DNA-TECHNOLOGY

For research use only

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MycosoScreen REAL-TIME PCR Detection Kit

REF **R1-P023-S3/5EU**

General information

Intended use:

MycosoScreen REAL-TIME PCR Detection Kit is intended for detection and typing of pathogens causing mycoses from genus *Candida*, *Malassezia*, *Saccharomyces* and *Debaryomyces* in samples from human biological material by Real-Time PCR method.

The kit is for research use only.

Method:

Multiplex Real-Time PCR, semi-quantitative analysis.

Samples:

Blood, phlegm, urine, swabs/scrapes from respiratory tract, gastrointestinal and urogenital tracts, faeces, bioptates, catheter and endotracheal tube washings, and fungal cultures.

DNA extraction:

Nucleic acid extraction kits that have a license as a medical product and are intended for the extraction of NA from corresponding biomaterial with subsequent analysis by PCR (for example, "DNA-Technology" made **PREP-NA DNA/RNA Extraction Kit**).

⚠ DNA extraction from catheter washings is carried out only with **PREP-NA** extraction kit.

Features:

Multiplex analysis – simultaneous detection of multiple targets in the one tube.
Internal control sample (IC) – is contained in №1-7 tubes with amplification mixture and is required for the control of amplification run. Sample intake control (SIC, in tube №8) is required for the assess of quality of DNA extraction from samples containing human cells and allows to determine if the amount of acquired DNA is sufficient for analysis.

For the control of strip positioning in the thermoblock of the detecting thermocycler the mixture in tube №3 contains a fluorescent probe Rox – "Marker". After the amplification the software compares the predetermined position of tubes with real position of marker and warns an operator in case of mismatch.

We also recommend including in assay the negative control (C-) which is not supplied but very helpful for contamination control purposes. Use deionized water or sterile buffered saline instead of sample, starting from extraction step.

Devices:

The automatic analysis for **MycosoScreen REAL-TIME PCR Detection Kit** is available on "DNA-Technology" made DTlite[1] and DTprime[2] REAL-TIME Thermal Cyclers; software version is not lower than 7.9.5.39; the current version of the software is available for download at http://www.dna-technology.ru/eng/support/.

Time of analysis (excluding sample preparation procedure):

from 1.5 hours.

The number of tests: 24 (including one positive control and one negative control in each run).

Kit contents:

Reagent	Organoleptic parameters	Quantity	
• Paraffin sealed PCR-mixes	Colorless/blue transparent liquid under white wax layer	20 µL in each	24 8-tubes strips
• Taq-polymerase solution	Colorless transparent liquid	500 µL in each	4 tubes
• Mineral oil	Colorless transparent viscous oily liquid	1.0 mL in each	4 tubes
• Positive control	Colorless transparent liquid	320 µL	1 tube
Associated accessories:			
Strip's caps			24 8-caps strips

[1] - supported by 4S1, 4S2, 5S1, 5S2, 6S1, 6S2 instruments.
[2] - supported by 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 instruments.

Strip content, color codes and detection channels

№ of tube	Detection channel				Color labeling of the buffer
	Fam	Hex	Rox	Cy5	
1	*Meyerozyma guilliermondii (C. guilliermondii)*	IC	-	-	Blue
2	*Candida albicans*	IC	-	*Pichia kudriavzevii (C.krusei)*	Colorless
3	*Saccharomyces cerevisiae*	IC	Marker	*Candida auris*	
4	*Candida tropicalis*	IC	-	*Clavispora lusitaniae (Candida lusitaniae)*	
5	*Debaryomyces hansenii (C.famata)*	IC	-	*Candida dubliniensis*	
6	*Candida glabrata*	IC	-	*Candida parapsilosis*	
7	*Malassezia sp.*	IC	-	*Malassezia furfur*	
8	*Kluyveromyces marxianus (C.kefyr)*	SIC	-	-	

Procedure

1 DNA extraction

⚠ Regardless of the nucleic acid extraction kit used, a negative control sample must undergo all the DNA extraction procedure stages (a sterile physiological solution or transport medium for clinical samples in the volume specified in the user manuals for the nucleic acid extraction kit can be used).

