



**EUROPEAN COMMISSION**  
JOINT RESEARCH CENTRE  
Institute for Reference Materials and Measurements (Geel)  
**Standards for Food Bioscience**  
European Union reference Laboratory for Feed Additive - Authorisation

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**EURL Evaluation Report on the Analytical Methods submitted  
in connection with the Application for Authorisation of a Feed  
Additive according to Regulation (EC) No 1831/2003**

**Quantum Blue**  
*(FAD-2012-0015; CRL/120000  
FAD-2012-0033; CRL/120040)*



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Dossier related to: **FAD-2012-0015 - CRL/120000  
FAD-2012-0033 - CRL/120040**

Name of Product: **Quantum Blue (5 L, 10 L, 5 G, 40 P)**

Active Substance(s): **6-phytase (EC 3.1.3.26)**

Rapporteur Laboratory: **European Reference Laboratory for Feed  
Additives (EURL-FA)**

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Date: **23/04/2013**

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Date: **26/04/2013**

## EXECUTIVE SUMMARY

In the current two applications (FAD-2012-0015 and FAD-2012-0033) authorisation is sought under article 4(1) for *Quantum Blue 5 L, 10 L, 5 G* and *40 P* under the category/functional group 4(c) "zootechnical additives"/"substances which favourable affect the environment", according to the classification system of Annex I of Regulation (EC) No 1831/2003. According to the Applicant, the active agent of all *Quantum Blue* products is *6-phytase* (EC 3.1.3.26) produced by the strain *Trichoderma reesei* (CBS 126897). The authorisation is sought for the use of the *feed additive* for a variety of animal species. According to the Applicant, *Quantum Blue 5 L* and *10 L* are liquid preparations with a guaranteed minimum enzyme activity of 5000 and 10000 FTU/g, respectively, while *Quantum Blue 5 G* and *40 P* are a granulate (G) or powder (P) preparation with a guaranteed minimum enzyme activity of 5000 and 40000 FTU/g, respectively. *Quantum Blue* preparations are intended to be used in *premixtures* and/or complete *feedingstuffs* to obtain minimum enzyme activities for 6-phytase of 150 and 250 FTU/kg *feedingstuffs* depending on the target species. The dry products can be mixed with the final feed or with the mineral *premixtures*, while the liquid product is to be sprayed onto feed pellets.

The Applicant used the enzyme activity units as defined in the EN ISO 30024:

- One *6-phytase* unit (FTU) is the amount of enzyme which liberates 1 micromole of inorganic phosphate from sodium phytate in one minute at 37°C and pH 5.5.

For the determination of the activity of *6-phytase* in the *feed additive, premixtures* and *feedingstuffs*, the Applicant proposed an in-house developed and validated colorimetric method measuring the inorganic phosphate released by the enzyme from the sodium phytate substrate. On the request of the EURL the Applicant applied the internationally recognised ring-trial validated colorimetric CEN method (EN ISO 30024) for the determination of *6-phytase* in the *feed additive, premixtures* and *feedingstuffs* samples containing *Quantum Blue* products and provided performance characteristics similar to those reported in the EN ISO 30024 standard: - a precision (repeatability and reproducibility) ranging from 1.2 to 5.5 %; - a *recovery* rate ranging from 80.4 to 102.4 %; and - a limit of quantification of 101 FTU/kg *feedingstuffs*.

Based on the experimental evidence and the performance characteristics presented, the EURL recommends for official control the EN ISO 30024 method, for the determination of the activity of the *6-phytase* in the *feed additive, premixtures* and *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

## KEYWORDS

"Quantum Blue 5 L, Quantum Blue 10 L, Quantum Blue 5 G, Quantum Blue 40 P, 6-phytase, zootechnical additives, substances which favourably affect the environment, chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening and breeding, piglets, pigs for fattening, sows, laying hens, minor laying poultry species,

## 1. BACKGROUND

In the current two applications (FAD—2012-0015 and FAD-2012-0033) authorisation is sought under article 4(1) (new use) for *Quantum Blue 5 L, 10 L, 5 G* and *40 P* under the category/functional group 4(c) "zootechnical additives"/"substances which favourable affect the environment", according to the classification system of Annex I of Regulation (EC) No 1831/2003.

