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Instructions for use

DERMATOPHYTE AND *C. ALBICANS* REAL TIME PCR KIT

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DERMATOPHYTE AND C. *ALBICANS* REAL TIME PCR KIT

For *in vitro* diagnostic use

Intended use

The Dermatophyte and *C. albicans* Real Time PCR Kit is an *in vitro* diagnostic kit for the quantitative detection of general dermatophytes (pan-dermatophytes), *Trichophyton (T.) rubrum* and *Candida (C.) albicans* in nail, skin, and hair specimens.

Description

The Dermatophyte and *C. albicans* Real Time PCR Kit is a Real Time polymerase-chain-reaction based kit. The Dermatophyte and *C. albicans* Real Time PCR Kit is for use by laboratory professionals and/or healthcare professionals only.

The primers and probes included are designed for a specific region of the its2 gene (see table 1) for identification of pan-dermatophytes, specific to *T. rubrum* and *C. albicans,* respectively. The pan-dermatophytes amplification is detected by the fluorescence dye HEX, the *T. rubrum* amplification

is detected by the fluorescence dye FAM and the *C. albicans* amplification is detected by the fluorescence dye CAL Fluor Red 610 (CAL-610) which performs like TEXAS RED, ROX or ALEXA FLUOR 594 dyes. The Internal Plasmid Control is detected by the fluorescence dye Cy5/Quasar 670.

Table 1. Target detection of the Primer and Probe Mix

Target detection	Gene	Dye	nm
Pan-dermatophytes[a]	its2	HEX	556
T. rubrum	its2	FAM	520
C. albicans	its2	CAL Red 610	610
Internal Plasmid Control	Synthetic fragment	Cy5/Quasar 670	669/670

[a]Dermatophytes in general e.g. *T. rubrum, T. mentagrophytes, T. interdigitale, T. tonsurans, T. schoeuleinii, T. violaceum, T. soudanense, T. verrucosum, T. erinacei, M. canis, M. audouinii, E. floccosum*.

Principle

Fungal nail and skin infections are mainly caused by dermatophytes or the yeast *C. albicans*, with the dermatophyte *T. rubrum* being the most common cause. The traditional identification method with culturing and microscopic examination is time-consuming and varies from 10 days to 4 weeks. With this multiplex Real Time PCR-based method, dermatophytes in general and specifically *T. rubrum* and *C. albicans* can be detected in specimens of nails, hair, and skin within 2-3 hours.

Precautions

- If the kit is used with DNA template from cultured fungi, the DNA template must be diluted at least 100 times with RNase/DNase free water.
- Use a new negative control (buffer A/B or purified RNase/DNase free water) for each run.
- Contamination might occur if you run the PCR in the same room where you purify your samples.
- Avoid placing Primer and Probe Mix under direct light, as this might reduce the stability and affect the efficiency.
- The kit should be stored in a dark place at -20 °C. If the kit is stored at room temperature the results might be invalid.
- Do not use the kit after it has expired.
- Do not use components from other kits.
- Incorrect use of reagent A and B causes incorrect purification of DNA. Thus, the results will be invalid.
- Inspect the kit content before use to ensure it is intact. Any damaged vials should be discarded.

Materials provided

The reagents supplied in the kit are listed in table 2. The kit comprises reagents enough to perform 100

multiplex Real Time PCR reactions. The control vial contains 150 µL corresponding to at least 50 PCR tests.

Table 2. Reagents provided

Reagent	Cap Color	Volume
Primer and Probe Mix [1]	Green	950 µL
PCR Positive DNA Control 1, *T. rubrum*	White	150 µL
PCR Positive DNA Control 2, *C. albicans*	Black	150 µL
RT Supermix [2]	Red	1.1 mL
Buffer A		20 mL
Buffer B		20 mL

[1] Primer and Probe Mix for detection of pan-dermatophytes, *T. rubrum, C. albicans*, and Internal Plasmid Control. The plasmid used as internal control is included in the mix.
[2] Enzyme master mix for probe-based Real Time PCR.

