Vitamin testing solutions

from R-Biopharm

Determination of natural and/or synthetic vitamins, single or multi-vitamin testing options

ELISA, IAC, microbiological test kits or reusable cartridges

Global availability for test kits and equipment

More information:

https://r-b.io/vitamins_EN

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Vitamin testing solutions

What are vitamins?

Vitamins are essential nutrients for human development and metabolism. Vitamins can be separated into water soluble vitamins (e.g. vitamin C and the vitamins of the B group) and fat soluble vitamins (e.g. vitamin A, D, E and K). The human body cannot synthetize vitamins (except vitamin K and D). Therefore, vitamins have to be provided by food or supplements (see figure 1).

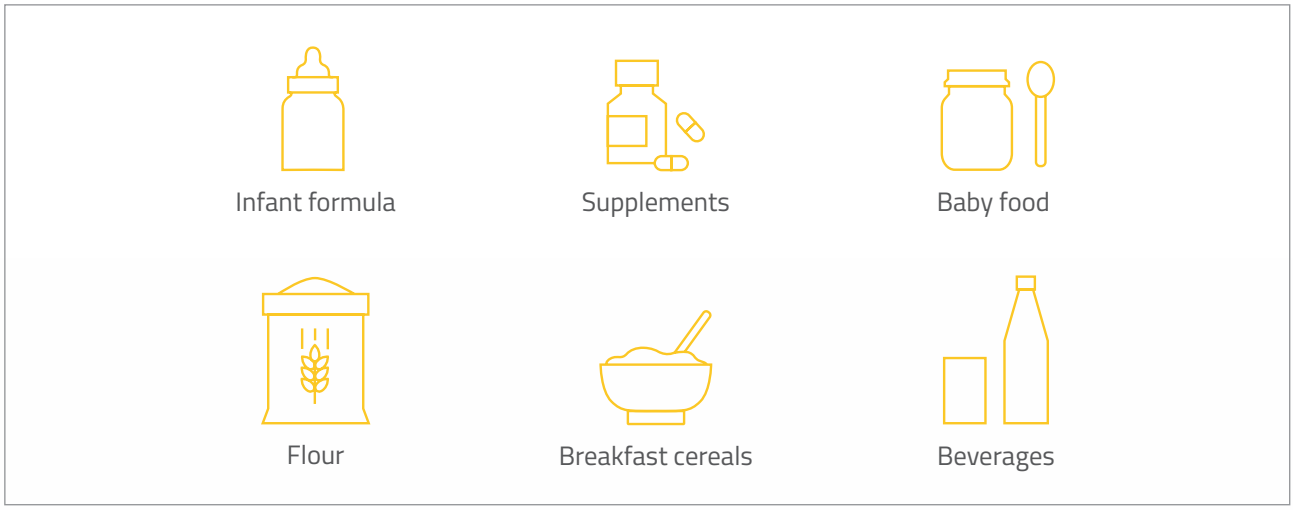


Figure 1: Frequently tested samples for vitamins are infant formula, baby food, nutritional supplements, flour, breakfast cereal and beverages.

Vitamin testing solutions

Why are food products tested for vitamins?

Accurate measurement of vitamin levels in foods is critical to comply with labelling requirements, product quality and internal product specifications. Additionally, by conducting regular testing production errors (e.g. problems during the addition of vitamin pre-mixes) are recognized early. Vitamins are susceptible to degradation, meaning that the levels in the food's formulation may not be the same as what is present in the final product due to heat, UV light, incorrect pH and certain metals. Hence, it is necessary that manufacturers as well as governmental labs control vitamin contents.

Quantify added or natural vitamins?

Food can contain natural as well as synthetic vitamins (see table 1). Fortification with synthetic vitamins is usually done by adding pre-mixes into the product. Knowing the form(s) of the vitamin in a food is critical to ensure the correct sample preparation and choosing the respective analytical method.

Table 1: Some examples for natural and/or synthetic vitamin forms.

