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**Textiles—Determination of
antifungal activity and efficacy of
textile products**

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Foreword

This translation has been made based on the original Japanese Industrial Standard established by the Minister of Economy, Trade and Industry through deliberations at the Japanese Industrial Standards Committee according to the proposal for establishment of Japanese Industrial Standard submitted by Japan Textile Evaluation Technology Council (JTETC)/Japanese Standards Association (JSA) with the draft being attached, based on the provision of Article 12 Clause 1 of the Industrial Standardization Law.

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Textiles—Determination of antifungal activity and efficacy of textile products

Introduction

This Japanese Industrial Standard has been prepared based on the first edition of **ISO 13629-1** published in 2012 with some modifications of the technical contents to correspond to technical trend and actual situation in Japan.

The portions given sidelines or dotted underlines are the matters in which the contents of the corresponding International Standard have been modified. A list of modifications with the explanations is given in Annex JA.

1 Scope

This Standard specifies a test method for the quantitative determination of the antifungal activity by measuring the intensity of luminescence produced by an enzymatic reaction of adenosine triphosphate (hereafter referred to as “ATP”), and antifungal efficacy.

This Standard is applicable to various kinds of textile products, such as yarns, woven and nonwoven fabrics, clothing, bedclothes, home furnishings and other miscellaneous goods.

Based on the intended application and on the environment in which the textile product is to be used, the user can select the most suitable evaluation method from the following methods.

- a) **Absorption method** An evaluation method in which spore suspension is inoculated directly onto the specimens.
- b) **Transfer method** An evaluation method in which test fungi are placed on an agar plate and printed onto the specimens.

NOTE : The International Standard corresponding to this Standard and the symbol of degree of correspondence are as follows.

ISO 13629-1:2012 *Textiles—Determination of antifungal activity of textile products—Part 1: Luminescence method* (MOD)

In addition, symbols which denote the degree of correspondence in the contents between the relevant International Standards and **JIS** are IDT (identical), MOD (modified), and NEQ (not equivalent) according to **ISO/IEC Guide 21-1**.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this Standard. The most recent editions of the standards (including amendments) indicated below shall be applied.

JIS K 8150 Sodium chloride (Reagent)

JIS L 0803 *Standard adjacent fabrics for staining of colour fastness test*

NOTE : Corresponding International Standard: ISO 105-F02 *Textiles—Tests for colour fastness—Part F02: Specification for cotton and viscose adjacent fabrics* (MOD)

JIS Z 8401 *Guide to the rounding of numbers*

3 Terms and definitions

For the purpose of this Standard, the following term and definition apply.

3.1 control specimen

specimen which is the same textile product as the textile product to be tested, but without antifungal treatment, used to validate the condition of test effectiveness

If control specimen is not available, a 100 % cotton standard adjacent fabric (cotton No. 3-1) specified in **JIS L 0803** is used as the control specimen, after 10 cycles of washing for 10 min at a temperature of 60 °C without detergents or any brighteners, rinsing twice for 5 min and air-drying.

3.2 antifungal agent

agent to prevent or mitigate the growth of fungus or to reduce the number of fungus

3.3 antifungal treatment

treatment to prevent or mitigate the growth of fungus or to reduce the number of fungus

3.4 spore suspension

liquid with evenly dispersed fungal spores in sterilized water containing an anionic surfactant (see 8.3)

3.5 ATP (adenosine triphosphate)

a multifunctional nucleotide present in living fungi

3.6 antifungal activity

activity to prevent or mitigate the growth of fungus

Expressed as the difference of growth value in logarithm of ATP between the control and test specimen.

3.7 antifungal efficacy

efficacy of antifungal activity shown by antifungal treatment

Evaluated by antifungal activity value.

3.8 luminescence method

method in which the concentration of ATP contained in fungal cells is measured by luminometer to obtain the amount of ATP (moles)

4 Principle

A test specimen and control specimen are inoculated with a spore suspension of a reference fungus and incubated at 25 °C for 42 h.

In this Standard, fungal growth or antifungal activity is determined quantitatively, by comparison with the result of a test specimen and control specimen, by measuring the amount of intracellular ATP.

5 Safety precaution

The test methods specified herein require the use of fungus.

This test shall be performed only by personnel with training and experience in microbiological techniques. Safety precautions shall be checked against and comply with regulations.

6 Reference fungi

The fungi to be used shall be selected from Table A.1.

Equivalent fungi types obtained from other agencies of the World Federation for Culture Collection (WFCC) may be used as agreed upon between the interested parties.

The preservation number and supply source of the fungus used shall be stated in the test report.

7 Apparatus

7.1 Gauze, for biochemical testing or glass wool (FR specification).

7.2 Petri dishes, that have been sterilized, internal diameter of approximately 90 mm. For the specimen of transfer method, internal diameter of 55 mm to 60 mm.

7.3 Dry sterilizer, capable of maintaining the temperature at 160 °C to 180 °C.

7.4 Autoclave, capable of maintaining the temperature at $(121 \pm 2) ^\circ\text{C}$ (equivalent to 103 kPa).

7.5 Safety cabinet, for biochemical testing, or one that offers equivalent performance.

7.6 Platinum colony loop, with a loop of 2 mm to 4 mm in diameter.

7.7 L-shaped platinum colony hook, capable of scraping spore.

7.8 Incubator, capable of maintaining the target temperature range of 20 °C to 37 °C with a margin of $\pm 2 ^\circ\text{C}$.