Materials required but not provided

- Tubes (1.5 mL)
- Heat block or water bath (95 °C)
- Vortex mixer
- Pipettes and tips for 2-20µL, 20-200µL, and 100-1000µL
- RNase/DNase free water (for dilution and negative control)
- 96-well plates and seal
- Real Time Thermal Cycler*, which contains fluorescent FAM, HEX, Cy5 (or Quasar 670), and

CAL-610 channels detection (such as Bio-Rad CFX96TM)
- Computer connected to the Real Time Thermal Cycler for analysis of the results

*The Dermatophyte and *C. albicans* Real Time PCR Kit has been validated on the following instruments: Bio-Rad CFX96™, Qiagen Rotor-Gene® Q, ABI 7500, and Roche LightCycler® 480.
Bio-Rad CFX96™ has been validated in-house.
Qiagen Rotor-Gene® Q, ABI 7500, and Roche LightCycler® 480 have been validated by external partners. SSI Diagnostica can only provide templates and support for Bio-Rad CFX96™.

Storage and stability

Please find the information on the box and labels.
The Dermatophyte and *C. albicans* Real Time PCR Kit is shipped with cooling elements but should be stored in a dark place at -20 °C immediately upon receipt. Avoid repeated thawing/freezing and direct light exposure of the Primer and Probe Mix, as this might reduce the stability of the reagent and thus affect the efficiency of the DNA amplification. If you plan to freeze/thaw the Primer and Probe Mix more than 10 times, aliquot in tubes. The expiry date of the kit is printed on the label.

Preservatives

There are no preservatives in this kit.

Sample collection and storage

Sample material can be nail, hair, and skin for the Real Time PCR analysis. The kit has been validated using samples (nail) stored for a longer duration at -20 °C.

Collected DNA from nail samples that have been purified in Buffer A and Buffer B have a durability of up to five years when stored at -20 degrees celsius.

Quality control

Positive controls included in the kit function as the kit quality control. For the negative control use RNase/DNase free water or the A/B buffer (A and B need to be mixed 1:1 ratio). Please include positive controls and the negative control for each run.

Before use check the vial to ensure there is no damage and/or leaks. In case of damage or leaks discard the vials.

Procedure

Template preparation and PCR setup should be performed in dedicated areas free of possible contamination.

DNA Preparation

1. Add 100 µL of Buffer A to the specimen*. Incubate the sample at 95 °C for 10 minutes.
2. Immediately add 100 µL of Buffer B* and vortex. The sample is ready for PCR.

*If the nail specimen is large, either cut it into small pieces or increase the volume of Buffer A to cover the sample. Increase the volume of Buffer B equally.

PCR Set-up

3. Prepare the master mix as described in table 3. Multiply with the number of samples and add 10% to ensure sufficient volume.
4. Dispense 18 µL of the master mix and 2 µL of template DNA (sample or positive/negative control) in each tube.
5. Set up the PCR protocol on a Real Time Thermal Cycler as described in table 4.
6. Select the fluorescence channel FAM *(T. rubrum)*, CAL-610 *(C. albicans)*, HEX (pan-dermatophyte), and Cy5/Quasar 670 (Internal Plasmid Control) as described in table 5.
7. Run the PCR amplification in the Real Time Thermal Cycler.

Table 3. Preparation of master mix

Reagent	Volume/reaction per sample
Primer and Probe Mix	8 µL
RT Supermix	10 µL
Total volume	18 µL

Table 4. Amplification

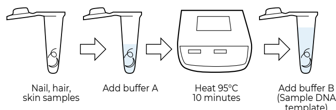
Step	Temp. (°C)	Time	Cycle(s)
Pre-denaturation	95 °C	2 minutes	1
Denaturation	95 °C	10 seconds	40
Annealing/extension	64 °C	1 minute	

Table 5. PCR target, dyes, and detection wavelength (nm)

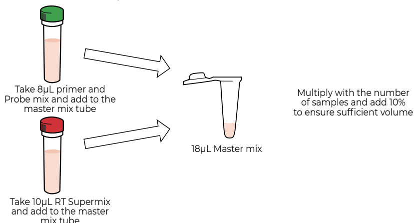
Target	Dye	Nm
T. rubrum	FAM	520
C. albicans	CAL Red 610	610
Pan-dermatophytes	HEX	556
Internal Plasmid Control	Cy5/Quasar 670	669/670

Quick guide for one sample

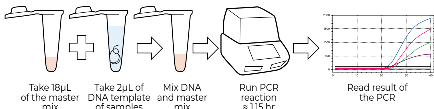
DNA Preparation



Master Mix Preparation



PCR Set-up



Interpretation of results

In **figure 1**, the result of a **positive *T. rubrum* sample** is shown by three amplification curves.

Amplification curve detected by FAM (shown as a **blue curve**): The specimen is positive for *T. rubrum* DNA.