Vitamin	
B1	Thiamin, thiamin hydrochloride, thiamin mononitrate
B2	Riboflavin, riboflavin-5'-phosphate
B3	Niacin, niacinamide
B5	Pantothenic acid, calcium pantothenate
B6	Pyridoxine, pyridoxamine, pyridoxal, pyridoxine hydrochloride
B7	Biotin
B9	Folic acid, multiple naturally-occurring folates
B12	Hydroxocobalamin, methylcobalamin, cyanocobalamin
C	Ascorbic acid, deoxyascorbic acid, multiple synthetic forms

Vitamin testing solutions

What analytical methods are offered by R-Biopharm?

R-Biopharm AG offers different test kits for the detection of water soluble vitamins in food and supplements.

VitaFast®

Microbiological tests

- Samples with an added or natural vitamin content can be analyzed
- Method in conformity with official guidelines (§ 64 of the German Food & Feed Act, AOAC)
- AOAC-RI certification for some VitaFast® tests
- Ready-to-use reagents and standards for 96 determinations
- Results available within 24 - 48 hours

RIDASCREEN®

ELISA

- Determination of total vitamin B12 content
- Determination of added folic acid vitamin
- One sample preparation procedure and one identical sample buffer for RIDASCREEN®FAST Vitamin B12 and Folic Acid
- Results within 1 - 2 h
- Ideal for process control

EASI-EXTRACT®

Immunoaffinity columns

- Isolation and concentration of the vitamin
- Pigments and interfering compounds are removed
- High recovery and low coefficient of variation
- Products referenced in AOAC Official Final Action Methods
- Unique multi-vitamin immunoaffinity column for the simultaneous detection of vitamin B12, folic acid and biotin

Vitamin testing solutions

What do the different analytical methods detect?

The RIDASCREEN®FAST Vitamin brand contains two different **ELISAs**, which are designed for vitamin testing. The antigen-antibody reaction of the respective ELISA is evaluated by a microtiterplate photometer. The immunoassay kit is suitable for multi-vitamin tablets, capsules, multi-vitamin juices, multi-vitamin jam, grain products, multivitamin sweets etc. RIDASCREEN®FAST Vitamin B12 (Art. No. R2103) detects total vitamin B12 and RIDASCREEN®FAST Folic acid (Art. No. R3203) detects added synthetic folic acid.

The brand VitaFast® includes **different microbiological test kits**, which detect added or natural vitamins. The wells of the microtiter plate are coated with lyophilized microorganisms. The growth of microorganisms is measured turbidimetrically with a microtiterplate reader. Different vitamin parameter are available for all water-soluble vitamins.

HPLC/LC-MS/MS in combination with **immunoaffinity columns** quantifies total vitamin content (vitamin B12 and biotin) or added vitamin content (folic acid). EASI-EXTRACT® MULTI-VIT B (LGE) allows to measure multiple vitamins (biotin, vitamin B12 and folic acid) simultaneously.

Table 2: Comparison of the vitamin testing methods for the analysis of vitamin B12.

	RIDASCREEN®FAST	VitaFast®	EASI-EXTRACT®
Method	ELISA	Microbiological test	Immunoaffinity column prior to HPLC or LC-MS/MS detection
Vitamins measured	Vitamin B12 total content, Folic acid added content	Added or total vitamin content depending on sample preparation	Vitamin B12 total content, biotin total content or folic acid added content
Benefits	Easy to use, no toxic chemicals for total vitamin B12	AOAC-RI	AOAC-OMA
Time to result (e.g. 10 samples B12)	65 min	48 h	7.5 h
Hands-on time (e.g. 10 samples B12)	40 min	4 h	4.5 h

Vitamin testing solutions

What equipment is required for the different vitamin test kits?

Depending on the analytical method, different equipment is needed. The graphic below combines the method with the necessary equipment for three different lab sizes (starting lab, lab with HPLC, fully automized lab).

