INTERNATIONAL STANDARD

ISO 21571

First edition
2005-02-15

Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Nucleic acid extraction

Produits alimentaires — Méthodes d'analyse pour la détection des organismes génétiquement modifiés et des produits dérivés — Extraction des acides nucléiques

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 21571:2005
https://standards.iteh.ai/catalog/standards/sist/02043d6d-ed20-45d1-a0e6-2c21c5619f9d/iso-21571-2005

Reference number
ISO 21571:2005(E)

© ISO 2005

PDF disclaimer

This PDF file may contain embedded typefaces. In accordance with Adobe's licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing the editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. The ISO Central Secretariat accepts no liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

Details of the software products used to create this PDF file can be found in the General Info relative to the file; the PDF-creation parameters were optimized for printing. Every care has been taken to ensure that the file is suitable for use by ISO member bodies. In the unlikely event that a problem relating to it is found, please inform the Central Secretariat at the address given below.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 21571:2005
https://standards.iteh.ai/catalog/standards/sist/02043d6d-ed20-45d1-a0e6-2c21c5619f9d/iso-21571-2005

© ISO 2005

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

Page

Foreword ... iv

Introduction ... v

1 Scope ... 1

2 Normative references ... 1

3 Principle ... 1
3.1 General ... 1
3.2 DNA extraction ... 2
3.3 DNA quantitation ... 2

4 General laboratory requirements ... 2

5 Procedure ... 2
5.1 Preparation of the test portion ... 2
5.2 DNA extraction/purification ... 4
5.3 Quantitation of the extracted DNA ... 5
5.4 Stability of extracted DNA ... 6

6 Interpretation ... 6

7 Test report ... 6

Annex A (informative) **Methods for DNA extraction** ... 7

Annex B (informative) **Methods for the quantitation of the extracted DNA** ... 34

Bibliography ... 41

iTeh STANDARD PREVIEW
(standards.iteh.ai)
ISO 21571:2005
https://standards.iteh.ai/catalog/standards/sist/02043d6d-ed20-45d1-a0e6-2c21c5619f9d/iso-21571-2005

© ISO 2005 – All rights reserved

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

ISO 21571 was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 275, *Food analysis — Horizontal methods*, in collaboration with Technical Committee ISO/TC 34, *Food products*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 21571:2005
https://standards.iteh.ai/catalog/standards/sist/02043d6d-ed20-45d1-a0e6-2c21c5619f9d/iso-21571-2005

 © ISO 2005 – All rights reserved

Introduction

The search for genetically modified origin of ingredients is performed by means of the following successive (or simultaneous) steps. After sample collection, nucleic acids are extracted from the test portion. Extracted nucleic acids can be further purified, simultaneously or after the extraction process. Afterwards, they are quantified (if necessary), diluted (if necessary) and subjected to analytical procedures (such as PCR). These steps are detailed in this and the following International Standards:

ISO 21568, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Sampling.*

ISO 21569, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Qualitative nucleic acid based methods.*

ISO 21570, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Quantitative nucleic acid based methods.*

Further information about definitions and general items involving the steps cited above are collected in:

ISO 24276, *Foodstuffs — Nucleic acid based methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions.*

iTeh STANDARD PREVIEW
(standards.iteh.ai)

The International Organization for Standardization (ISO) draws attention to the fact that it is claimed that compliance with this document may involve the use of a patent concerning the silica-based extraction method (No. EP 0389063/USP 5,234,809) given in Clause A.4.

ISO 21571:2005
https://standards.iteh.ai/catalog/standards/sist/02043d6d-ed20-45d1-a0e6-2c21c5619f9d/iso-21571-2005

ISO takes no position concerning the evidence, validity and scope of this patent right.

The holder of this patent right has assured the ISO that he/she is willing to negotiate licences under reasonable and non-discriminatory terms and conditions with applicants throughout the world. In this respect, the statement of the holder of this patent right is registered with ISO. Information may be obtained from:

Jean Deleforge,
BioMérieux
Chemin de l'Orme,
69280 Marcy-l'Étoile, France.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights other than those identified above. ISO shall not be held responsible for identifying any or all such patent rights.

© ISO 2005 – All rights reserved

iTeh STANDARD PREVIEW
(standards.iteh.ai)
ISO 21571:2005
https://standards.iteh.ai/catalog/standards/sist/02043d6d-ed20-45d1-a0e6-2c21c5619f9d/iso-21571-2005

Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Nucleic acid extraction

1 Scope

This International Standard provides general requirements and specific methods for DNA extraction/purification and quantitation. These methods are described in Annexes A and B.

