DNA-TECHNOLOGY

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RUO

For research use only

MycosoResista Trichophyton

REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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1. INTENDED USE

MycosoResista Trichophyton REAL-TIME PCR Detection Kit is an *in vitro* Nucleic Acid Test (NAT). **MycosoResista Trichophyton REAL-TIME PCR Detection Kit** is designed for detection of DNA of *Trichophyton* genus fungi with identification of *Trichophyton mentagrophytes* var. *indotineae* species and mutations in *ERG1* gene (Phe397Leu, Leu393Ser, Leu393Phe, Ser436Ala, His440Tyr, Ala448Thr), determining antifungal resistance, in human biological material (skin and its appendages: hair and nails) by real-time PCR.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for using the **MycosoResista Trichophyton REAL-TIME PCR Detection Kit**.

The **MycosoResista Trichophyton REAL-TIME PCR Detection Kit** can be used in research practice.

Potential users: qualified personnel trained in molecular research methods.

Apply the kit only as directed in the present instruction for use.

2. METHOD

Method: polymerase chain reaction (PCR) with results detection in real-time; quantitative multiplex analysis.

The amplification process consists of a series of repeated cycles of temperature denaturation of DNA, annealing of primers with complementary sequences, and subsequent elongation of polynucleotide chains from these primers with Taq polymerase.

To increase the sensitivity and specificity of the amplification reaction, hot start is used. For package S, hot start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin. The PCR starts only when paraffin is melted.

The Internal Control (IC) is included in the **PREP-DERM DNA Extraction Kit** and must be used. It allows for monitoring all stages of the sample testing process, assessing the effect of inhibitors on the results, and tracking the quality of real-time polymerase chain reaction (PCR) performance in each individual tube.

DNA probes, each containing a fluorescent label and a fluorescence quencher, are introduced into the PCR mix. When a specific product is formed, the DNA probe is destroyed and the quencher stops affecting the fluorescent label, which leads to an increase in the fluorescence level. The number of hydrolyzed probes (and hence the fluorescence level) increases in proportion to the number of specific amplicons formed. The fluorescence level is measured at each amplification cycle in real time.

The DNA probe used for detection of amplification products of genome fragments of the detected fungi and the corresponding mutations, include fluorescent tags Fam, Rox and Cy5. The DNA probe used for detection of the amplification product of internal control includes fluorescent dye Hex.

To monitor the position of strips in the thermoblock of the detecting thermal cycler, an oligonucleotide with a Cy5 fluorescent label (the “Marker”) has been added to the PCR mix in tubes No. 4, 8. It is used by the instrument to determine the position of the strip in the thermoblock. After amplification is complete, the software compares the operator-defined marker location with its actual position. If a mismatch is found (due to incorrect strip placement), it warns the operator of this mismatch. In this case, it is necessary to check the placement of the strips in the thermoblock (the first tube is marked with blue buffer) and correct the tube IDs in the protocol.

The use of several fluorescent dyes allows to reduce the number of tubes and biomaterial required for the assay by simultaneously registering the results of multiple amplification reactions.

Table 1 shows detection channels for amplification products.

Table 1. Detection channels of amplification products

Number of tube in the strip	Detection channels, target analytes				Color marking of PCR mix
	Fam	Hex	Rox	Cy5	
1, 5	*Trichophyton mentagrophytes* var. *indotineae*	IC	*Trichophyton* spp.	-	Blue
2, 6	Phe397Leu	IC	-	Leu393Phe	Colorless or pink
3, 7	His440Tyr	IC	Leu393Ser	-	
4, 8	Ser436Ala	IC	Ala448Thr	Marker	

The automatic analysis is available on DNA-Technology instruments: DTlite, DTprime or DTprime II real-time thermal cyclers (see https://www.dna-technology.com for available supply options). The current software version is available for download at https://www.dna-technology.com/software.

The **MycosoResista Trichophyton REAL-TIME PCR Detection Kit**. is also approved for use with CFX96 (Bio-Rad) real-time thermal cyclers.

3. CONTENTS

MycosoResista Trichophyton REAL-TIME PCR Detection Kit comes in package S (strips).