Lab starting with vitamin analysis (minimal equipment)

- VitaFast®
- RIDASCREEN®FAST ELISA

MTP reader
- RIDA®ABSORBANCE 96

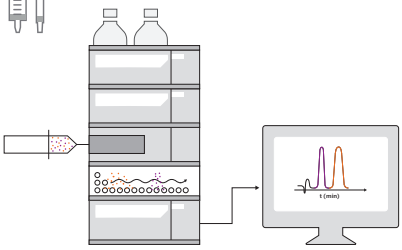
Accessories
VitaFast® Spiking Standard Folic acid, Vitamin B12, Biotin and Pantothenic acid

Lab equipped with HPLC

- VitaFast®
- RIDASCREEN®FAST ELISA

MTP reader
- RIDA®ABSORBANCE 96

- EASI-EXTRACT® immunoaffinity columns

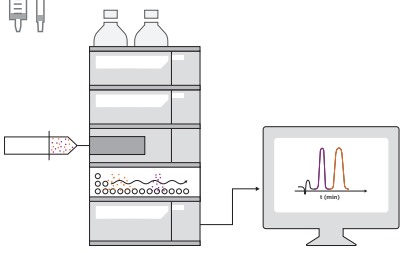


HPLC

Accessories
VitaFast® Spiking Standard Folic acid, Vitamin B12, Biotin and Pantothenic acid

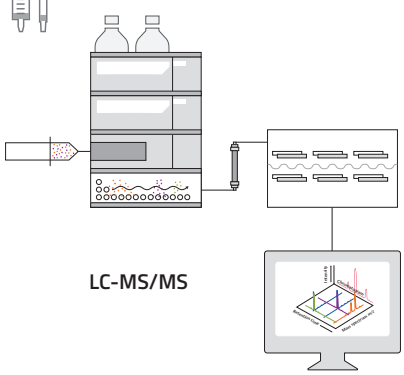
Fully automized lab

- EASI-EXTRACT® immunoaffinity columns



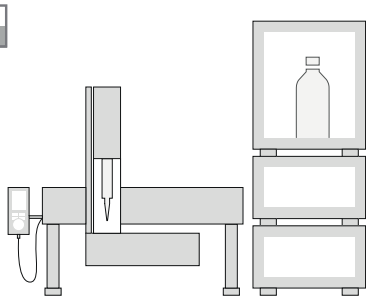
HPLC

- EASI-EXTRACT® immunoaffinity columns



LC-MS/MS

IMMUNOPREP® ONLINE cartridges



Automated system for use with HPLC or LC-MS/MS
- CHRONECT Symbiosis RIDA®CREST

Vitamin testing solutions

What equipment is available from R-Biopharm AG?

For the evaluation of the ELISA and microbiological test kits R-Biopharm AG offers the RIDA®Absorbance 96 (Art. No. ZRA96FF). The RIDA®CREST could be used for immunoaffinity cartridges for vitamin B12.

RIDA®ABSORBANCE 96

Absorbance reader

Innovative microtiter plate photometer including RIDASOFT® Win.NET software

CHRONECT Symbiosis RIDA®CREST

UHPLC system for automated, online clean-up using the IMMUNONPREP® ONLINE immumoaffinity cartridges

Vitamin testing solutions

What products have been externally validated?

The AOAC-PTM program certifies that a method performs according to the manufacturer's documented claims. The manufacturer submits its internal validation. The AOAC accepts the data, and an independent lab carries out verification with exemplary samples from the internal validation (typically 2 or 3 matrices are verified). The report from the independent lab is reviewed by the AOAC experts.

Methods having an AOAC-OMA validation have been more rigorously tested than methods having only an AOAC-PTM certificate. A collaborative study with a minimum of 5 test materials and at least 8 independent labs needs to be carried out and strict method performance criteria need to be met.

Table 3: Overview about the approvals of the vitamin test kits.