7.9 Vial, 30 ml screw-top glass vial with polytetrafluoroethylene or silicone gasket and polypropylene cap.

7.10 Glass rod, between 5 mm and 18 mm in diameter and 1 g to 50 g in mass.

7.11 Glass funnel, 60 mm in inside diameter.

7.12 Pipettes, of capacity 0.05 ml, 0.1 ml, 0.2 ml, 1 ml, 5 ml and 10 ml with a tolerance of under 5.0 % of nominal capacity.

- 7.13 Pasteur pipette**, for microbiological testing.
- 7.14 Conical flask**, of capacity 100 ml to 500 ml.
- 7.15 Tweezers**, made of a material which can be sterilized.
- 7.16 Plastic test tube**, especially for the luminometer.
- 7.17 Test-tube agitator**, capable of agitating vial and test tube.
- 7.18 Centrifugal separator**, with a centrifugal acceleration of approximately 2 000 G.
- 7.19 Centrifuge tube**, used in a centrifugal separator.
- 7.20 Hemocytometer**, capable of measuring $1 \times 10^6/\text{ml}$ to $3 \times 10^6/\text{ml}$.
- 7.21 Microscope**, magnification 200 $\times$.
- 7.22 Ultrasonic cleaner**, compact, for experimental tools, with a frequency of approximately 30 kHz to 50 kHz.
- 7.23 Luminometer**, measuring at a wavelength of 300 nm to 650 nm, and capable of ATP measurement at 1×10^{-11} mol/L to 1×10^{-5} mol/L, under the assay conditions defined in 8.4, clauses 11 and 13.
- 7.24 pH-meter**, with an accuracy of ± 0.1 at 25 °C.
- 7.25 Refrigerator**, capable of maintaining a temperature of between 2 °C and 10 °C.
- 7.26 Freezers**, one adjustable to a temperature below -80 °C and another to a temperature below -20 °C.

Test tubes, vials, flasks, pipettes and tweezers shall be carefully washed in alkaline or neutral detergent, rinsed, dried, and processed by dry sterilization or high-pressure steam sterilization before use.

8 Reagents and culture media

8.1 General Reagents used in tests shall be of analytical quality and/or suitable for microbiological purposes.

Dehydrated products available on the commercial market are recommended for use in preparing the culture media, strictly in accordance with the manufacture's instructions.

8.2 Pure water Analytical-grade water for microbiological media and reagent preparation, which is freshly distilled and/or ion-exchanged and/or ultra-filtered and/or filtered with reverse osmosis (RO). It shall be free from all toxic or fungus-inhibitory substances.

8.3 Anionic surfactant Dioctyl sodium sulfosuccinate to prepare the spore suspension.

8.4 Luminescent reagents, reagents and buffer solutions

8.4.1 General Use reagents and buffer solutions prepared as shown in 8.4.2 to 8.4.8. Commercially prepared items shall be used after appropriate validation.

8.4.2 ATP standard stock solution (10^{-3} mol/L) (hereafter referred to as “ATP standard”) Dissolve 60.5 mg of adenosine-disodium 5'-triphosphate trihydrate ($C_{10}H_{14}O_{13}P_3Na_2 \cdot 3H_2O$) to water to make 100 ml.

Place the prepared solution in a tightly sealed container and freeze at a temperature of -20°C or lower for storage of up to six months.

Do not refreeze frozen solution that has thawed.

8.4.3 Buffer solution for ATP luminescent reagent, prepared from the following compositions to be $\text{pH } 7.5 \pm 0.2$.

— N-[Tris(hydroxymethyl)methyl] glycine	1 117 mg
— Disodium salt of ethylenediaminetetraacetic acid dihydrate	183 mg
— Magnesium acetate tetrahydrate	808 mg
— DL-dithiothreitol	6.7 mg
— Dextrin	25 000 mg
— Sucrose	925 mg
— Pure water	250 ml (final volume)

8.4.4 ATP luminescent reagents ATP luminescent reagents shall enable a luminometer (7.23) to measure the ATP of 1×10^{-11} mol/L to 1×10^{-5} mol/L, under the assay conditions defined in 8.4, clause 11 and clause 13, and is prepared from the following compositions.

— Luciferase	0.7 mg
— D-luciferin	12.6 mg
— Bovine serum albumin	56 mg
— Buffer solution (see 8.4.3)	30 ml

Once fully dissolved, stand at room temperature for 15 min before use. Use within 3 h of preparation.

The use of any unspecified ATP reagent in the composition shall be recorded in the test report.

8.4.5 ATP extraction reagents ATP extraction reagents must be able to extract intracellular ATP from the incubated fungus with an efficiency of 80 % or higher, and prepared from the following compositions to be $\text{pH } 12.0 \pm 0.5$.

Use sodium hydroxide to adjust pH.

— N-[Tris(hydroxymethyl)methyl] glycine	45 mg
— Benzalkonium chloride, 10 % aqueous solution	0.2 ml