The content of the reagent kit is presented in Table 2.

Table 2. **MycosoResista Trichophyton REAL-TIME PCR Detection Kit** content, package S (strips) for R1-P038-S3/4ER

Reagent	Description	Total volume	Amount
Paraffin-sealed PCR mix	Colorless, pink or blue transparent liquid under waxy white fraction	20 µL in each	tubes, 24 strips of 8
Taq polymerase solution	Colorless transparent liquid	1.0 mL in each	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL in each	4 tubes
Positive control No. 1[1]	Colorless transparent liquid	320 µL	1 tube
Positive control No. 2[2]	Colorless transparent liquid	320 µL	1 tube
Strip caps	24 strips of 8		

All components are ready for use.

MycosoResista Trichophyton REAL-TIME PCR Detection Kit is designed for 48 tests (no more than 12 runs), which includes analysis of test samples, negative and positive controls.

[1] - the component is indicated as “C+ No. 1” on the label

[2] - the component is indicated as “C+ No. 2” on the label

4. ADDITIONAL REAGENTS AND EQUIPMENT REQUIRED

List of required equipment, reagents and consumables includes:

- UV PCR cabinet;
- Real-time detecting thermal cycler[3];
- Vortex mixer[4];
- Vortex rotor for 0.2 mL strips;
- Refrigerator with freezer;
- Tube rack for 0.2 mL strips;
- Tube rack for 1.5 mL tubes;
- Single channel pipettes (dispensers covering 2.0-20 µL; 20-200 µL; 200-1,000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 10 µL; 20 µL; 200 µL; 1,000 µL);
- Pipette rack;
- Powder-free surgical gloves;
- Container for used pipette tips, tubes and other consumables;
- **PREP-DERM DNA Extraction Kit** (DNA-Technology, Russia).

The following detecting thermal cyclers are validated for work with the **MycosoResista Trichophyton REAL-TIME PCR Detection Kit**:

- DTprime in DTprime *M* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime;
- DTprime II in DTprime II *M* modification (manufactured by "DNA-Technology R&P", LLC), hereinafter – DTprime II;
- DTlite in DTlite *S* modification (manufactured by "DNA-Technology R&P", LLC), (only for package S, and package U, manual dosing if using tubes), hereinafter – DTlite;
- CFX96 (Optical Reaction Module CFX96) (manufactured by Bio-Rad Laboratories, USA), hereinafter – CFX96.

For detection thermal cyclers not mentioned above please contact the manufacturer of the reagent kit.

Software:

The most recent version of the DT thermal cyclers software can be downloaded from https://www.dna-technology.com/software.

Supported OS: Windows 7 and above.

[3] - hereinafter – detecting thermal cycler; the required parameters are indicated below

[4] - laboratory shaker DTspin (DNA-Technology, Russia) is recommended

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of manufacture.

5.1. Storage conditions

- All components of **MycosoResista Trichophyton REAL-TIME PCR Detection Kit** must be stored at temperatures from 2°C to 8°C over the storage period.
- Paraffin-sealed PCR mixes must be stored away from light over the storage period.

WARNING! Protect components from light and excessive temperatures, as they are detrimental to product performance.

5.2. Transport conditions

Transportation of the reagent kit is carried out in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container corresponding to the storage conditions of the kit components.

- It is allowed to transport the kit in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermoboxes from 2°C to 25°C for no longer than 5 days.

WARNING! Reagent kits transported with violation of temperature conditions must not be used.

5.3. Shelf-life of the kit following the first opening of the primary container

- All components of the kit must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C over the storage period.
- Paraffin-sealed PCR mixes must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C and out of light over the storage period.

WARNING! The kits stored under undue regime must not be used.

An expired **MycosoResista Trichophyton REAL-TIME PCR Detection Kit** must not be used.

We strongly recommend following the current instructions for use in order to obtain accurate and reliable results.

The manufacturer guarantees the conformity of **MycosoResista Trichophyton REAL-TIME PCR Detection Kit** to the technical documentation if the storage, transportation and handling requirements are fulfilled.

