



SLOVENSKI STANDARD
oSIST prEN ISO 30024:2023
01-maj-2023

Krma - Določanje aktivnosti fitaze (ISO/DIS 30024:2023)

Animal feeding stuffs - Determination of phytase activity (ISO/DIS 30024:2023)

Futtermittel - Bestimmung der Phytaseaktivität (ISO/DIS 30024:2023)

Aliments des animaux - Détermination de l'activité phytasique (ISO/DIS 30024:2023)

Ta slovenski standard je istoveten z: prEN ISO 30024

<https://standards.iteh.ai/catalog/standards/sist/608ae4bd-d306-417f-a729-3f675ec4ed94/osist-pren-iso-30024-2023>

ICS:

65.120

Krmila

Animal feeding stuffs

oSIST prEN ISO 30024:2023

en,fr,de

DRAFT INTERNATIONAL STANDARD

ISO/DIS 30024

ISO/TC 34/SC 10

Secretariat: INSO

Voting begins on:
2023-03-02

Voting terminates on:
2023-05-25

Animal feeding stuffs — Determination of phytase activity

Aliments des animaux — Détermination de l'activité phytasique

ICS: 65.120

iTeh STANDARD PREVIEW
(standards.iteh.ai)

[oSIST prEN ISO 30024:2023](https://standards.iteh.ai/catalog/standards/sist/608ae4bd-d306-417f-a729-3f675ec4ed94/osist-pren-iso-30024-2023)

<https://standards.iteh.ai/catalog/standards/sist/608ae4bd-d306-417f-a729-3f675ec4ed94/osist-pren-iso-30024-2023>

This document is circulated as received from the committee secretariat.

THIS DOCUMENT IS A DRAFT CIRCULATED FOR COMMENT AND APPROVAL. IT IS THEREFORE SUBJECT TO CHANGE AND MAY NOT BE REFERRED TO AS AN INTERNATIONAL STANDARD UNTIL PUBLISHED AS SUCH.

IN ADDITION TO THEIR EVALUATION AS BEING ACCEPTABLE FOR INDUSTRIAL, TECHNOLOGICAL, COMMERCIAL AND USER PURPOSES, DRAFT INTERNATIONAL STANDARDS MAY ON OCCASION HAVE TO BE CONSIDERED IN THE LIGHT OF THEIR POTENTIAL TO BECOME STANDARDS TO WHICH REFERENCE MAY BE MADE IN NATIONAL REGULATIONS.

RECIPIENTS OF THIS DRAFT ARE INVITED TO SUBMIT, WITH THEIR COMMENTS, NOTIFICATION OF ANY RELEVANT PATENT RIGHTS OF WHICH THEY ARE AWARE AND TO PROVIDE SUPPORTING DOCUMENTATION.

ISO/CEN PARALLEL PROCESSING



Reference number
ISO/DIS 30024:2023(E)

© ISO 2023

iTeh STANDARD PREVIEW (standards.iteh.ai)

[oSIST prEN ISO 30024:2023](https://standards.iteh.ai/catalog/standards/sist/608ae4bd-d306-417f-a729-3f675ec4ed94/osist-pren-iso-30024-2023)

<https://standards.iteh.ai/catalog/standards/sist/608ae4bd-d306-417f-a729-3f675ec4ed94/osist-pren-iso-30024-2023>