1.1 Sample taking from phlegm, urine, swabs/scrapes from respiratory tract, gastrointestinal and urogenital tracts, faeces, bioptates, endotracheal tube washings, and fungal cultures is carried out according to the extraction kit instruction.

1.2 Sample taking and DNA extraction from washings from intravenous catheter

⚠ DNA extraction from catheter washings is carried out only with **PREP-NA** extraction kit.

1.2.1. Cut with sterile scissors a 5.0-10 mm piece of catheter and place it in a 1.5 mL tube.

1.2.2. Add in the tube 100 µL of deionized water or sterile saline.

1.2.3. Vortex the tube for 3-5 s and spin down the drops for 3-5 s.

1.2.4. Add in the tube 300 µL of lysis buffer from **PREP-NA** extraction kit.

1.2.5. Vortex the tube for 3-5 s and spin down the drops for 3-5 s.

1.2.6. Termostate the tube on 65 °C for 15 min.

1.2.7. Spin down the drops for 3-5 s and transfer the supernatant in a new tube.

Further processing of the samples should be done starting with the addition of the precipitation buffer.

2 PCR amplification

⚠ The reagents and tubes should be kept away from direct sun light.

Strictly observe the completeness of the strips and caps to them. Do not use the caps to the strips of the other kits!

2.1 Mark the required number of strips with paraffin sealed PCR-mix: 1 strip for each sample to be tested, 1 strip for positive control (C+) and 1 strip for negative control (C-).

Example. If you need to test 2 samples, mark 2 strips for analyzed samples, 1 strip for "C-" and 1 strip for "C+". Total number of strips – 4 (Table 1).

Table 1.Labeling of strips for PCR

Sample	№ of strip	№ of tube in the strip
1	1	1-8
2	2	1-8
"C–"	3	1-8
"C+"	4	1-8

2.2 Vortex the Taq-polymerase solution thoroughly for 3-5 s, then spin briefly for 1-3 s.

2.3 Add 10 µL of Taq-polymerase solution into each tube of the strip. Avoid paraffin layer break.

2.4 Add one drop (~20 µL) of mineral oil into each tube. Close the tubes.

2.5 Vortex the tubes with samples and "C-" and "C+" for 3-5 sec and spin down the drops by centrifuging on vortex-microcentrifuge for 1-3 s.

⚠ Open the cap of the strip, add DNA sample (or control sample), then close the strip before proceeding to the next strip to prevent contamination. Use filter tips.

2.6 Add 10 µL of the DNA sample into corresponding strips. Close the strips tightly. Do not add DNA into the "C-", "C+" tubes.

2.7 Add 10 µL of negative control sample (C-), which passed whole NA extraction procedures into corresponding tube. Add 10 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break. Close the strips tightly.

2.8 Spin the strips for 3–5 s to collect drops.

2.9 Set the strips to the real-time PCR thermal cycler.

2.10 Launch the RealTime_PCR application in "Device operation" mode. Upload "Mycosis_screen_en.ini" file before the first run. In subsequent runs add the appropriate test to the protocol, specify the number and ID's of the samples, specify the position of the strips in the thermal unit (2.9) and the number of positive and negative controls, and run PCR.

3 Data collection and data analysis

Registration and interpretation of the PCR results are held in automatic mode.

3.1 On analysis of the results it is necessary to consider the values of internal control (IC, tubes №1-7, Hex detection channel) and sample intake control (SIC, tube №8, Hex detection channel):

- If one or more tubes in the strip lack IC and at the same time lack specific positive results, the result in those tubes is considered invalid due to incorrect conduction of PCR. In this case repeating of PCR amplification, or reextraction of DNA, or resampling is needed (performed sequentially).
- For the control of sample intake containing human cells the parameter SIC is used (the sufficient amount of human DNA).The value of SIC less than 3.0 in case of absence of specific results in all tubes of the strip is considered as insufficient amount of biomaterial. In this case resampling is recommended. If the origin of sample does not contain human biological material, the value of SIC is not considered.