This International Standard has been established for food matrices, but could also be applicable to other matrices, such as grains and feed.

It has been designed as an integral part of nucleic-acid-based analytical methods, in particular ISO 21569 on qualitative analytical methods, and ISO 21570 on quantitative analytical methods.

2 Normative references

iTeh STANDARD PREVIEW
(standards.iteh.ai)

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 21571:2005
https://standards.iteh.ai/catalog/standards/sist/02043d6d-ed20-45d1-a0e6-
2e21e5619f9d/iso-21571-2005

ISO 24276:—[1)], *Foodstuffs — Nucleic acid based methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions*

ISO 21568, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Sampling*

3 Principle

3.1 General

The objective of nucleic acid extraction methods is to provide nucleic acids suitable for subsequent analysis (see ISO 24276).

NOTE The "quality" of DNA depends on the average length of the extracted DNA molecules, the chemical purity and the structural integrity of the DNA sequence and of the double helix (e.g. intra-, inter-strand linking between DNA bases, single-strand gaps, cross-linking with polyols, haemin, etc). Moreover, such alterations are often sequence-specific and consequently not randomly distributed all over the genome (see References [1], [2], [3] and [4]).

Users of this International Standard should note that some methods (e.g. all silica-based methods), might be covered by patent rights.

1) To be published.

© ISO 2005 – All rights reserved

3.2 DNA extraction

The basic principle of DNA extraction consists of releasing the DNA present in the matrix and further, concurrently or subsequently, purifying the DNA from polymerase chain reaction (PCR) inhibitors.

DNA extraction/purification methods are described in Annex A. Method-selection is an experience-based choice of the user, taking into account the scope and examples of matrices as given in each method.

Alternative protocols are suitable provided that the method has been validated on the respective matrix under investigation.

3.3 DNA quantitation

Quantitation of extracted DNA could be useful for subsequent PCR analysis.

It may be performed by either physical (e.g. measure of absorbance at a specific wavelength), chemical-physical (e.g. use of intercalating or binding agents able to emit fluorescence), enzymatic (e.g. bioluminescence detection) methods or by quantitative PCR. The latter method is especially suitable for composite matrices or for samples with a low DNA content or whose DNA is degraded.

There are several methods available to quantify the DNA present in a solution, as described in Annex B. It is for the user to choose the most appropriate one to be applied, depending on the amount and quality of DNA to be quantified and, consequently, on the matrix from which the DNA has been extracted.

Alternative protocols are suitable, provided that the method has been validated on the respective matrix under investigation.

iTeh STANDARD PREVIEW
(standards.iteh.ai)
ISO 21571:2005

4 General laboratory requirements

Accidental contamination of DNA can originate from dust and spreading aerosols. As a consequence, the organization of the work area in the laboratory is logically based on

— the systematic containment of the methodological steps involved in the production of the results, and

— a “forward flow” principle for sample handling.

The latter ensures that the DNA to be analysed and the amplified DNA generated by PCR remain physically segregated.

Further details can be found in ISO 24276.

5 Procedure

5.1 Preparation of the test portion

5.1.1 General

Commodity-specific variables (e.g. humidity) and processing can impact the amount and quality of DNA extracted from the material under investigation. Therefore the performance characteristics of a given DNA extraction method depend on the matrix.

Take appropriate measures to ensure that the test portion is representative of the laboratory sample.

The test portion shall be of sufficient size and shall contain a sufficient number of particles to be representative of the laboratory sample (e.g. 3 000 particles at an LOD of 0,1 %) to allow a statistically valid conclusion to be made (see ISO 21568).

 © ISO 2005 – All rights reserved

For practical/technical reasons, it is not recommended to exceed a size of 2 g.

Any restrictions that arise from the size of the test portion which prevent it from being representative shall be reported and taken into consideration in the interpretation of the analytical results. The methods for DNA extraction in Annex A describe test portions from 200 mg to 500 mg, which are usually adequate for DNA-rich raw materials (e.g. ground grains, flour). However, for certain matrices containing very low amounts or degraded DNA, insufficient DNA suitable for analysis can be extracted. In these cases, the test portion may be increased.

DNA extractions shall be carried out at least on two test portions.

Storage of standards, samples and test portions shall comply with ISO 24276 and shall be organized in such a way as to preserve the biochemical parameters to be analysed (for details, see ISO/IEC 17025).

