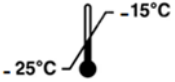
Sina FungiReal Cryptococcosis

(Real-Time PCR Kit)

REF PQ3691 Σ 25 TESTS



RUO

Components

Kit Contents	Quantity/ Volume	Storage Condition
PCR Master Mix	625µl	-20°C
Primer Mix	375µl	-20°C
IC* (10ng/µl)	50µl	-20°C
Positive control	250µl	-20°C

*IC: Internal Control

Description

Sina FungiReal Cryptococcosis real time PCR Kit is an in vitro diagnosis kit for the qualitative detection of Cryptococcus neoformans DNA, extracted from biological sample such as: respiratory tract specimens (BAL, BL, sputum) and body fluids peritoneal, pleural and CSF. The assay is compatible with common laboratory real time PCR equipment and result would be ready in less than 2 hours. This kit is ready to use reporting format.

The rapid, and reliable detection provided by this kit is guaranteed by quality control processes that ensures the user can have full confidence in assay quality and reproducibility.

Warnings and Precautions

A. Chemical risks

Please follow, the GLP rules and carefully read any warnings, instructions, or Safety Data Sheets provided by the supplier and follow general safety regulations when you hand chemicals, biohazards, or other materials.

B. Biological risks

Sina FungiReal Cryptococcosis real time PCR Kit involves safe material, but working with Cryptococcus is potentially dangerous and transmissible. All personnel are responsible for reading and following all necessary health and safety precautions.

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

Storage and Stability

Sina FungiReal Cryptococcosis Kit should be stored at -20°C upon arrival. Check the package for any possible damages.

The performance of the kit will be guaranteed until the printed expiration date, when the contents keep in an appropriate condition.

Required materials and tools that are not supplied with the kit

Title	Description of the requirement
Materials and Tools	Nucleic acid extraction kit SinaPure DNA (EX6011)
	Validated real-time thermal cycler instrument
	Class II hood / PCR station
	Vortex mixer
	Mini centrifuge
	Fridge (2 to 8°C) and freezers (-25°C to -15°C)
	Micropipettes for volumes of 1 to 1000µl
Reagents	Negative extraction control, for example nuclease free water (recommended)
Consumables	Appropriate DNase / RNase free plastic-ware for PCR preparation
	DNase / RNase free pipette tips
	Disposable gloves, powder-Free
	PCR plastic-ware compatible with the thermal cycler of choice

Important notes: please read before starting

1. Follow GLP rules when performing nucleic acid extraction from biological species.
2. Check the procedure with a known Positive Control, if use the kit for the first time.
3. It is important to consider the background signal while you analyse the samples and then its measurement should be excluded (in table analysis).

Protocol

Take the Sina FungiReal Cryptococcosis real time PCR Kit from the freezer and allow to thaw. Quickly vortex the tubes once defrosted. Prepare the final reaction mix as follows: The IC is external and to confirm the accuracy of the extraction, 2µl is added to the patient sample at the beginning of the extraction process and the extraction proceeds with the patient sample.

A. PCR Regent

Reagent	Volume		
	PC	NC	Sample
Master Mix 2X	25µl	25µl	25µl
Primer/probe mix	15µl	15µl	15µl
Template	2µl	-	10µl
DNase free water	8µl	10µl	-
Total	50µl	50µl	50µl

B. Channel for acquisition

No	Name of channel	Target	Source wavelength	Detection wavelength
FAM	Crypto*	470 **nm**	510 **nm**	FAM
2	HEX	IC*	535 **nm**	556 **nm**

*Crypto: Cryptococcus *IC: internal control

C. qPCR program

Step	Temperature (°C)	Time (sec)	Number of cycles	Analysis mode	Data collection
Pre-incubation	95	600	1	None	None
Denaturation	95	25	40	Quantification	None Acquire
Annealing-extension	58	70			

Analysis

Cryptococcus (FAM)	Internal Control (HEX)	Result
≤ 40	*	Cryptococcus detected.
Undet/blank or >40	≤ 35	Cryptococcus not detected.
Undet/blank or >40	Undet/blank or > 35	Invalid. Sample needs to be re-tested by repeating PCR if there is sufficient extracted DNA. Otherwise, sample needs to be re-tested by re-extraction or re-collection from the original source.

Undet: Undetermined. *: Not required on the Ct value.

Quality Control

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

Signs

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

Tips and Suggestions

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. 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.

To use as an internal DNA extraction control

The internal control can be added to the lysis/extraction buffer. Correctly, use the kit before expiration date and taking extreme care during PCR set-up.

1. Add 2µl of internal control DNA template (BLUE) to each sample in lysis/extraction buffer or elution buffer.
2. Complete DNA extraction according to the manufacturer's protocol.
3. When using Primer design 2X qPCR Master Mix, make up a mix containing all reagents according to the protocol below.

Troubleshooting

This guide may help solve problems that may arise.

Problem	The assay is Insufficient amplification
Possible Causes	Thermal cycler was not at correct temperature
Solution	Check optional conditions for your thermal cycler
Possible Causes	The initial amount of template is too low.
Solutions	•Increase the number of amplification cycles, •Increase the amount of template. •Repeat your extraction with a new sample. •Concentrate extracted DNA.

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.

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