6. WARNINGS AND PRECAUTIONS

- Molecular biology procedures, such as nucleic acid extraction, PCR-amplification and detection require qualified staff to avoid the risk of erroneous or unreliable results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.
- Wear powder-free single-use surgical gloves. Wear work clothes and personal protective equipment while working with pathogenic microorganisms. The work clothes and personal protective equipment must be suitable for work to be performed and comply with health and safety requirements.
- Avoid any direct contact with the biological samples, reagents and materials used to carry out the test. Avoid producing spills or generating aerosols. Do not eat/drink components of the kit. Do not inhale gas/fumes/vapor/aerosols produced by the components of the kit. Avoid contact with eyes.
- Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time.
- Pipettes used to handle samples must only be used for one purpose. The pipettes must be of positive displacement type or be used with aerosol barrier pipette tips. The tips employed must be sterile, free from DNases and Rnases, and free from DNA and RNA.

- The reagents must be handled under a laminar flow hood. Amplification reagents must be prepared in aliquots sufficient for a single session.
- Handle and dispose of all biological samples, reagents and materials used to carry out the assay as potentially infectious[5, 6]. Any material that has been exposed to biological samples must be treated with disinfecting solution for at least 30 minutes or autoclaved for 1 hour at 121°C before disposal.
- All of the liquid solutions are designed for single use and cannot be used more than once in amplification reactions.
- Only use the reagents provided in the kit and those recommended by the manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits.
- All laboratory equipment and tools, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, gloves, etc., as well as reagents must be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Never introduce amplification products in the area designed for extraction/preparation of amplification reactions.
- Do not open the tubes after amplification. Avoid producing accidental spills of the amplification products. Dispose of all PCR waste materials (tubes, tips etc.) only in a closed form in a specialized sealed container with disinfectant solution. Waste materials must be removed in accordance with laboratory internal procedures, and with national and international standards.
- Working surfaces, as well as rooms where NA extraction and PCR are performed, must be disinfected with bactericidal irradiators (UVGI) for 30 minutes before and after the assay. All surfaces in the laboratory (test tube racks, equipment, tools, etc.) must be treated with disinfecting solution daily.

Emergency actions

Eye Contact: If any component of the kit enters the eyes, flush the eyes gently using potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, seek medical attention.

Skin Contact: If any component of this kit comes into contact with the skin and causes discomfort, remove any contaminated clothing. Rinse the affected area with plenty of soap and water. If pain or irritation occurs, seek medical attention.

Ingestion: If any component of this kit is ingested, rinse the mouth with plenty of potable water. If irritation or discomfort occurs, seek medical attention.

Do not use the kit:

- If the transportation and storage conditions have been violated;
- If the appearance of the reagents does not correspond to the product documentation;
- If the packaging of the kit components is breached;
- After the expiry date.

Adverse health effects are **NOT** anticipated from routine use of this kit in compliance with the current instruction for use.

[5] - All oligonucleotide components are produced by artificial synthesis in compliance with internal quality control protocol. They do not contain blood or products of blood processing.

[6] - Positive control is produced using artificial DNA synthesis technology, it does not contain parts of infectious agents.

7. SAMPLES

MycosoResista Trichophyton REAL-TIME PCR Detection Kit is designed to detect DNA extracted from skin and its appendages: hair and nails.

7.1. Interfering substances

The presence of PCR inhibitors in a sample may cause controversial (uncertain) results.

The maximum concentrations of common interfering substances that do not inhibit amplification of laboratory control and internal control are: hemoglobin – 0.35 mg/mL of DNA sample.

To assess possible interference of medicines, those that could potentially be present in leftover quantities in human biological samples taken from the corresponding biotopes were selected (Chlorhexidin bigluconate, Terbinafine, Clotrimazole, Ketoconazole, Amorolfine, Naftifine hydrochloride).

For all the medicines, no influence in concentration of up 10% in biomaterial sample was shown.

Assessment of the influence of interfering substances in biological samples on test results is presented in the table below.