5.1.2 Samples

All operations for the preparation of test samples (e.g. grinding, homogenization, division, drying) shall be carried out in accordance with the procedures described in ISO 24276, taking care to prevent all contamination of the sample or modification of its composition.

Laboratory samples shall be sufficiently homogeneous before reducing the laboratory sample and taking the test portion.

For liquid samples, shake the vessel containing the sample to improve the homogenization of the product. In the case of non-homogenous products like raw oils, check that the sediments have been completely removed from the walls of the vessel.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

For solid matrices that cannot easily be suspended, the matrix shall be ground to reduce the particle size and/or facilitate the extractability of DNA. In such a case, attention shall be paid to the particle size. The test portion subjected to extraction shall contain a minimum number of particles as specified in ISO 21568. Milling/grinding devices should be capable of being thoroughly cleaned and shall be selected in order to achieve the expected particle number and particle size distribution within the test portion as defined in ISO 21568.

ISO 21571:2005
https://standards.iteh.ai/catalog/standards/sist/0204d0bd-ed26-45d1-a0e6-2c2fc5619bd/iso-21571-2005

If components of the laboratory sample have been removed prior to extraction, then such procedures shall be reported.

Final food products that are solid or paste and have high lipid contents are often not easy to grind to the desired particle size in a single step. Several procedures may therefore be added, such as lipid removal using hexane after intermediate grinding, freezing or freeze-drying before grinding.

In order to facilitate the grinding of paste or viscous products, it is possible to apply one of the following treatments to certain matrices:

— heating to a maximum temperature of 40 °C;

— dissolving in an appropriate liquid such as water;

— freezing at a temperature below or equal to –20 °C.

Homogenize the whole laboratory sample. Sample the two test portions, taking into account possible dilutions or concentrations.

During milling/grinding, precautions should be taken to ensure that the heating of the sample is kept to a minimum since heating can have a negative impact on the quality of the extracted DNA.

Milling/grinding techniques with a high risk of cross-contamination (such as the combined use of liquid nitrogen and mortar) shall be avoided as far as possible. As a rule of good practice, any dust-producing methodological step should be contained from all other analytical steps.

© ISO 2005 – All rights reserved

If salts, spices, powdered sugars and/or other substances that could potentially interfere with the extraction or analytical method are present, appropriate purification steps should be considered according to the selected method (see Annex A).

For example, in samples from composite matrices, the target matrix (e.g. the breading layer of fish sticks) can be isolated for DNA extraction.

5.2 DNA extraction/purification

5.2.1 General

The following considerations apply for the design of extraction methods.

The quality and yield of nucleic acid extracted using a given method on a given matrix should be both repeatable and reproducible in terms of analysis, provided sufficient nucleic acid is present in the matrix from which it has been extracted.

In order to obtain a good quality DNA, it is advisable, where relevant, to remove the following:

— polysaccharides (pectin, cellulose, hemi-cellulose, starch, thickeners, etc.) using appropriate enzyme treatments (e.g. pectinase, cellulase, hemi-cellulase, α-amylase) or organic extraction (e.g. CTAB/chloroform);

— RNA and/or proteins using an appropriate treatment, such as enzymatic treatment by RNase and proteinase, respectively;

— the lipid fractions using for example enzyme treatments, or solvents (e.g. *n*-hexane);

— salts (e.g. from the extraction/lysis buffer, from the precipitation step) able to interfere with the subsequent analysis.

iTeh STANDARD PREVIEW
(standards.iteh.ai)
ISO 21571:2005
https://standards.iteh.ai/catalog/standards/sist/02043d6d-ed20-45d1-a0e6-2c21c5619f9d/iso-21571-2005

In particular for solid or dried samples, the volume of lysis/extraction buffer should be adapted to guarantee the DNA is dissolved.

NOTE 1 DNA purification can be performed by different means such as fractionated precipitation, using solvents like phenol, chloroform, ethanol, isopropanol, and/or by adsorption on solid matrices (anion exchange resin, silica or glass gel, diatomaceous earth, membranes, etc.). Several DNA purification principles may be combined. If appropriate, extraction and purification can be performed within the same step.

Should a DNA co-precipitant such as glycogen, PEG or t-RNA be used to improve the DNA recovery during the precipitation steps, it should neither contain any detectable level of nuclease activity or PCR inhibitors/competitors, nor bear any sequence similarity with the potential PCR target under study. For genetically modified plants, a carrier DNA may be used (e.g. salmon or herring sperm DNA).

