

## DermatoPlex

Product Code: 7117192  
 Reactions: 192

**Storage and Stability:**

Reagents are shipped on dry ice or ice packs. All kit components are stable between -25°C to -15°C; refer to expiry on the label. Excessive freeze/thawing is not recommended. Store protected from light between -25°C to -15°C.

**Notes:**

This product is for Research Use Only, not for use in diagnostic procedures.

## RESEARCH USE ONLY



Store between -25°C to -15°C

### Description

DermatoPlex is a 1-well real-time PCR multiplex for detection of common fungi causing dermatophytosis (*Trichophyton mentagrophytes* complex and *Trichophyton rubrum* complex), and a selection of *Candida* species including *C. albicans*, *C. glabrata*, *C. orthopsilosis*, *C. tropicalis* and *C. parapsilosis*.

The reagents are compatible with the following real-time detection systems: Roche LightCycler® 480 Instrument II (LC480 II) (96- or 384-well format), Bio-Rad CFX96™ Dx (CFX96 Dx), CFX96 Touch™ (CFX96 Touch), Bio-Rad CFX384 Touch™ (CFX384 Touch) Real-time PCR Detection Systems, the Applied Biosystems® 7500 Fast (7500 Fast) and Applied Biosystems® 7500 Fast Dx (7500 Fast Dx).

The DermatoPlex kit is for research use only (RUO) and is not intended for use in diagnostic procedures.

### Components

Reagents	192 reactions	Cap colour
Plex Mastermix, 2x	1 x 1.2 mL	Blue
DermatoPlex Mix, 20x	1 x 150 µl	Yellow
Internal Control DNA	1 x 500 µl	Red
Nuclease Free Water	1 x 1 mL	Neutral

### Recommended procedures:

#### Sample extraction

Pre-digestion step:

To improve release of DNA from fungi in samples, it is recommended to include a pre-digestion step.

Example protocol:

- Place sample into the following lysis/digestion solution:
  - 300 µl sterile PBS
  - 180 µl Lysis buffer containing N-Laurylsarcosin, sodium salt
  - 20 µl Proteinase K
- Incubate the solution at 65°C for 30 minutes.
- Vortex and centrifuge, and collect 200 µl of supernatant for extraction.

Extraction step when using the Internal Control DNA:

- Example protocol:
  - Dilute Internal Control DNA 1 in 200 in 1x PBS
  - 20 µl of the diluted Internal Control DNA is loaded into 200 µl of sample prior to extraction (extraction can be performed on an automated platform such as MP24 or MP96)
  - Collect 100 µl of eluate from extraction
- Use 2.5 µL of the sample elution volume for a 10 µL qPCR reaction.

### Post extraction setup

- RT-qPCR Master mix setup 10.0 µl

Component	Supplied	Volume
Plex Mastermix, 2x	Yes	5.0 µl
DermatoPlex Mix, 20x	Yes	0.5 µl
Nuclease Free Water	Yes	2.0 µl
Total volume (for 1 reaction)		7.5 µl

*Recommended to Vortex and centrifuge the components before making up the master mix.*

Add 7.5 µl of the RT-qPCR Master mix to each well.

Add 2.5 µl extracted sample to each well.

### Programming and Data Analysis

- Roche LightCycler® 480 Instrument II (LC480 II)  
Refer to LC480 II Instrument Operator's Manual
- Bio-Rad CFX96™ Dx (CFX96 Dx) and CFX96 Touch™ (CFX96 Touch)  
Refer to CFX96 Dx and CFX96 Touch Real-Time PCR Detection Systems manual
- Bio-Rad CFX384 Touch™ (CFX384 Touch)  
Refer to CFX384 Touch Real-time PCR Detection Systems manual.
- Applied Biosystems® 7500 Fast (7500 Fast), Applied Biosystems® 7500 Fast Dx (7500 Fast Dx)  
Refer to Applied Biosystems 7500 FAST/7500 FAST Dx manual

## 2. Instrument Detection Formats

The channels used for LC480 II instrument are shown below.

Channel	Target
465-510	<i>T. mentagrophytes</i>
533-580	<i>T. rubrum</i>
533-610	Candida
618-660	IC

The channels used for CFX96 Dx, CFX96 Touch and CFX384 Touch are shown below.

Channel	Target
FAM	<i>T. mentagrophytes</i>
HEX	<i>T. rubrum</i>
Texas Red	Candida
Cy5	IC

The channels used for ABI 7500 Fast and ABI 7500 Fast Dx are shown below.

Channel	Target
FAM	<i>T. mentagrophytes</i>
JOE	<i>T. rubrum</i>
Texas Red	Candida
Cy5	IC

## 3. Thermocycling Program

Create the following **Cycling program**

- Touch down cycling is for specific amplification of target
- Quantification cycling is for PCR amplification and fluorescence acquisition

Program Name	Cycles	Target °C	Hold
Polymerase activation	1	95°C	2 min
Touch down cycling: Step down - 0.5°C/Cycle	10	95°C	5 s
		61°C – 56.5°C <sup>δ</sup>	30 s
Quantification cycling <sup>+</sup> : Acquisition/Detection	40	95°C	5 s
		52°C <sup>+</sup>	40 s
Cooling	1	40°C	30 s

<sup>δ</sup> **Step size:** -0.5 °C/Cycle, **Sec Target:** 56 °C

<sup>+</sup> **Analysis mode:** Quantification, **Acquisition mode:** Single

## 4. Data Analysis

Perform data analysis, as described in the instrument's operator manual.

**For LC480II SpeedX Colour Compensation (CC) must be run and applied before analysis.**