Table 3. Maximum concentration of interfering substances

Biomaterial	Interfering substance	Concentration of interfering substance
Endogenous substances		
Skin	Hemoglobin	**0,35 mg/mL**
Exogenous substances		
Skin	Chlorhexidin bigluconate	10%
	Clotrimazole	10%
	Terbinafine	10%
	Ketoconazole	10%
Nails	Amorolfine	10%
	Naftifine hydrochloride	10%
Hair	Ketoconazole	10%

To decrease the amount of PCR inhibitors, please follow the rules of biomaterial collection.

7.2. Sample collection and preparation

Sample collection is performed in accordance with the **PREP-DERM** instruction for use.

7.3. Transport and storage of the samples

Transport and storage conditions for biomaterial samples are outlined in the **PREP-DERM** instruction for use.

8. PROCEDURE

DNA extraction from biological material

PREP-DERM DNA Extraction Kit is validated for DNA extraction. NA extraction is performed in accordance with the **PREP-DERM** instruction for use.

WARNING! Negative control must go through all stages of sample preparation simultaneously with DNA extraction according to **PREP-DERM** instruction for use.

8.1. PCR

WARNING!

- The reagents and tubes must be kept away from direct sunlight.
- Closely monitor the completeness of the strips and caps for them. Do not use strip caps from other kits!

8.1.1 Mark 4 strip tubes with paraffin-sealed PCR mixes for each test sample, negative control (“C-”), positive control No. 1 (“C+ No. 1”) and positive control No. 2 (“C+ No. 2”) (see Table 4).

WARNING! The volume of reagents is calculated for no more than 12 runs assuming a variable number of test samples, 1 negative control and 2 positive controls per run.

Note.

1. 1 strip is intended for test of 2 samples.
2. It is allowed to use 2 positive controls (“C+ No. 1” and “C+ No. 2”) in the first run and 1 positive control (“C+ No. 1”) for further runs with **the same batch of reagent kits.**

Table 4. Example of tube marking to test 2 samples

Test samples	**Marking of tubes in the strip**
Test sample No. 1	Strip No. 1, tubes 1–4
Test sample No. 2	Strip No. 1, tubes 5–8
“C-”	Strip No. 2, tubes 1–4
“C+ No. 1”	Strip No. 2, tubes 5–8
“C+ No. 2”	Strip No. 3, tubes 1–4

8.1.2 Shake the tubes with Taq polymerase solution on vortex for 3–5 seconds and centrifuge on vortex for 1–3 seconds.

8.1.3 Add 10 µL of Taq polymerase solution to each marked tube (including “C-”, “C+ No. 1” and “C+ No. 2”). Avoid paraffin layer break.

8.1.4 Add one drop of mineral oil (~20 µL) to each tube. Cover the strips loosely with caps.

8.1.5 Shake the tubes with positive controls for 3–5 seconds and centrifuge on vortex for 1–3 seconds.

WARNING!

1. Before introducing DNA preparation and negative control into tubes with PCR mix, fulfill the recommendations for DNA preparation use listed in the **PREP-DERM DNA Extraction Kit** instruction for use.
2. To prevent contamination, only open the cap of the strip into which the sample is to be added and close it before adding the next sample. Close the strips tightly. Use filter tips.

8.1.6 Add 5.0 µL of DNA sample into corresponding marked strip tubes. Do not add DNA to the “C-”, “C+ No. 1” and “C+ No. 2” tubes. Avoid paraffin layer break.

8.1.7 Add 5.0 µL of negative control (“C-”) which passed whole DNA extraction procedure into strip tubes marked “C-”. Avoid paraffin layer break.

8.1.8 Add 5.0 µL of positive control No. 1 (“C+”) into the corresponding strip tubes marked “C+ No. 1”. Avoid paraffin layer break.

8.1.9 Add 5.0 µL of positive control No. 2 (“C+”) into the corresponding strip tubes marked “C+ No. 2”. Avoid paraffin layer break.

8.1.10 Centrifuge the strips for 3–5 seconds on a vortex mixer.

8.1.11 Set the strips into the real-time thermal cycler.

8.1.12 For DT instruments: Launch the operating software for DT instrument[7]. Add corresponding test[8], specify the number and IDs of the samples, positive and negative controls. Specify position of the strips in thermal unit (see 8.1.11) and run PCR. See Table 5.