Amplification curve detected by HEX (shown as a **green curve**): The specimen is positive for pan-dermatophytes.

Amplification curve detected by Cy5/Quasar 670 (shown as a **purple curve**): Detection of the Internal Plasmid Control. This signal should be observed in all tests, both positive and negative.

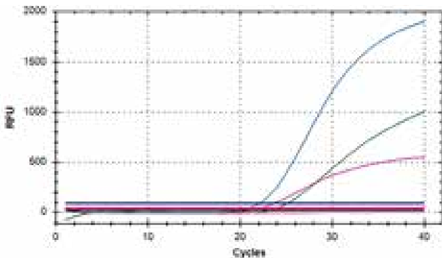


Figure 1: A *T. rubrum* positive specimen showing amplification curves for *T. rubrum* (FAM, **blue**), pan-dermatophytes (HEX, **green**) and Internal Plasmid Control (Cy5/Quasar 670, **purple**).

In **figure 2**, the result of a **positive *C. albicans* sample** is shown by two amplification curves.

Amplification curve detected by CAL-610 (shown as a **pink curve**): The specimen is positive for *C. albicans* DNA.

Amplification curve detected by Cy5/Quasar 670 (shown as a **purple curve**): Detection of the Internal Plasmid Control. This signal should be observed in all tests, both positive and negative. However, **notice** that the positive control decreases in signal when *C. albicans* is present in the sample.

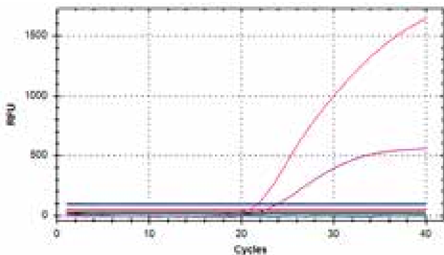


Figure 2: A *C. albicans* positive sample showing amplification curves for *C. albicans* (CAL Fluor Red 610, **pink**) and Internal Plasmid Control (Cy5/Quasar 670, **purple**).

In **figure 3** a **pan-dermatophyte positive result** is shown by two amplification curves.

Amplification curve detected by HEX (shown as a **green** curve): The specimen is positive for pan-dermatophytes. In this case the nail infection is not caused by *T. rubrum* since an amplification curve detected by FAM (**blue** curve) is not observed.

Amplification curve detected by Cy5/Quasar 670 (shown as a **purple** curve): Detection of the Internal Plasmid Control. This signal should be observed in all tests, both positive and negative.

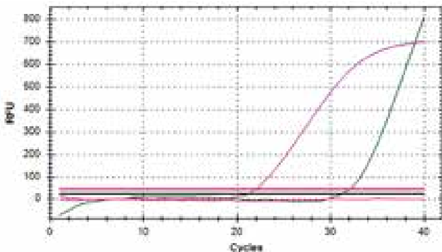


Figure 3: A pan-dermatophyte positive specimen showing amplification curves for, pan-dermatophytes (HEX, **green**) and Internal Plasmid Control (Cy5/Quasar 670, **purple**).

In **figure 4** a **negative result** is shown by one amplification curve.

Amplification curve detected by Cy5/Quasar 670 (shown as a **purple** curve): Detection of the Internal Plasmid Control. This signal should be observed in all tests, both positive and negative.

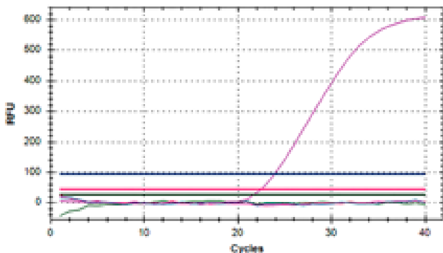


Figure 4: A negative specimen showing an amplification curve for the Internal Plasmid Control (Cy5/Quasar 670, **purple**).

In **figure 5** an **invalid result** is shown by no amplification curves.

The amplification curve detected by Cy5/Quasar 670 is shown as a **purple** curve. This signal should be observed in all tests, both positive and negative.

The test below outlines an invalid test result as there are no positive results for the Internal Plasmid Control.

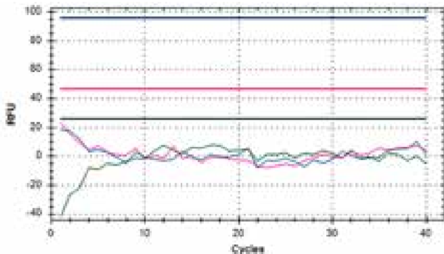


Figure 5: An invalid test result for a specimen showing no amplification curve for the Internal Plasmid Control (Cy5/ Quasar 670, **purple**).