COPYRIGHT PROTECTED DOCUMENT

© ISO 2023

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

	Page
Foreword.....	iv
Introduction.....	v
1 Scope.....	1
2 Normative references.....	1
3 Terms and definitions.....	1
4 Principle.....	3
5 Reagents.....	3
6 Apparatus.....	5
7 Sampling and sample preparation.....	6
8 Sample extractions.....	6
8.1 For compound feeds (excluding mineral feeds).....	6
8.2 For mineral feeds and premixtures.....	6
8.3 For feed additives.....	7
9 Procedure.....	7
9.1 Blank solution.....	7
9.2 Standards.....	7
9.2.1 Phosphate standard solution.....	7
9.2.2 Phytase level control.....	8
9.3 Standard curve.....	8
9.4 Phytase level control.....	9
9.5 For compound Feed (excluding mineral feeds).....	9
9.6 For mineral feeds and premixtures.....	10
9.7 For feed additives.....	11
10 Calculations.....	11
10.1 Formulation standard curve.....	11
10.2 Calculation phytase activity.....	12
10.3 Correction for phytic acid purity and water content.....	13
10.4 Interference with blank values.....	13
11 Precision.....	14
11.1 Limit of detection and limit of quantification.....	14
11.2 Interlaboratory tests.....	14
11.3 Repeatability.....	14
11.4 Reproducibility.....	14
12 Test report.....	15
Annex A (informative) Interlaboratory study results for compound feeds (excluding mineral feeds).....	16
Annex B (informative) Interlaboratory study results for mineral feeds and premixtures.....	19
Annex C (informative) Interlaboratory study results for feed additives.....	20
Bibliography.....	22

ISO/DIS 30024:2023(E)

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, Food products, Subcommittee SC 10, Animal feeding stuffs.

This second edition cancels and replaces the first edition (ISO 30024:2009), which has been technically revised.

The main changes compared to the previous edition are as follows:

- extension of the scope to premixtures, mineral feeds and feed additives
- adaptation of phytic acid (phytate substrate) specifications
- editorial modifications (new normative reference paragraph, new annexes.....)

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This International Standard has been developed to quantify phytase products in feedingstuff samples to enable the European Commission to control the phytase content of animal feed products. However, the method cannot be used to evaluate the in vivo efficacy of the phytase products.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

[oSIST prEN ISO 30024:2023](https://standards.iteh.ai/catalog/standards/sist/608ae4bd-d306-417f-a729-3f675ec4ed94/osist-pren-iso-30024-2023)

<https://standards.iteh.ai/catalog/standards/sist/608ae4bd-d306-417f-a729-3f675ec4ed94/osist-pren-iso-30024-2023>

Animal feeding stuffs — Determination of phytase activity

1 Scope

This International Standard specifies the determination of phytase activity in feeding stuff samples, including feed raw materials from plant origin, compound feeds (complete, complementary, mineral feeds), premixtures and feed additives.

The method is collaboratively validated for the determination of phytase activity in complete feed, complementary feed including mineral feed, premixtures and feed additives.

The method does not distinguish between phytase added as a feed additive and endogenous phytase already present in the feed materials. Therefore, the method is also applicable for feed materials from plant origin.

The method cannot be used to evaluate or compare the in vivo efficacy of the phytase product. It is not a predictive method of the in vivo efficacy of phytases present on the market as they can develop different in vivo efficacy per unit of activity.

Note 1 Products which are not included in the validation studies in the Annexes have to be checked especially on their linearity of response (release of phosphorus)

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

phytase unit (U, FTU, FYT)

amount of enzyme that releases 1 μmol of inorganic phosphate from phytate per minute in acetate buffer at pH 5,5 and 37 °C under the reaction conditions specified in this International Standard

3.2

premixture

mixtures of feed additives or mixtures of one or more feed additives with feed materials or water used as carriers, not intended for direct feeding to animals

[SOURCE: Regulation (EC) No 1831/2003 article 2 par. 2 e)]

3.3

premixture (or premix)

uniform mixture of one or more micro-ingredients/feed additives with a diluent and/or carrier and that is not intended for direct feeding to animals

Note 1 to entry: Premixtures are used to facilitate the uniform dispersion of the micro-ingredients/additives in a larger mix.

