
**Textiles — Identification of some
animal fibres by DNA analysis method
— Cashmere, wool, yak and their
blends**

*Textiles — Identification de certaines fibres animales par la
méthode d'analyse de l'ADN — Cachemire, laine, yack et leurs
mélanges*

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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).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT), see the following URL: [Foreword — Supplementary information](#).

The committee responsible for this document is ISO/TC 38, *Textiles*.

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Introduction

The composition of fibres in textile products is one of the most important properties. Labelling of composition of textile products is required globally by legislation or by voluntary regulation for fair trade.

The testing method to determine the composition of some animal fibres in the textile products has been developed as ISO 17751[3]. This is only one method to determine the animal fibre composition currently available. In this method, animal fibres are observed by microscope and identified from the shape of scales by experienced examiners. Many samples can be tested with a high degree of efficiency using this method. However, even experienced examiners have difficulties in identifying fibres, because textile products have a broad variety of colours and finishings, and there are many blends in animal fibres.

Given this situation, several testing methods to obtain the more accurate results have been investigated and developed. Among those methods, the DNA (deoxyribonucleic acid) analysis method has been found to be a practical and feasible method to identify the inherent type of animal fibres.

As it is well known, DNA is specific for animals. The DNA-PCR (polymerase chain reaction) method has recently been developed with high accuracy. A very trivial quantity of the DNA extracted from animal fibres is amplified by PCR to yield a huge quantity of copy DNA. Mitochondrial DNA is used for this analysis because it provides greater numbers than nuclear DNA.

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Textiles — Identification of some animal fibres by DNA analysis method — Cashmere, wool, yak and their blends

WARNING — The use of this International Standard can involve hazardous materials, operations, and equipment. This International Standard does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this International Standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations and supplier's requirement for safety prior to use.

1 Scope

This International Standard specifies a testing method for DNA analysis of some animal fibres to identify cashmere, wool, yak, and their blends by using extraction, amplification by the polymerase chain reaction (PCR) method and DNA detection processes.

This International Standard is applicable to cashmere, yak, and wool and their blends as a qualitative method.

2 Caution

Test results for fibre identification by the DNA analysis method can be obtained with a high accuracy for the above-mentioned textile products which were processed at lower dye concentration levels or dyed in light colours.

However, when such textile products were processed under severe conditions or high temperatures, the mitochondrial DNA could have been damaged. In such cases, identification can be difficult because amplification of DNA by PCR cannot take place. If textile products were contaminated by using products from another species, such as cashmere grease on wool fibres, this situation may be solved by checking using microscopy techniques.

3 Normative references

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

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 8655-2, *Piston-operated volumetric apparatus — Part 2: Piston pipettes*

4 Terms and definitions

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

4.1

DNA

deoxyribonucleic acid, that exists in nuclei and in mitochondria of animal fibre cells and is composed of a linear array of 4 bases (Adenine: A, Thymine: T, Guanine: G and Cytosine: C)

Note 1 to entry: The DNA sequence is identical and intrinsic for each animal fibre.

4.2

animal fibres

cashmere, wool, or yak fibres

4.3

buffer solution

solution used to maintain pH of reaction solution at required value

4.4

reducing agent

agent that degrades animal fibres through reductive cleavage of S-S bonds in the fibres

4.5

DNA amplification

amplification of the specific fragment of DNA by the PCR method

4.6

PCR method

method of the polymerase chain reaction

Note 1 to entry: The amplification process of the DNA fragment with a constant length is explained in [Annex A](#).

4.7

DNA polymerase for PCR method

heat-stable DNA polymerase that is used for PCR and has no proof reading activity

4.8

primer

short length fragment of a single strand DNA which is a reaction initiator and designed as the identical sequence of 18-30 bases to DNA of the animal fibre

4.9

primer set

set of primer with the reaction direction of forward and reverse

4.10

primer for cashmere

primer with an identical base sequence of mitochondrial DNA of cashmere

Note 1 to entry: The sequence of base for primers will be submitted to the public database.

4.11

primer for wool

primer with an identical base sequence to mitochondrial DNA of wool

4.12

primer for yak

primer DNA with an identical base sequence to mitochondrial DNA of yak

Note 1 to entry: Information concerning the primers may be obtained from ISO/TC 38 secretariat.

4.13

gel electrophoresis migration

method to detect the amplified constant length DNA fragments

5 Principle

Mitochondrial DNA is extracted from animal fibre samples by using a chemical and enzyme reaction. The extracted DNA is purified by using a precipitation method and centrifuge. The purified DNA is applied for the amplification reaction of PCR method. In the PCR method, primers for cashmere, yak and wool are respectively tested. If the sample is cashmere, only cashmere primer can amplify the constant length of DNA fragments. Then, the constant length of DNA fragments is detected by the electrophoretic migration method.

The sample fibres are identified by knowing whether amplification was observed or not for the tests using all primers respectively.

6 Apparatus and equipment

6.1 Pipettes, capable of measuring and taking (0 to 20) μl ($\pm 0,20 \mu\text{l}$), (20 to 200) μl ($\pm 1,60 \mu\text{l}$), (200 to 1 000) μl ($\pm 8 \mu\text{l}$) within systematic errors defined in ISO 8655-2.

6.2 Micro tube, capable of withstanding the centrifugation of 14 000g and autoclave.

The capacity is 2 ml for purification and 0,2 ml for PCR method. A tube of 0,2 ml for PCR method should follow the manufacturer's recommendation of the PCR instrument.

6.3 Cap lock, use for micro tube.

6.4 Heat block, with mounting holes for micro tubes and capable of heating up to about 80 °C ($\pm 1,0$ °C).

6.5 Shaking agitator, capable of heating up to 50 °C and maintaining at the temperature of 50 °C and shaking micro tube at around 500 r/min or higher.

6.6 Shaking machine, capable of mounting micro tubes and shaking at around 500 times/min or higher.

This machine can be replaced by an equivalent instrument such as microtube rotator which is possible to rotate at 30 r/min or higher.

6.7 Centrifuge, capable of centrifuging of 14 000g or higher, setting up temperature from 0 °C to room temperature and mounting micro tubes or units of centrifugal ultrafiltration.

6.8 Unit of centrifugal ultrafiltration¹⁾, capable of capturing molecules with the molecular weight of 100 kDa (Dalton) or more.

6.9 PCR instrument²⁾, capable of programing for temperature and time.

6.10 UV illuminator, UV irradiator.

6.11 Photo booth.

6.12 Generic plastic box with a resealable lid.

6.13 Comb, used for making wells in the agarose gel of the gel electrophoresis migration test.

6.14 Mixer mill, used for mixing and homogenizing animal fibres.

6.15 Erlenmeyer flask, with capacity 200 ml.

1) Amicon Ultra is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

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