When using vacuum freeze dryers to dry the DNA pellets obtained after a precipitation step, the risk of cross contamination should be taken into account.

Re-suspend the DNA in water or in a buffer solution that prevents DNA from degradation.

When setting up a new type of DNA extraction, or when applying one of the methods described in Annex A to a new matrix, the potential quality and integrity of the extracted DNA using the chosen protocol should be estimated by the following approach. A known quantity of a tracer DNA is added to the lysis buffer plus sample used for DNA extraction. When the chosen tracer is a predetermined amount of DNA or represents a predetermined number of copies of a particular DNA-sequence mixed to a matrix at start of DNA extraction, attention shall be paid to ascertain the lack of DNA sequence similarity between the tracer DNA and the target DNA sequence under study.

NOTE 2 The use of a tracer DNA is a good approximation to a real situation where DNA of a given matrix, complexed to other components (e.g. proteins) is expected. Such a method may also be used to estimate the presence of soluble and *trans*-acting PCR inhibitors in the extracted DNA (see ISO 24276, ISO 21569 and ISO 21570).

 © ISO 2005 – All rights reserved

However, tracer DNA may give a misleading impression of recovery, since tracer DNA may be much easier to separate from matrix than the target DNA.

5.2.2 Controls

The controls to be included are described in Table 1 of ISO 24276:—. These should as a minimum include an extraction blank control and a positive extraction control, but may also include an environment control.

5.2.3 Control of DNA purity: Internal PCR control

When setting up a new type of extraction, the presence of PCR inhibitors in the extracted DNA may be estimated using DNA spikes (see ISO 24276, ISO 21569 and ISO 21570). The amount of added DNA shall not exceed the maximum level supported by PCR and shall contain a definite number of target sequence copies. This number should be determined individually for each target sequence and indicated as a multiple of the existing lower limit of detection. Ideally, the target concentration of the positive control PCR should correspond to the sensitivity needed in the analysis. Care shall be taken when using highly concentrated cloned target DNA. As far as possible, the positive controls shall conform to the conditions of the test material with regard to the nucleic acids they contain.

5.3 Quantitation of the extracted DNA

5.3.1 General

The quality, integrity and amount of the nucleic acid template influences the performance of the analytical method, and hence the analytical results obtained. The limit of detection of a specific method may therefore depend on the amount of nucleic acids used.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

Quantitation of DNA is helpful

ISO 21571:2005
https://standards.iteh.ai/catalog/standards/sist/02045d0d-cd26-45d1-a0e6-2c21c5619f9d/iso-21571-2005

— to compare the efficiency of different DNA extraction protocols for a given matrix (repeatability), and

— to measure the concentration of nucleic acids prior to analysis.

5.3.2 Range of application

Each method of quantitation shall be applied within its dynamic range, also considering its level of precision.

5.3.3 Quantity standards

The accuracy of the quantitation methods depends on the nucleic acid standards used to calibrate the method.

If using a method that is sensitive to the size and/or quality of the nucleic acid fragments, then the nucleic acid standards that match the size and/or quality of the expected nucleic acid as extracted from the sample shall be used.

The reference material used should ensure traceability to stated references, usually national or international Standards, through an unbroken chain of comparison [see ISO Guide 30].

When a method using intercalating agents is employed, high molecular mass DNA standard should be used when high molecular mass DNA is to be quantified. Low molecular mass DNA should be used when low molecular mass DNA is to be quantified. High molecular mass nucleic acid usually also contains a certain amount of lower molecular mass fragments. This means that many methods for DNA quantitation suffer from a certain degree of inaccuracy, which should be taken into account.

NOTE Additionally, depending on the matrix and type of extraction method, a certain portion of the extracted DNA may be recovered as single-stranded DNA (with much poorer intercalation capacity), leading to an underestimation of the overall DNA content. In contrast, single-stranded DNA is equally well detected by physical measurements.

© ISO 2005 – All rights reserved

At least three points (preferably replicated) are required for the construction of a good calibration curve. The amount of standard DNA used for each calibration point depends on the sensitivity of the method and on the dynamic range under consideration.

5.4 Stability of extracted DNA

The DNA extracted shall be stored under such conditions that the stability is ensured to perform the subsequent analyses.

Repeated freezing and thawing of DNA solutions should be avoided.

6 Interpretation

The DNA extraction method employed shall be appropriate to obtain the quality and quantity of nucleic acid required for the subsequent analysis.

