



CERTIFIED REFERENCE MATERIAL BCR[®] – 487

CERTIFICATE OF ANALYSIS

PIG LIVER			
Vitamin	Mass fraction (dry matter)		Number of accepted sets of results p
	Certified value ¹⁾ [mg/kg]	Uncertainty ²⁾ [mg/kg]	
B ₁ (thiamine) ³⁾	8.6	1.1	18
B ₂ (riboflavine)	106.8	5.6	12
B ₆ (total pyridoxine) ⁴⁾	19.3	2.9	11
B ₁₂	1.12	0.09	8
Folate (total)	13.3	1.3	17
¹⁾ This value is the unweighted mean of the means of p accepted sets of results obtained by different sample preparation procedures and analytical techniques. The values are traceable to the International System of Units (SI). ²⁾ The uncertainty is taken as the half-width of the 95 % confidence interval of the mean defined in (1). ³⁾ Expressed as thiamin chloride hydrochloride. ⁴⁾ Expressed as pyridoxine hydrochloride.			

This certificate is valid for one year after purchase.

Sales date:

The minimum amount of sample to be used is 2.5 g each for all vitamins.

NOTE

This material has been certified by BCR (Community Bureau of Reference, the former reference materials programme of the European Commission). The certificate has been revised under the responsibility of IRMM.

Brussels, March 1998
Revised: September 2013

Signed: _____

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Additional Material Information	
	Mass fraction (dry matter)
	Value ¹⁾ [mg/kg]
5-methyltetrahydrofolic acid (5-MTHF)	2.6
¹⁾ This value is the unweighted mean of the means of 3 sets of results obtained by a common sample preparation procedure (sample digestion with hog kidney enzyme followed by solid-phase extraction) and analytical technique (HPLC with fluorescence detection). The values are traceable to this methodology	

DESCRIPTION OF THE SAMPLE

CRM 487 is a lyophilised pig liver powder which is packaged into rubber-stoppered penicillin vials under an inert atmosphere. Each sachet contains approximately 15 g of material.

ANALYTICAL METHOD USED FOR CERTIFICATION

- Normal & reverse phase HPLC with fluorimetric detection (vitamins B₁, B₂ & B₆)
- Fluorimetry (vitamin B₁)
- Microbiological assay using *Lactobacillus Fermenti* (ATCC 8014) & *Lactobacillus viridescens* (ATCC 12706) (vitamin B₁)
- Microbiological assay using *Saccharomyces calshbergensis* or *uvarum* (ATCC 9080), or *Neurospora Sitophila* (ATCC 9776) (vitamin B₆).
- Microbiological assay using *Lactobacillus rhamnosus* (ATCC 7469) (vitamin B₁₂ & folate).
- Competetive Radio-protein binding assay (vitamin B₁₂).

A detailed description of the employed methods can be found in the certification report.

PARTICIPANTS

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- National Food Administration, Uppsala (SE)
- National Food Agency of Denmark, Soborg (DK)
- Paediatrics & Child Health, University of Leeds (GB)
- Pedigree Pet Foods, Melton Mowbray (GB)
- Schweizerisches Vitam Institut, Basel (CH)
- TNO Nutrition & Food Research Institute, Zeist (NL)
- Unilever Research Colworth Laboratory, Bedford (GB)
- University College, Cork (IE)
- University Louis Pasteur, Strassburg (FR)

SAFETY INFORMATION

The usual laboratory safety measures apply.

INSTRUCTIONS FOR USE

The material is intended to be used for calibration and for performance verification of an analytical method.

1. Sachets should be allowed to equilibrate to room temperature before opening. Contents should be used on the day of opening only.
2. Before removing a sample for analysis, the material in the sachet should be thoroughly mixed. The recommended sample sizes are 2.5 g for all vitamins.
3. Methods used for the determination for vitamin B₁ should include extraction with dilute acid with heating, followed by dephosphorylation with a suitable takadiastase enzyme, and determination using either a microbiological assay, or HPLC with fluorimetric detection (after pre- or post-column conversion to thiochrome).
4. Methods used for the determination for vitamin B₂ should include extraction with dilute acid with heating, followed by dephosphorylation with a suitable takadiastase enzyme, and determination using either a microbiological assay, or HPLC with fluorimetric detection.
5. Methods used for the determination for vitamin B₆ should include extraction with either dilute acid with heating, or trichloroacetic acid at ambient temperature, followed by dephosphorylation with suitable takadiastase & phosphatase enzymes, and determination using either a microbiological assay, or HPLC with fluorimetric detection. For microbiological procedures, an extraction time of > 4 h at 121 °C is required to give equivalent results to the HPLC procedures.
6. Methods used for the determination of vitamin B₁₂ should include extraction using buffer, followed by treatment with cyanide, and determination using either radioassay kit, or microbiological assay.
7. Methods used for the determination of total folate should include extraction using buffer and heating, deconjugation with a hog kidney deconjugase enzyme (or any other suitable deconjugase preparation such as human plasma or chicken pancreas), and determination using a microbiological assay. A media pH of 6.2 should be used for organism growth and a suitable antioxidant (such as ascorbic acid) added to the samples prior to extraction.
8. The certified values are expressed on dry matter bases. The dry matter correction must be made on a separate sub-sample of the contents of the same sachet taken for vitamin analyses, and should be made in parallel to the latter (see Certification Report for details).
9. The stated uncertainty applies when the reference material is used for calibration, or for verifying the validity of a calibration curve. When it is used to assess the performance of an analytical technique, the user may refer to the recommendations in the chapter "Instructions for Use" of the certification report.

Dispose in accordance with good laboratory practice.

STORAGE

Each sachet should be stored unopened, and at temperatures not exceeding –20 °C. However, the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened samples.

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NOTE

A technical report on the production of BCR-487 is available on the internet (<http://www.irmm.jrc.be>). A paper copy can be obtained from IRMM on request.