[7] - Please, apply to Operation Manual for DTprime, DTprime II and DTlite Real-Time PCR instruments PART II.
[8] - Instructions for uploading "files with test parameters" can be found on DNA-Technology’s website https://www.dna-technology.com/assaylibrary.

8.1.13 For CFX96 thermal cyclers: perform PCR considering reaction mixture volume of 35 µL according to amplification program shown in Table 6.

Table 5. PCR program for DTprime, DTprime II and DTlite thermal cyclers

Step	Temperature, °C	Min	Sec	Number of cycles	Optical measurement	Step type
1	80	0	30	1		Cycle
	94	1	30			
2	94	0	30	5		Cycle
	64	0	15		√	
3	94	0	10	45		Cycle
	64	0	15		√	
4	94	0	5	1		Cycle
5	25[9]	…	…	Temperature hold		Temperature hold
√ - optical measurements						

Table 6. PCR program for CFX96 thermal cycler

Step	Temperature, °C	Time, min:sec	Number of cycles (repeats)
1	80	01:00	1
2	94	01:30	1
3	94	00:15	50
4	64 **√**	00:20	
√ - optical measurements (Plate Read), set the fluorescence measurement on the Fam, Hex, Rox, Cy5 channels at 64°C			

9. CONTROLS

MycosoResista Trichophyton REAL-TIME PCR Detection Kit contains a positive control which is produced using genetic engineering techniques and contains the target DNA sequence.

To reveal possible contamination, a negative control is required.

WARNING! The negative control must undergo all stages of DNA extraction simultaneously with the test samples.

The test result is considered invalid when the exponential growth of the fluorescence level for the specific amplification product and for internal control (IC) are not observed.

The test result is considered valid if:

- For positive control the test result must be displayed as “+”, meaning “detected”. In case of a negative result (“-”, “not detected”) for the positive control the results of the current run are considered invalid. In this case it is required to repeat amplification of all DNA samples.
- For negative control the test result must be displayed as “-”, meaning “not detected” for specific amplification target and as “+”, or “detected” for internal control. Otherwise, the results of the current run are considered invalid; decontamination procedure is required.

[9] - temperature hold at 10**°**C is allowed

10. DATA ANALYSIS

10.1. Amplification results are calculated automatically by the software that is provided with the thermal cycler.

10.2. When using CFX96 detection thermal cyclers, use regression type analysis (Cq Determination Mode: Regression). In the “Baseline Setting” tab select “Baseline Subtracted Curve Fit”.

10.3. Result interpretation is carried out according to Table 7. The results are valid if the conditions for the interpretation of results obtained for controls are met.

WARNING!

- The test result is considered **invalid** if DNA of *Trichophyton spp*. is not detected, but DNA of *Trichophyton mentagrophytes var. indotineae* is identified.
- The detection of any mutation in the *ERG1* gene is considered **valid** only upon simultaneous detection of *Trichophyton spp*. DNA.

10.4. Invalid result may be due to the presence of inhibitors in the DNA preparation obtained from biological material; incorrect execution of the analysis protocol; noncompliance with the amplification temperature regime, etc. In this case it is necessary to repeat PCR with the available DNA preparation, or to re-extract DNA and perform PCR for this sample, or to re-collect biological material from the patient (performed sequentially).

10.5. If a positive result is obtained for a negative control, the results of the entire run batch are considered invalid. In this case it is necessary to carry out special measures to identify and eliminate possible contamination.

10.6. If a negative result is obtained for a positive control, the results of the entire run batch are considered invalid. In this case it is necessary to repeat amplification of the whole batch of samples.