	VitaFast®	EASI-EXTRACT®	RIDASCREEN®	IMMUNOPREP®
	Microbiological / enzymatic tests	Immunaffinity columns	ELISA	Online immunoaffinity cartridges
Vitamins				
Folic Acid	AOAC-PTM	●	●	
Vitamin B12 (Cyanocobalamin)	AOAC-PTM	AOAC-OMA	●	●
Vitamin B7 (Biotin)	AOAC-PTM	AOAC-OMA		
Vitamin B3 (Niacin)	●			
Pantothenic Acid	AOAC-PTM			
Vitamin B1 (Thiamin)	●			
Vitamin B2 (Riboflavin)	AOAC-PTM			
Vitamin B6 (Pyridoxin)	●			
Inositol	●			
Vitamin C (L-Ascorbic Acid)	●			
Multi-Vitamin B		●		

Vitamin testing solutions

How can you check for matrix effects in food sample ?

Different spiking standards are available from R-Biopharm AG to identify matrix effects.

Table 4: Spiking standard for VitaFast® test kits.

	VitaFast®
	Spiking standards
Vitamins	
Folic Acid Spiking standard	●
Vitamin B12 Spiking standard	●
Vitamin B7 (Biotin) Spiking standard	●
Pantothenic Acid Spiking standard	●

Vitamin testing solutions

Which testing approach is right for me?

Medium sized contract labs

Equipment

RIDA®ABSORBANCE 96

Test kits

VitaFast® test kits

"Microbiological assays are recognized by international official institutions as the gold standard for many years. Microbiological methods allow the determination of both native and added vitamins.

In the past we have used traditional microbiology for vitamins. However, we had problems with media production, contamination, maintaining of stock cultures and procurement of raw materials. The lab staff are enthusiastic about VitaFast® test kits, where specific microorganisms are pre-coated in the microtiter plate wells. The test reagents are ready to use and standardized. The medium and standard only have to be reconstituted with sterile water contained in the kit.

Depending on the food matrix different vitamins can be determined from the same sample extract (see table 5). Compared to traditional microbiological methods, VitaFast® test kits significantly reduce sample processing time and provide greater reliability, higher productivity and better accuracy."

Table 5: The sample preparation within the same food category and having the same color code are in principle combinable (white color = methods are not combinable).

Parameter	Art. No.	Total vitamin content in infant food/baby food	Added vitamins in capsules, pills, vitamin mixes	Added vitamins in liquid samples e.g. multivitamin juices, fitness drinks	Added vitamins in cereals, flour
Folic Acid	P1001				
Vitamin B12 (Cyanocobalamin)	P1002				
Vitamin B7 (Biotin)	P1003				
Vitamin B3 (Niacin)	P1004				
Pantothenic Acid	P1005				
Vitamin B1 (Thiamin)	P1006				
Vitamin B2 (Riboflavin)	P1007				
Vitamin B6 (Pyridoxin)	P1008				

Vitamin testing solutions

Ingredients manufacturer

Equipment

RIDA®ABSORBANCE 96

Test kits

- VitaFast® test kits
- RIDASCREEN®FAST Vitamin B12
- RIDASCREEN®FAST Folic Acid

"We produce intermediate products containing synthetic vitamins. We used to send our vitamin samples (bars, powders, liquids, capsules and baked goods) to an external lab, but this was very time consuming and took at least 10 days. Investment in HPLC and staff was too expensive and complicated. We decided to use VitaFast® test kits. When starting with VitaFast® we had to get used to sterile working techniques like wearing gloves, sterile filtering and cleaning the workspace before starting. VitaFast® is really user friendly and can be established quickly.

VitaFast® provides a high analytical performance.

Sometimes the impact of food processing might have an impact on vitamins. For a quick process control we use RIDASCREEN®FAST Vitamin B12 and RIDASCREEN®FAST Folic Acid as they have the same sample preparation and results are available within two hours. With the ELISA we also check for vitamin homogeneity in large food batches. The flexible microtiter plate format is suited for small as well as large number of samples."

Vitamin testing solutions

Govermental lab

Equipment

RIDA®ABSORBANCE 96

Test kits

- VitaFast® test kits
- RIDASCREEN®FAST Vitamin B12

"As a government lab our major role is to verify the concentration of vitamins in the food with the product label. Sometimes at the end of the shelf life the vitamins have degraded. This is the reason why food producers add more vitamins than labelled. Depending on the product we detect total or added vitamins.