ISO/DIS 30024:2023(E)

[SOURCE: ISO 20588 Animal feeding stuffs — Vocabulary; point 3.2.39]

3.4 **mineral feed**

complementary feed containing at least 40 % crude ash

[SOURCE: Regulation (EC) No 767/2009 article 3 par. 1 k]

3.5 **mineral mix (or mineral supplement or mineral feed)**

feed that mainly consists of mineral elements, which is as an entire mix free-flowing

[SOURCE: ISO 20588 Animal feeding stuffs — Vocabulary; point 3.2.37]

3.6 **feed additive**

substances, micro-organisms or preparations, other than feed material and premixtures, which are intentionally added to feed or water in order to perform, in particular, one or more functions.

[SOURCE: Regulation (EC) No 1831/2003 article 2 par. 2 a)]

3.7 **feed additive**

substance intentionally added to feed and/or water, not consumed as feed by itself, whether or not it has a nutritional value, that affects the characteristics of feed including organoleptic properties, animal products, animal production or performance or welfare, or the environment

Note 1 to entry: Microorganisms, enzymes, acidity regulators, trace elements, vitamins and other products fall within the scope of this definition, depending on the purpose of use and the method of administration.

Note 2 to entry: Coccidiostats and histomonostats are a category of feed additives.

Note 3 to entry: Feed additive does not include feed materials and premixtures

[SOURCE: ISO 20588 Animal feeding stuffs — Vocabulary; point 3.2.18]

3.8 **compound feed**

mixture of at least two feed materials, whether or not containing feed additives, for oral animal-feeding in the form of complete or complementary feed

[SOURCE: Regulation (EC) No 767/2009 article 3 par. 1 h)]

3.9 **compound feed (or formula feed or feed mixture)**

mixture of at least two feed materials, whether or not containing feed additives, for oral animal feeding in the form of a complementary feed or a complete feed

Note 1 to entry: Complete feed is a form of compound feed

Note 2 to entry: Complementary feed is a form of compound feed.

[SOURCE: ISO 20588 Animal feeding stuffs — Vocabulary; points 3.2.9; 3.2.10; 3.2.11]

3.10 **Feed materials**

products of vegetable or animal origin, whose principal purpose is to meet animals' nutritional needs, in their natural state, fresh or preserved, and products derived from the industrial processing thereof, and organic or inorganic substances, whether or not containing feed additives, which are intended for use in oral animal-feeding either directly as such, or after processing, or in the preparation of compound feed, or as carrier of premixtures;

[SOURCE: Regulation (EC) No 767/2009 article 3 par. 1 g)]

3.11

Feed materials

products of vegetable or animal origin, whether or not containing *feed additives*, that are intended for use in oral animal feeding to meet animals' nutritional needs

Note 1 to entry: Feed materials can be in their natural state, fresh or preserved, or products derived from industrial processing, either organic or inorganic substances.

Note 2 to entry: Feed materials may be fed to animals either directly as such, or after processing, or in the preparation of compound feed, or as carrier of premixtures.

[SOURCE: ISO 20588 Animal feeding stuffs — Vocabulary; points 3.2.23]

4 Principle

Phytase releases phosphate from the substrate myo-inositol hexakisphosphate (phytate). The released inorganic phosphate is determined by forming a yellow complex with an acidic molybdate/vanadate reagent. The optical density (OD) of the yellow complex is measured at a wavelength of 415 nm and the inorganic phosphate released is quantified from a phosphate standard calibration curve.

5 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

WARNING — This method requires the handling of hazardous substances. Observe local regulations for potentially hazardous chemicals to minimize risks to organizational, technical, and personal safety.

5.1 **Ammonia solution**, 25 % mass fraction; NH_3 .

<https://standards.iteh.ai/catalog/standards/sist/608ae4bd-d306-417f-a729-33554b446146/iso-30024:2023>

5.2 **Ammonium heptamolybdate tetrahydrate**, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$.

5.3 **Ammonium monovanadate**, NH_4VO_3 .

5.4 **Hydrochloric acid**, 25 % mass fraction; HCl .

5.5 **Nitric acid**, 65 % mass fraction; HNO_3 .