The quality of the extracted nucleic acid should be assessed using an analytical method that is influenced by the same quality parameters as the analysis to be performed on the extracted nucleic acid (e.g. if the analysis to be performed is PCR, then an additional PCR should be used for the assessment of the quality of the extracted DNA).

Further parameters for method compatibility can be found in ISO 21569, ISO 21570 and ISO 24276.

7 Test report

iTeh STANDARD PREVIEW
(standards.iteh.ai)

When issuing the final test report in accordance with ISO 24276, the following additional information to document the activity of the laboratory shall be included:

ISO 21571:2005
https://standards.iteh.ai/catalog/standards/sist/02043d6d-ed20-45d1-a0e6-2c21c5619f9d/iso-21571-2005

— a statement describing the derivation of the test portions, and any preliminary processing of the sample before nucleic acid extraction;

— the size of the test portions used for the nucleic acid extraction;

— the nucleic acid extraction method used;

— any special observations made during testing;

— any operation not specified in the method or considered to be optional but that can have an effect on the results;

— the interpretation of the results;

— the experimentor's name.

Handling and storage of raw data are described in ISO/IEC 17025 and related quality assurance schemes. Consistency should be achieved.

© ISO 2005 – All rights reserved

Annex A
(informative)

Methods for DNA extraction

A.1 Preparation of PCR-quality DNA using phenol/chloroform-based DNA extraction methods

A.1.1 Basic phenol/chloroform method

A.1.1.1 General

This routine method (see Reference [5]) is suitable for the extraction of DNA from a wide variety of matrices (see A.1.1.8).

Phenol is usually very suitable for nuclease destruction and protein denaturation.

When foliar or green material (e.g. chicory leaves, dried alfalfa) is investigated, many PCR inhibitors may also be co-precipitated together with DNA. For this reason, difficulties may be encountered in obtaining PCR-amplifiable DNA reproducibly.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

The corrosive hazardous properties of phenol must be taken into consideration, thus the use of DNA extraction methods based on CTAB and/or PVP and/or silica adsorption are favoured as primary alternatives.

ISO 21571:2005
https://standards.iteh.ai/catalog/standards/sist/02043d6d-ed20-45d1-a0e6-2c21c5619f9d/iso-21571-2005

A.1.1.2 Validation status

The method has been widely applied in all areas of biology, agronomy and medicine, over the past 40 years, but has never been evaluated through interlaboratory studies for GMO detection in foodstuffs.

A.1.1.3 Principle

The method consists of a lysis step (thermal lysis in presence of sodium dodecyl sulfate and a high EDTA content) followed by the removal of contaminants (such as lipophylic molecules, polysaccharides and proteins) and nucleases from the DNA-containing aqueous phase using phenol and chloroform. A final DNA precipitation with ethanol concentrates the DNA and eliminates salts and residual chloroform. The critical step of the method is the lysis step [5].

A.1.1.4 Safety precautions

A fume hood is necessary for handling organic chemicals.

A.1.1.5 Reagents

A.1.1.5.1 Ethanol, volume fraction $\phi(C_2H_5OH) = 96\ \%$

Store and use at –20 °C.

© ISO 2005 – All rights reserved

A.1.1.5.2 Glacial acetic acid (CH_3COOH).

A.1.1.5.3 Potassium acetate ($C_2H_3O_2K$).

A.1.1.5.4 Hydrochloric acid, ϕ(HCl) = 37 %.

A.1.1.5.5 Isoamyl alcohol [$(CH_3)_2CHCH_2CH_2OH$].

A.1.1.5.6 Phenol (C_6H_5OH).

A.1.1.5.7 Chloroform ($CHCl_3$).

A.1.1.5.8 Tris(hydroxymethyl)-aminomethane (Tris) ($C_4H_{11}NO_3$).

A.1.1.5.9 Ethylenediaminetetraacetic acid dipotassium salt (K_2EDTA) ($C_{10}H_{14}N_2O_8K_2$).

A.1.1.5.10 Potassium hydroxide (KOH).

A.1.1.5.11 Potassium chloride (KCl).

A.1.1.5.12 Sodium dodecyl sulfate (SDS) ($C_{12}H_{25}O_4SNa$).

A.1.1.5.13 Proteinase K, approximately 20 Units/mg lyophilisate.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

A.1.1.5.14 RNase-A, DNase-free, from bovine pancreas, approximately 50 Kunitz Units/mg of lyophilisate.