We stopped with traditional microbiology, because we had quality problems and we needed to save lab space. We buy a whole range of VitaFast® test kits. We do not carry out the sample preparation to determine the total vitamin B12 content using VitaFast® Vitamin B12 as it uses the toxic cyanide and we want to protect our lab staff. Instead we use RIDASCREEN®FAST Vitamin B12 to determine the total vitamin B12 content, the test kit has no toxic sample preparation and results are available within 1 - 2 hours."

Vitamin testing solutions

Infant formula manufacturer

Equipment

HPLC

Test kits

- EASI-EXTRACT® VITAMIN B12 (LGE)
- EASI-EXTRACT® BIOTIN
- VitaFast® test kits

"Babies are vulnerable consumers and as a result infant formula is the most regulated food in the world. It is therefore, vital for us to follow Official Methods where possible. Both EASI-EXTRACT® VITAMIN B12 (LGE) and EASI-EXTRACT® BIOTIN are referenced in AOAC Final Action Official Methods therefore we felt confident that these products could meet with the strict performance criteria required and would provide accurate and reliable results at the low levels we work at ensuring that we could confirm the claim levels of our products.

The R-Biopharm LGE format column is unique and is the only column of this format on the market. LGE columns are larger format column which allows the sample to mix directly with the gel. We have observed slightly higher recoveries and better % RSD value."

For pantothenic acid we use VitaFast® Pantothenic acid as it gives better results compared to HPLC.

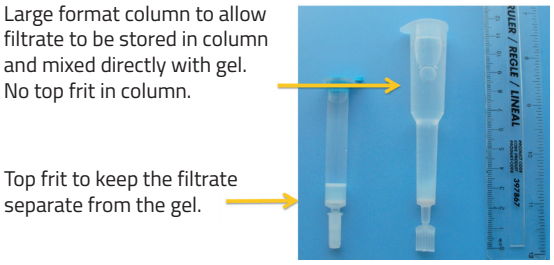


Figure 2: Immunoaffinity products

Vitamin testing solutions

Large contract lab

Equipment

HPLC

Test kits

- EASI-EXTRACT® VITAMIN B12
- EASI-EXTRACT® BIOTIN

"Although a high percentage of our vitamin testing is conducted on infant milk formula/dairy industry we routinely get a range of other samples to test for our customers. We therefore require some versatility in our methods to ensure we obtain optimum results and as a result implemented immunoaffinity column methods (EASI-EXTRACT® VITAMIN B12, EASI-EXTRACT® FOLIC ACID and EASI-EXTRACT® BIOTIN).

Extraction methods can easily be slightly modified if required and technical support is always available from R-Biopharm if we come across an unusual sample. The range of application notes available is essential to us and we routinely rely on these which helps us to meet our quick turnaround times."

Vitamin testing solutions

Multi-national dairy company

Equipment

CHRONECT Symbiosis RIDA®CREST

Test kits

IMMUNOPREP® ONLINE VITAMIN B12

"We previously only conducted small numbers of vitamin analysis in-house using the R-Biopharm immunoaffinity columns despite having our own in-house laboratory and trained technical staff. The majority of samples were sent to an external lab for analysis.

Costs for the external analysis were increasing therefore we wanted to bring analysis in-house, focusing on vitamin B12 as over 70 % of our samples were for this analyte. The aim was to reduce overheads and to improve the speed of results. We currently had to wait 4 - 6 working days. We routinely sent 25 samples per week for analysis with a cost of >100 € per test.

It was important that the method implemented had excellent performance and sensitivity as the majority of samples tested were infant formulas. Using the CHRONECT Symbiosis RIDA®CREST and IMMUNOPREP® ONLINE VITAMIN B12 cartridges we have been able to significantly reduce annual spend on outsourcing and have been able to provide results to our customers quicker. Overall, we have been impressed that testing samples in-house is more cost effective, with the return on investment for the CHRONECT Symbiosis RIDA®CREST being achieved with a short time frame of around 18 months."