According to the Applicant, the active agent of all *Quantum Blue* products is *6-phytase* (EC 3.1.3.26) produced by the strain *Trichoderma reesei* (CBS 126897). The authorisation is sought for the use of the *feed additive* for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, minor poultry species for fattening and breeding, piglets, pigs for fattening, sows (FAD 2012-0015 [1a]) and laying hens, minor laying poultry species (FAD 2013-0033 [1b]).

According to the Applicant, *Quantum Blue 5 L* and *10 L* are liquid preparations with a guaranteed minimum enzyme activity of 5000 and 10000 FTU/g, respectively, while *Quantum Blue 5 G* and *40 P* are a granulate (G) or powder (P) preparation with a guaranteed minimum enzyme activity of 5000 and 40000 FTU/g, respectively [2a, b].

*Quantum Blue* preparations are intended to be used in *premixtures* and/or complete *feedingstuffs* to obtain minimum enzyme activities for 6-phytase of 150 [2b] and 250 FTU/kg [2a] *feedingstuffs*. The dry products can be mixed with the final feed or with the mineral *premixtures*, while the liquid product is to be sprayed onto feed pellets [3a,b].

The Applicant used the enzyme activity units [2a,b] as defined in the EN ISO 30024 [4]:

- One *6-phytase* unit (FTU) is the amount of enzyme which liberates 1 micromole of inorganic phosphate from sodium phytate in one minute at 37°C and pH 5.5.

## 2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Quantum Blue 5 L, 10 L, 5 G* and *40 P* products and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

## 3. EVALUATION

### *Qualitative and quantitative composition of impurities in the feed additive*

When required by EU legislation, analytical methods for official control of undesirable substances in the additive such as heavy metals (arsenic, cadmium, lead and mercury), dioxins, microbiological agents and mycotoxins are available from the respective European Union Reference Laboratories [5].

### *Description of the analytical methods for the determination of the active agents in the feed additive, premixtures and feedingstuffs*

For the determination of the activity of *6-phytase* in the *feed additive, premixtures* and *feedingstuffs* the Applicant proposed an in-house developed and validated colorimetric method measuring the inorganic phosphate released by the enzyme from the sodium phytate substrate [6a]. Acetate buffer (pH 5.5) is used for extraction of *feed additive* and *premixtures* samples while borate buffer (pH 10.0) is used for extraction of *feedingstuffs* samples. After extraction the sample is incubated for 60 min at 37 °C (*feed additive* and *premixtures*) or at 60 °C (*feedingstuffs*). The released inorganic phosphate is determined measuring at 415 nm the yellow complex formed after the reaction of molybdate and vanadate ions with inorganic phosphate. The released inorganic phosphate is quantified with a phosphate standard curve.. The measurement results of feedingstuff samples (expressed in QPU units) are divided by 5.67 to be reported in the international units (FTU).

In order to check and confirm the applicability of the internationally recognised ring-trial validated colorimetric CEN method (EN ISO 30024) [4] the EURL requested the Applicant to apply this method for the determination of *6-phytase* in the *feed additive, premixtures* and *feedingstuffs* samples containing *Quantum Blue* products. The standard method measures the

inorganic phosphate released by the *6-phytase* enzyme from the sodium phytate substrate. At first, the enzyme is extracted. Then, the enzyme and the substrate are incubated at pH = 5.5 and 37 °C for 30 min. The released inorganic phosphate is determined measuring at 415 nm the yellow complex formed after addition of the acidic molybdate/vanadate reagent. The released inorganic phosphate is quantified with a phosphate standard curve. The Applicant applied the CEN method as such to feeding stuff samples and to the feed additive (after appropriate dilution) [7a]. Premixture samples were first extracted with an EDTA/BSA buffer for 45 minutes. The extracts were then centrifuged at 500 g for 5 minutes before further dilution and assay [7a].