Table 7. PCR results interpretation

№ tube on the strip	**Detection channel**				**Result interpretation**
	Fam, Cp/Cq	Hex, Cp/Cq	Rox, Cp/Cq	Cy5, Cp/Cq	
Test samples					
1,5	**≤35**	Not considered	**Specified**	Not specified	***Trichophyton mentagrophytes* var. *indotineae* DNA is detected**
	>35	Not considered	**Specified**	Not specified	***Trichophyton mentagrophytes* var. *indotineae* DNA is detected**, but is insufficient to detect mutations in gene *ERG1*[10]
	Not specified	Not considered	**≤35**	Not specified	***Trichophyton* spp. DNA is detected**
	Not specified	Not considered	**>35**	Not specified	***Trichophyton* spp. DNA is detected,** but is insufficient to detect mutations in gene *ERG1*[10]
	Specified	Not considered	Not specified	Not specified	Invalid result
2,6	**Specified**	Not considered	Not specified	Not specified	**Phe397Leu substitution is detected**
	Not specified	Not considered	Not specified	**Specified**	**Leu393Phe substitution is detected**
3,7	**Specified**	Not considered	Not specified	Not specified	**His440Tyr substitution is detected**
	Not specified	Not considered	**Specified**	Not specified	**Leu393Ser substitution is detected**
4,8	**Specified**	Not considered	Not specified	Not specified	**Ser436Ala substitution is detected**
	Not specified	Not considered	**Specified**	Not specified	**Ala448Thr substitution is detected**
For all tubes	Not specified	**Specified**	Not specified	Not specified	DNA of target microorganisms is not detected, mutations in *ERG1* gene are not detected
	Not specified	Not specified	Not specified	Not specified	Invalid result
Negative control					
1,5	Not specified	**Specified**	Not specified	Not specified	**Negative result** Run results are valid
2,6	Not specified	**Specified**	Not specified	Not specified	
3,7	Not specified	**Specified**	Not specified	Not specified	
4,8	Not specified	**Specified**	Not specified	Not specified	
Positive control No. 1					
1,5	**Specified**	**Specified**	**Specified**	Not specified	**Positive result** Run results are valid
2,6	**Specified**	**Specified**	Not specified	Not specified	
3,7	**Specified**	**Specified**	**Specified**	Not specified	
4,8	Not specified	**Specified**	**Specified**	Not specified	

[10] - Low DNA quantity (Cp > 35) may lead to a false-negative result in the detection of *ERG1* mutations

Positive control No. 2					
1,5	Not specified	**Specified**	Not specified	Not specified	**Positive result** Run results are valid
2,6	Not specified	**Specified**	Not specified	**Specified**	
3,7	Not specified	**Specified**	Not specified	Not specified	
4,8	**Specified**	**Specified**	Not specified	Not specified	

11. SPECIFICATIONS

a. Analytical specificity

For biomaterial samples containing DNA of the target analytes, the software of the detecting thermal cycler records exponential fluorescence growth in the corresponding tube on the specified detection channels during amplification.

For biomaterial samples that do not contain DNA of the target analytes, the software of the detecting thermal cycler records absence of exponential fluorescence growth in the corresponding tubes on the specified detection channels and exponential fluorescence growth for the internal control on the Hex detection channel.

The **MycosoResista Trichophyton REAL-TIME PCR Detection Kit** detects DNA of the following fungi targets:

Genus: *Trichophyton* spp.;

Species: *Trichophyton mentagrophytes* var. *indotineae*;

Mutations of *ERG1* gene corresponding to amino acid substitutions: Phe397Leu, Leu393Ser, Leu393Phe Ser436Ala, His440Tyr, Ala448Thr

There were no nonspecific cross-reactions of any kit component with any target.

There were no nonspecific positive amplification results in the presence of DNA of *Nannizzia incurvata, Epidermophyton floccosum, Candida albicans, Malassezia furfur,* Human simplex virus 1, Human simplex virus 2, *Enterobacter cloacae, Enterobacter aerogenes, Neisseria gonorrhoeae, Aspergillus niger, Geotrichum candidum*, as well as human DNA in concentration up to 1.0×10^8 copies/mL of sample.

b. Analytical sensitivity (limit of detection)

Limit of detection (LoD) is 5 copies of each analyte's DNA per amplification tube.

LoDs of microorganisms detected with the **MycosoResista Trichophyton REAL-TIME PCR Detection Kit** were established by analysis of serial laboratory controls' dilutions and are presented in Table 8.