Vitamin testing solutions

Pharmaceutical company – validating the multi-vitamin column

Equipment

LC-MS/MS

Test kits

EASI-EXTRACT® MULTI-VIT B (LGE)

"We have historically been using other methods for the analysis of vitamins however we are re-assessing our internal methods with the aim of improving our workflow. We therefore were looking at introducing a range of methods for multi-analytes if possible.

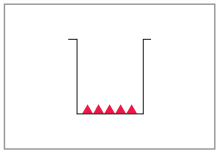
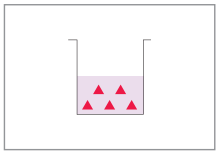
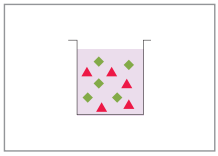
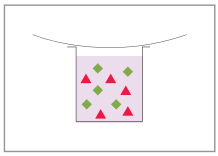
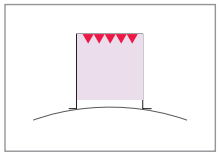
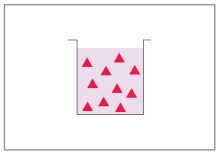
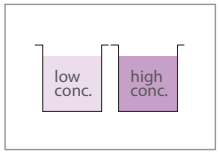
We are using LC-MS/MS detection where possible and one area that we were looking to improve was the analysis of certain B-vitamins; namely vitamin B12 and biotin. Our products are a range of multi-vitamins which contain a range of minerals and antioxidants – many of which can cause issues during our current extraction.

We found out online that R-Biopharm had a range of immunoaffintiy columns, some of which were recommended in AOAC Official Methods. This gave us the confidence that the products were robust and could meet with the strict recovery requirements that we were looking for. After a consultation with the team at R-Biopharm, we started to consider the EASI-EXTRACT® MULTI-VIT B (LGE) columns instead of the single vitamin products. EASI-EXTRACT® MULTI-VIT B (LGE) is the only multi-vitamin column on the market.

One of our initial challenges was to ensure we had a suitable sample preparation for our specific range of products. The technical team in Glasgow helped us to develop a simple protocol which met with our specifications. We are now in the final stages of validating the method. We are looking forward to finally implementing this method as we feel that it will bring many benefits to us whilst still ensuring that we reach our required performance specifications."

VitaFast® – workflow

For this microbiological assay sterile working and a good pipetting technique is important. It is strongly recommended to pipette samples and standards in triplicate. Depending on the sample preparation natural or synthetic vitamins can be quantified. It is important to suspend the sample completely. Sample preparation has to be done as outlined in the respective Instruction for Use. Important note: This workflow should describe the general test procedure and the test principle. It does not replace reading of the respective IFU.

Test procedure		Test principle	
1	Place the required number of microwell strips in the frame		Microwells are coated with specific microorganisms
2	Add 150 µL of assay medium		The addition of the assay medium leads dissolves the microorganisms
3	Add 150 µL of standard or sample		The growth of microorganism is dependent on the supply of the respective vitamin in the sample
4	Cover the strips with adhesive foil		It is important that the foil sticks to the grid at every single well to prevent evaporation
Incubate the vitamin test according to the respective temperature and time (ref. IFU) in the dark			
5	Press down the adhesive foil once more. Place the microtiter plate upside down on the table and dissolve the microorganisms by shaking the plate on the surface of the desk		As the microorganisms have settled to the bottom of the well during incubation, it is important to mix the content of the well
6	Invert the plate to the regular position and remove the adhesive foil and remove remaining air bubbles before measurement		The intensity of growth of the microorganisms is correlated to the extracted vitamin and compared to a standard curve for quantitative turbidimetrical measurement
Read the plate in a microtiter plate photometer at 540 - 550 nm or 610 - 630 nm, use 4 parameter software			
7	For evulation use the RIDASOFT® Win.NET		The turbidity in each well corresponds to the growth of the microorganism

◆ Vitamin ▲ Microorganisms