Table 6: Ct cut-off values for nail, hair and skin specimens and culture samples

Interpretation	Channel FAM **Blue**	Channel HEX **Green**	Channel CAL-610 **Pink**	Channel Cy5/Quasar 670 **Purple**
Positive ***T. rubrum*** *****	Ct < 37	Ct < 35	-	Ct < 37
Positive Dermatophyte	-	Ct < 35	-	Ct < 37
Positive ***C. albicans***	-	-	Ct < 37	Ct < 37
Internal plasmid control (Negative)	-	-	-	Ct < 37

*If the concentration of *T. rubrum* is very low the CT value on the HEX channel might be higher than 35. Compare with your Internal plasmid control.

Disposal

Follow local procedures and/or guidelines from national authorities for disposal of biological materials.

Limitations

- Samples with very high amount of DNA should be diluted to provide a valid result.
- Samples with DNA amount under the limit of detection might not be detected.

Clinical performance

The clinical performance was performed by testing 518 human nail, skin, and hair samples suspected of having a fungal infection. These samples were tested using the Dermatophyte and *C. albicans* Real Time PCR Kit as well as the conventional method of culture (sabouraud and dermatophytes agar). The conventional method of culture was used as the reference method. The clinical samples came from two different external sites; Germany (N=468) and Austria (N=50). The method: increased detection, was used to show the increased ability of the RT-PCR kit to detect positive cases of fungal infections as compared to the conventional method of culture.

The Pan-Dermatophytes in table 7 is a representation of all pan-dermatophytes except for *T. rubrum* which is tested separately in the RT-PCR kit. With regards to the Pan-Dermatophytes, the RT-PCR kit was able to detect 35 (6.8%) positive cases as opposed to 28 (5.4%) positive cases by the conventional method of culture. This is an increased detection of 1.4% (N=7).

For *T. rubrum*, the RT-PCR kit was able to detect 199 (38.4%) positive cases as compared to 116 (22.4%) positive cases by the conventional method of culture. This is an increased detection of 83 positive cases (16%) when compared to the conventional method of culture (table 7).

For *C. albicans*, there was no difference in the total number of positive cases detected between the RT-PCR kit and the conventional method of culture. However, this shows that the RT-PCR kit is equally as capable of detecting *C. albicans* as the conventional method of culture.

Overall, the clinical performance data shows that the RT-PCR kit has an increased ability to detect Pan-Dermatophytes and *T. rubrum* positive cases when compared to the conventional method of culture.

Table 7 Clinical Performance data for Pan-Dermatophytes, *T. rubrum* and *C. albicans* (N=518 samples)

	Pan-Dermatophytes*		*T. rubrum*		*C. albicans*	
	(N)	(%)	(N)	(%)	(N)	(%)
Conventional methods (culture)	28	5.4	116	22.4	12	2.3
RT-PCR	35	6.8	199	38.4	11	2.1
Increased Detection	7	1.4	83	16.0	0	0

(N) = Total Numbers
(%) = Percentages
*This includes all pan-dermatophytes except *T. rumbrum.*

Analytical studies

Limit of Detection (LoD)

The Dermatophyte and *C. albicans* Real Time PCR Kit LoD (copies of genomic DNA) for pan-dermatophyte, *T. rubrum* and *C. albicans* (under the condition of using buffer A and buffer B for DNA template preparation) can be found in the table below.

Table 8. Limit of Detection

Strains	**LoD (copies of genomic DNA)**
Pan-dermatophyte	90
T. rubrum	42
C. albicans	11

Strain reactivity

The Dermatophyte and *C. albicans* Real Time PCR Kit showed an **analytical sensitivity of 93.1%** for pan-dermatophytes and an **analytical sensitivity of 100%** for both *T. rubrum* and *C. albicans* detection. This was the case when testing a panel of DNA samples purified from 2 *T. rubrum*, 2 *C. albicans* and 27 other dermatophytes *(E. floccosum, M. canis, M. audouinii, M. gypseum, T. erinaceid, T. interdigitale, T. mentagrophyte, T. schoeuleinii, T. soudanense, T. tonsurans, T. verrucosum, T. violaceum).*