5.6 **Potassium dihydrogenphosphate**, KH_2PO_4 .

5.7 **Phytate** (anionic form of phytic acid), **phytic acid**

5.7.1 phytate specifications

- All forms of phytate or phytic acid are authorized for use [for examples, phytic acid (PA), phytic acid dodecasodium salt (Na-PA), phytic acid dodecapotassium salt (K-PA), phytic acid hexamagnesium salt n-hydrate (Mg-PA)]
- $\leq 0,1$ % mass fraction of inorganic phosphorus
- assay ≥ 90 % phosphorus (P) basis (dry basis)
- The substrate should have a percentage of IP6 (= hexaphosphate inositol containing 6 phosphate groups) of more than 95.

ISO/DIS 30024:2023(E)

Note 1 to entry Information about IP6 ratio or percentage is available from supplier upon request. As phytic acid salt hydrates are supplied with different contents of crystallization water, ensure that the crystallization water is in the stoichiometric range of 10 to 13 mol. In case of deviation see [10.3](#)

5.7.2 control of the phytate

Blank Optical Density (OD) from the standard curve, see [\(9.3\)](#) shall be lower than 0,2; a higher OD value indicates phosphate or phytase contamination of used reagents.

5.8 Sodium acetate trihydrate, $\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$.

5.9 Polysorbate 20¹⁾.

5.10 Diluted nitric acid.

Dilute 1 volume nitric acid 65 % mass fraction ([5.5](#)) with 2 volumes water. Store at room temperature. The maximum storage time is indefinite.

5.11 Ammonium heptamolybdate reagent.

Dissolve 100,0 g ammonium heptamolybdate tetrahydrate ([5.2](#)) in approximately 800 ml water (hot water at 50 °C to 60 °C may be used to facilitate salt dissolution). Add 10 ml 25 % mass fraction ammonia solution ([5.1](#)) and make up with water to 1 000 ml. Store at room temperature in the dark. The maximum storage time is 2 months.

5.12 Ammonium vanadate reagent.

Dissolve completely 2,35 g of ammonium monovanadate ([5.3](#)) in approximately 400 ml water (hot water at 50 °C to 60 °C may be used to facilitate salt dissolution). Add 20 ml diluted nitric acid ([5.10](#)) and make up with water to 1 000 ml. Store at room temperature in the dark. The maximum storage time is 2 months.

5.13 Molybdate/vanadate STOP reagent.

Mix 1 volume ammonium vanadate reagent ([5.12](#)) with 1 volume ammonium heptamolybdate reagent ([5.11](#)) and add 2 volumes diluted nitric acid ([5.10](#)). Mix and store at room temperature. The maximum storage time is 1 day.

5.14 Polysorbate 20, 10 % mass fraction.

Dissolve 10,0 g of polysorbate 20 ([5.9](#)) with water and make up to 100 ml. Store at room temperature. The maximum storage time is 6 months.

5.15 Acetate buffer, pH 5,5; 0,25 mol/l.

Dissolve 34,0 g of sodium acetate trihydrate ([5.8](#)) in approximately 900 ml water. Adjust the pH with 25 % mass fraction hydrochloric acid ([5.4](#)) to $5,50 \pm 0,02$ and make up to 1 000 ml with water. Store at room temperature. The maximum storage time is 2 weeks.

5.16 Acetate buffer with 0,01 % mass fraction polysorbate 20, pH 5,5; 0,25 mol/l.

Dissolve 34,0 g of sodium acetate trihydrate ([5.8](#)) in approximately 900 ml water. Adjust the pH with 25 % mass fraction hydrochloric acid ([5.4](#)) to $5,50 \pm 0,02$. Add 1 ml 10 % mass fraction polysorbate 20 ([5.14](#)) and make up to 1 000 ml with water. Store at room temperature. The maximum storage time is 2 weeks.

1) Tween 20 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.