A.1.1.5.15 Equilibrated phenol, pH > 7,8.

ISO 21571:2005
https://standards.iteh.ai/catalog/standards/sist/02043d6d-ed20-45d1-a0e6-2c21fa5619f0/iso-21571-2005

Use phenol equilibrated against extraction buffer (A.1.1.5.18) without SDS, or prepared according to Reference [5], or according to the manufacturers recommendations.

A.1.1.5.16 Chloroform-isoamyl alcohol

Mix 24 volume parts of chloroform (A.1.1.5.7) with 1 volume part of isoamyl alcohol (A.1.1.5.5).

A.1.1.5.17 Phenol-chloroform-isoamyl alcohol

Mix 1 volume part of equilibrated phenol (A.1.1.5.15) with 1 volume part of the chloroform-isoamyl alcohol solution (A.1.1.5.16).

A.1.1.5.18 Extraction/lysis buffer, substance concentration c(Tris) = 0,050 mol/l, c(K_2EDTA) = 0,050 mol/l, mass concentration ρ(SDS) = 30 g/l.

Adjust the pH to 8,0 with HCl or KOH.

A.1.1.5.19 TE buffer, c(Tris) = 0,010 mol/l, c(K_2EDTA) = 0,001 mol/l.

Adjust the pH to 8,0 with HCl or KOH.

A.1.1.5.20 Proteinase-K solution, ρ = 20 mg/ml, dissolved in sterile water.

Do not autoclave. Store at −20 °C, but avoid repeated freezing and thawing.

© ISO 2005 – All rights reserved

A.1.1.5.21 RNase-A solution, ρ = 10 mg/ml lyophylisate.

Store at –20 °C, but avoid repeated freezing and thawing.

A.1.1.5.22 Ethanol solution, $\phi(C_2H_5OH)$ = 70 %.

Store and use at –20 °C.

A.1.1.5.23 Potassium acetate solution, $c(C_2H_3O_2K)$ = 3 mol/l.

Adjust the pH to 5,2 with glacial acetic acid. Do not autoclave. If necessary, filter through a 0,22 µm filter.

A.1.1.6 Apparatus and equipment

Usual laboratory equipment and, in particular, the following.

A.1.1.6.1 Centrifuge, capable of achieving a minimum acceleration of 10 000 g.

In some steps a refrigerated centrifuge is required.

A.1.1.6.2 Water bath or incubator, working in a temperature range from 60 °C to 70 °C.

A.1.1.6.3 Vacuum dryer (optional).

iTeh STANDARD PREVIEW
(standards.iteh.ai)

A.1.1.6.4 Freeze dryer (optional).

A.1.1.6.5 Mixer, e.g. Vortex®[2)]

ISO 21571:2005
https://standards.iteh.ai/catalog/standards/sist/02043d6d-ed20-45d1-a0e6-2c21c5619f9d/iso-21571-2005

A.1.1.6.6 Reaction vessels, resistant to freezing in liquid nitrogen.

A.1.1.7 Procedure

A.1.1.7.1 General

Once the matrix test portion has been prepared, apply the following DNA extraction/purification protocol. Scale-adaptation of masses and buffer volumes is required as a function of the selected size of the test portion.

A.1.1.7.2 Extraction procedure

Weigh 0,25 g of the test sample into a microtube.

Add 1,6 ml of extraction buffer (A.1.1.5.18) and, when necessary (e.g. in protein-rich matrices), 50 µl of proteinase K solution (A.1.1.5.20.) Incubate at 60 °C to 70 °C, usually for between 30 min to 2 h (overnight incubation is also possible). Add RNase A (A.1.1.5.21) up to a final concentration of 0,1 µg/ml. Centrifuge at 5 000 g for 30 min and recover the supernatant in a fresh tube. Add 1 volume of equilibrated phenol (A.1.1.5.15) to the supernatant, then mix gently and thoroughly. Centrifuge at 5 000 g for 15 min and recover the upper aqueous phase in a fresh tube. Add 1 volume of phenol-chloroform isoamyl alcohol (A.1.1.5.17) to the supernatant, then mix gently and thoroughly. Centrifuge at 5 000 g for 15 min and recover the aqueous phase in a fresh tube. Repeat this step once or more times (depending on the matrix) until the interface between the phases is clean.

---

2) Vortex 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. Equivalent products may be used if they can be shown to lead to the same results.

© ISO 2005 – All rights reserved