Table 9 Sensitivity - Strain Reactivity

Strains	**Pan-dermatophyte**	***T. rubrum***	***C. albicans***
T. rubrum (n=2)	Positive (n=2)	Positive (n=2)	N/A
Other Dermatophytes: *E. floccosum, M. canis, M. audouinii, M. gypseum, T. erinaceid, T. interdigitale, T. mentagrophyte, T. schoeuleinii, T. soudanense, T. tonsurans, T. verrucosum, T. violaceum* (n=27)	Positive (n=25)	N/A	N/A
C. albicans (n=2)	N/A	N/A	Positive (n=2)
Sensitivity	**93.1 % (27/29)**	**100 % (2/2)**	**100% (2/2)**

Specificity (Cross-reactions)

To determine the analytical specificity of the Dermatophyte and *C. albicans* Real Time PCR Kit for cross-reactivity it was tested with a panel of DNA samples purified from different fungal panels. The Dermatophyte and *C. albicans* Real Time PCR Kit showed an **analytical specificity of 100%, according to table 10.**

Table 10 Specificity - Cross-reactions

Strains	**Pan-dermatophyte**	***T. rubrum***	***C. albicans***
T. rubrum (n=2)	N/A	N/A	Negative (2/2)
Other Dermatophytes: *E. floccosum*, *M. canis*, *M. audouinii*, *M. gypseum*, *T. erinaceid*, *T. interdigitale*, *T. mentagrophyte*, *T. schoeuleinii*, *T. soudanense*, *T. tonsurans*, *T. verrucosum*, *T. violaceum* (n=27)	N/A	Negative (27/27)	Negative (27/27)
Non-dermatophytes: *A. flavus*, *A. fumigatus*, *A. niger*, *A. wentii*, *F. oxysporum*, *F. proliferatum*, *F. solani*, *Mallassezia furfur*, *S. brevicaulis* (n=11)	Negative (11/11)	Negative (11/11)	Negative (11/11)
C. albicans (n=2)	Negative (2/2)	Negative (2/2)	N/A
Other Candida species (n=5)	Negative (5/5)	Negative (5/5)	Negative (5/5)
Specificity	**100 % (18/18)**	**100 % (45/45)**	**100% (45/45)**

Repeatability & reproducibility

The results in figure 5 come from the repeatability and reproducibility study and was performed on three separate lots.

The recovery (percentage of samples giving the expected result) of pan-dermatophytes, *T. rubrum* and *C. albicans* was 100% for the positive samples across all three lots (Lot 1, Lot 2, and Lot 3). For the negative samples, several false positive results were initially detected resulting in the following recovery rates: 90.2%, 90.5% and 91.7% for Lot 1, Lot 2, and Lot 3 respectively. However, upon further analysis of the PCR curve, when compared against the respective controls, it was discovered that the majority of the false positive results were indeed negative. Therefore, the recovery for the true negative samples were 99.4%, 99.2% and 99.4% for Lot 1, Lot 2, and Lot 3 respectively.

The overall recovery was 99.7%, 99.6% and 99.7% for Lot 1, Lot 2, and Lot 3 respectively. The overall recovery is based upon the total number of PCR runs and thus includes both the positive and true negative samples.

	Lot 1		Lot 2		Lot 3	
	Total Samples Tested	Recovery	Total Samples Tested	Recovery	Total Samples Tested	Recovery
Positive Samples	672/672	100%	624/624	100%	624/624	100%
Negative Samples	606/672	90,2%	565/624	90,5%	572/624	91,7%
Total number of PCR runs	1344		1248		1248	
Recovery based upon total number of PCR runs	1278/1344	95,1%	1189/1248	95,3%	1196/1248	95,8%

True Negative Samples	668/672	99,4%	619/624	99,2%	620/624	99,4%
Recovery based upon total number of PCR runs	1340/1344	99,7%	1243/1248	99,6%	1244/1248	99,7%

Figure 5: The recovery data from Lot 1, Lot 2, and Lot 3.

Incident reporting

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the Competent Authority of the member state in which the user and/or patient is established.

Quality certificate

SSI Diagnostica's development, production and sales of *in vitro* diagnostics are quality assured and certified in accordance with ISO 13485.

REF 99462

IVD

Information and ordering

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Revision history

- SSI Diagnostica can only provide support for Bio-Rad CFX96™.
- Information about storage of samples revised.
- Change of logo of notified body

2nd edition • September 2023 • 10538

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