3.2 In the samples containing DNA of detected pathogens the software detects positive result on the corresponding detection channel (Fam or Cy5) in a corresponding tube. In the result table the result of the qualitative analysis ("+"), the value of threshold cycle (Cp) and logarithm of concentration (Lg, the number of copies of DNA target in 1.0 mL of sample) will be depicted. Interpretation of the result will be "detected (N Lg)".

3.3 In the samples not containing DNA of detected pathogens the software detects negative result on the corresponding detection channel (Fam or Cy5) in a corresponding tube. In the result table the result of the qualitative analysis ("-") will be depicted. Interpretation of the result will be "not detected".

3.4 In the tube №5 of the strip the value of Lg≤2.5 on Fam detection channel is not considered by the software. In the result table the result of qualitative analysis ("-") will be depicted. Interpretation of the result will be "not detected".

3.5 In the tube №7 of the strip the value of Lg≤2.5 on Fam detection channel is not considered by the software in case of the absence the exponential increase of fluorescence on Cy5 detection channel, and the in result table the result of qualitative analysis ("-") will be depicted. Interpretation of the result will be "not detected".

3.6 In the tube №8 of the strip the value of Lg≤2.0 on Hex detection channel is not considered by the software. In the result table the result of qualitative analysis ("-") will be depicted. Interpretation of the result will be "not detected".

3.7 For positive and negative control samples the results must correspond to those from the Table 2. In the negative control sample the IC value must be no less than 3.5. In the positive control sample the IC value is not considered.

Table 2. The results of the test for positive and negative control samples

№ of tube in the strip	Pathogen	C- (Lg)	C- result	C+ (Lg)	C+ result
1	*Meyerozyma guilliermondii*	-	-	3.5-5.5	+
2	*Candida albicans*	-	-	3.5-5.5	+
	Pichia kudriavzevii	-	-	3.5-5.5	+
3	*Saccharomyces cerevisiae*	-	-	3.5-5.5	+
	Candida auris	-	-	3.5-5.5	+
4	*Candida tropicalis*	-	-	3.5-5.5	+
	Clavispora lusitaniae	-	-	3.5-5.5	+
5	*Debaryomyces hansenii*	-	-	3.5-5.5	+
	Candida dubliniensis	-	-	3.5-5.5	+
6	*Candida glabrata*	-	-	3.5-5.5	+
	Candida parapsilosis	-	-	3.5-5.5	+
7	*Malassezia spp.*	-	-	3.5-5.5	+
	Malassezia furfur	-	-	3.5-5.5	+
8	*Kluyveromyces marxianus*	-	-	3.5-5.5	+
	SIC	-	-	3.5-5.5	+

Shipping, storage and handling requirements

The kit should be stored at the temperatures from 2 °C to 8 °C and out of light during the storage period.

PCR kit has to be transported in insulated shipping containers along with ice packs inside by all types of roofed transport at the temperature corresponding to the storage conditions of the PCR kit components for its entire shelf life or in insulated shipping containers along with ice packs inside at the temperature from 2° C to 25 °C but no longer than 5 days.

Shelf-life – 12 months since the date of production if all the conditions of transportation, storage and operation are met.

Contact our customer service department regarding quality issues with the kit:

8 800 200-75-15 (toll-free call for Russia)

+7 (495) 640-16-93 (chargeable call for CIS and foreign countries).

E-mail: hotline@dna-technology.ru, www.dna-technology.ru.

Address: 117587, Moscow, Varshavskoye sh., 125g building 6, floor 5, room 14, DNA Technology, LLC.

Key to symbols

	For research use only		Consult instructions for use		Caution
	Temperature limitation		Manufacturer		Batch code
	Expiration date		Number of tests		Do not expose to sunlight
	Date of manufacture		Catalogue number		Not sterile
	Single use				