Sina Fungiplex Universal

(Real-Time PCR Kit)

REF **PQ3681**

Σ **25 Tests**

RUO

-20 °C

Components

Contents	Quantity/ Volume	Store Temperature
Master Mix	625µl	-20°C
Primer Mix	375µl	-20°C
Positive control (concentrated)	50µl	-20°C

Description

The Sina Fungiplex Universal is a Ready-to-Use real-time PCR Kit, which detect a broad range of fungal pathogens. This kit is used for the qualitative detection of Fungal DNA extracted from clinical samples (serum, plasma, whole blood, respiratory tract specimens and body fluids). The assay is compatible with common laboratory real time PCR result in less than 1.5 hours. This kit is ready to use reporting format, as detailed below:

Apophysomyces	**Cunninghamella**	**Mucor**	**Saccharomyces**
Aspergillus	**Exophiala**	**Paecilomyces**	**Saksenaea**
Candida	**Fusarium**	**Penicillium**	**Scedosporium**
Cladosporium	**Histoplasma**	**Pneumocystis**	**Syncephalastrum**
Coccidioides	**Lichtheimia**	**Rhizomucor**	**Talaromyces**
Cryptococcus	**Microsporum**	**Rhizopus**	**Trichosporon**

Storage and Stability

Sina Fungiplex Universal real- time PCR kit is shipped on dry ice and should be stored at -20°C upon arrival. Check the package for any possible damage. The performance of the kit will be guaranteed until the printed expiration date, when the contents keep in an appropriate condition.

Warnings and Precautions

A. Chemical risks

There are no hazardous materials included in the manufacture of the Sina Fungiplex Universal real time PCR Kit. The composition of all reagents represents no specific risk to the user or to their property.

Additional chemicals and materials may be required for procedures described in these Instructions for Use. Carefully read any warnings, instructions, or Safety Data Sheets provided by the supplier and follow general safety regulations when handling chemicals, biohazards, or other materials.

B. Biological risks

Sina Fungiplex Universal real time PCR Kit involves safe material, but working with fungi samples are potentially dangerous and transmissible (samples received from patients). All personnel are responsible for reading and following all necessary health and safety precautions and all procedure should be hell in BSL2 lab.

It is very important to wear appropriate PPE at all times; a lab coat, protective gloves and safety glasses are minimum needed for working with these materials.

Important notes: please read before starting

1. It is recommended to extract DNA use sinapure DNA kit (Cat NO: EX6011).

2. At least one positive and negative control should be included in each analysis. All controls should be treated and tested in the same manner as patient samples.

3. Check the procedure with a known positive sample, if use the kit for the first time.

4. You need Nuclease Free Water for negative control (NTC).

Protocol

- Remove the Sina Fungiplex Universal real time PCR Kit from the freezer and allow to thaw. Quickly vortex the tubes once defrosted.

Prepare the final reaction mix as follows:

PCR Regent

Reagent	Volume (µl)		
	PC*	NTC**	Sample
Master Mix, 2X	25	25	25
Primer mix	15	15	15
Template	2	-	10
Nuclease Free Water	8	10	-
Total	50	50	50

*Positive Control

**Non Template Control

qPCR program

Step*	Temperature (°C)	Time (sec)	Number of cycles
Pre-incubation	95	300	1
Denaturation	95	60	40
Annealing	61	25	
Extension	72	20	

*Activate syber Green channel.

Analysis

Panfugi (Cyber)	-
≤ 40	Fungi detected.
Undet/blank or > 40	Fungi not detected.
Undet/blank or > 40	Invalid. Sample needs to be retested by repeating PCR if there is sufficient extracted DNA. Otherwise, sample needs to be retested by re-extraction or re-collection from the original source.

Tips and Suggestions

A negative control that yields a positive test result is indicative of cross contamination. The assay run should be repeated using a fresh aliquot of negative control material, ensuring that the work area and equipment are properly decontaminated. A false negative result is indicative of reagent failure or sample handling error. Ensure all reagents have been stored correctly and, where applicable, expiry date before repeating the assay run taking extreme care during PCR set-up. Expected results for the positive control are provided.

Quality Control

All components of this Kit are successfully tested in terms of DNA purification and amplification reaction by known positive sample.

Troubleshooting

This guide may help to solve problems that may arise.

Problem	The assay is Insufficient amplification
Possible Causes	• The initial amount of template is too low. • Thermal cycler was not at correct temperature
Diagnostic Test	• Check DNA absorbance ratio at 260 and 280nm (260/280); Pure DNA should have a 260/280 ratio of ≥ 1.8 • The quality of the extracted DNA is inherently linked to the sensitivity achieved by real-time PCR assays.
Solutions	• Increase the number of amplification cycles. • Concentrate extracted DNA • Repeat your extraction with a new sample

Uniformity of Ct values across different tests

The clinical significance of positive results with high Ct are difficult to interpret in the absence of clinical history and context. Positive results with low DNA load (high Ct) can be seen in the early stages of infection (before the person becomes capable of infection transmission) or late occurrence of infection when the risk of transmission is low.

Signs

Signs	Definitions
	Temperature range on product use
RUO	For Research Use Only
	Name and address of the manufacturer of the product
REF	Product technical code

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