LoD corresponds to the following DNA concentration values when using the **PREP-DERM DNA Extraction Kit** and the indicated final elution (dilution) volume of the extracted DNA.

Table 8. Limits of detection

No.	Analytes	LoD (copies per tube)	LoD (copies in 1.0 mL of extracted DNA)	LoD (copies/sample) with 300 µL of DNA elution
				PREP-DERM DNA Extraction Kit
1	*Trichophyton* spp.	5	$1.0x10^3$	$3.0x10^2$
2	*Trichophyton mentagrophytes* var. *indotineae.*	5	$1.0x10^3$	$3.0x10^2$
3	Phe397Leu	5	$1.0x10^3$	$3.0x10^2$
4	Leu393Phe	5	$1.0x10^3$	$3.0x10^2$
5	His440Tyr	5	$1.0x10^3$	$3.0x10^2$
6	Leu393Ser	5	$1.0x10^3$	$3.0x10^2$
7	Ser436Ala	5	$1.0x10^3$	$3.0x10^2$
8	Ala448Thr	5	$1.0x10^3$	$3.0x10^2$

c. Diagnostic characteristics

Biomaterial	Analyte	Diagnostic sensitivity	Diagnostic specificity
Skin	*Trichophyton* spp.	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	Trichophyton mentagrophytes var. *indotineae*	100% (95% CI: 78.20% – 100%)	100% (95% CI: 92.89% – 100%)
	ERG1 gene mutation (Phe397Leu substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
	ERG1 gene mutation (Leu393Phe substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
	ERG1 gene mutation (His440Tyr substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
	ERG1 gene mutation (Leu393Ser substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
	ERG1 gene mutation (Ser436Ala substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
	ERG1 gene mutation (Ala448Thr substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
Hair	*Trichophyton* spp.	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	Trichophyton mentagrophytes var. *indotineae*	100% (95% CI: 78.20% – 100%)	100% (95% CI: 92.89% – 100%)
	ERG1 gene mutation (Phe397Leu substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
	ERG1 gene mutation (Leu393Phe substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
	ERG1 gene mutation (His440Tyr substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
	ERG1 gene mutation (Leu393Ser substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
	ERG1 gene mutation (Ser436Ala substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)

Biomaterial	Analyte	Diagnostic sensitivity	Diagnostic specificity
	ERG1 gene mutation (Ala448Thr substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
Nails	*Trichophyton* spp.	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	Trichophyton mentagrophytes var. *indotineae*	100% (95% CI: 78.20% – 100%)	100% (95% CI: 92.89% – 100%)
	ERG1 gene mutation (Phe397Leu substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
	ERG1 gene mutation (Leu393Phe substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
	ERG1 gene mutation (His440Tyr substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
	ERG1 gene mutation (Leu393Ser substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
	ERG1 gene mutation (Ser436Ala substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
	ERG1 gene mutation (Ala448Thr substitution)	100% (95% CI: 29.24% – 100%)	100% (95% CI: 94.48% – 100%)
Total		100% (95% CI: 97.90% – 100%)	100% (95% CI: 98.13% – 100%)

d. Reproducibility and repeatability

Reproducibility is 100%.

Repeatability is 100%.

12. TROUBLESHOOTING

Table 9. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat the entire test Dispose of the current batch
C-	+	Contamination	Dispose of the current batch Perform decontamination procedures
IC	Invalid	PCR inhibition	Repeat the entire test Re-take sample

If you encounter any undescribed issues, contact our customer service department:

Phone: +7(495) 640-16-93

E-mail: hotline@dna-technology.ru

https://dna-technology.com/support

13. QUALITY CONTROL

The quality control procedures are performed in accordance with ISO 9001:2015 and ISO 13485:2016.

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

Manufacturer: "DNA-Technology Research & Production", LLC,

142281, Russia, Moscow Region,

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Phone/fax: +7(495) 640-17-71

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14. KEY SYMBOLS

RUO	For research use only		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
	Caution		Non-sterile