dilution 1:20)	

The reliability of VitaFast® has also been checked in spiking experiments, in general the recoveries for all kind of food samples lie between 90 - 105 %. The juice contains according to the label a concentration of 0.8 mg B2 / 100 ml. VitaFast® gave a higher concentration as the producer presumably overdosed.

## Discussion

The standard curve shows a good reproducibility with very low coefficients of variation. The validations with international recognized reference materials, method comparisons with HPLC and recovery experiments with spiked samples gave excellent results.

In conclusion, the VitaFast product line shows high precision and accuracy. Thus it provides a valuable tool for cost effective vitamin analysis in routine laboratories as the hands-on time of the user-friendly test system is very short.