The performance characteristics provided by the Applicant as supplementary information [7a] are presented in Table 1 together with those reported in the EN ISO 30024 standard [4]. As the experimental data provided are in good agreement with the performance characteristic reported in the EN ISO 30024 standard, the applicability of the standard method to determine *6-phytase* in samples containing *Quantum Blue* products is demonstrated. Furthermore, the Applicant reported a limit of quantification (LOQ) of 101 FTU/kg feedingstuffs [6a].

Based on the satisfactory experimental evidence available the EURL recommends for official control the EN ISO 30024:2009 standard method, for the determination of the activity of the *6-phytase* in the *feed additive, premixtures and feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

**Table 1:** Performance characteristics of analytical methods for the determination of *6-phytase* in *feed additive (FA), premixtures (PM) and feedingstuffs (FS)*, as provided by the Applicant [7a] and as reported in the ISO EN 30024 standard.

	Activity	RSD <sub>r</sub> (%)	RSD <sub>ip</sub> (%)	R <sub>rec</sub> (%)	Reference
FA	54000 FTU/g	1.9 *	2.5 *	80.4	[7a]
PM	533 FTU/g	2.9 *	5.5 *	84.9	[7a]
FS	1464 FTU/kg	1.2	2.3	102.4	[7a]
FS	500-1500 FTU/kg	2.2-10.6	3.3-12.7	77.2-108.1 #	[4]

RSD<sub>r</sub> and RSD<sub>ip</sub>: relative standard deviation for *repeatability* and *intermediate precision*; R<sub>rec</sub>: recovery rate

\* Recalculated by the EURL, excluding outliers.

# Calculated by the EURL.

#### **4. CONCLUSIONS AND RECOMMENDATIONS**

In the frame of this authorisation the EURL recommends for official control the ring trial validated colorimetric method (EN ISO 30024), for the determination of the activity of the 6-*phytase* in the *feed additive, premixtures and feedingstuffs*.

##### ***Recommended text for the register entry (analytical method)***

Determination of 6-*phytase* in the *feed additive, premixtures and feedingstuffs*:

- colorimetric method based on the enzymatic reaction of 6-*phytase* on the phytate - EN ISO 30024.

One 6-*phytase* unit (FTU) is the amount of enzyme which liberates 1 micromole of inorganic phosphate from sodium phytate in one minute at 37°C and pH 5.5.

#### **5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL**

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Quantum Blue 5 L, 10 L, 5 G* and *40 P* products have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

#### **6. REFERENCES**

- [1a] Reference SANCO/D/2 Forw. Appl. 1831/0039-2012
- [1b] Reference SANCO/D/2 Forw. Appl. 1831/0048-2012
- [2a] Application, Proposal for Register Entry
- [2b] Application, Proposal for Register Entry
- [3a] Technical dossier, Section II: Identity, characterisation and conditions of use of the additive; methods of analysis
- [3b] Technical dossier, Section II: Identity, characterisation and conditions of use of the additive; methods of analysis
- [4] ISO EN 30024:2009 "Animal feeding stuffs – Determination of phytase activity"
- [5] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories
- [6a] Technical dossier, Section II - Annex\_II\_095
- [7a] Supplementary information – SIN-QuantumBlue-6phytase
  - [a] refers to dossier no. FAD 2011-0015,
  - [b] refers to dossier no. FAD 2011-0033



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## **7. RAPPORTEUR LABORATORY**

The Rapporteur Laboratory for this evaluation was the European Union Reference Laboratory for Feed Additives, IRMM, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009.

## **8. ACKNOWLEDGEMENTS**

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- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Labore Landwirtschaft, Leipzig (DE)