WizDia-Q™ Blastomyces dermatitidis qPCR Kit

REF WQ0114
RUO Research Use Only
Σ 100

Description
WizDia-Q™ Blastomyces dermatitidis qPCR Kit constitutes a ready-to-use system for the detection of ***Blastomyces dermatitidis (Ajellomyces dermatitidis***) by one-step real-time polymerase chain reaction (PCR) which has more sensitivity and specificity than conventional methods.

Kit Storage and Stability
- This kit is stable at -20 ºC temperature.
- Wizbiosolutions does not recommend using the kit after the expiry date stated on the pack.
- Freeze/thawing should be avoided.

Kit Contents

Component	Volume	Cap color
qPCR Reaction Mix	500 µl x 2 vial	Natural
B. dermatitidis Detection Mix	250 µl x 2 vial	Yellow
Positive Control	100 µl x 1 vial	Red
PCR grade water	1,000 µl x 1 vial	Green

Reagent and Equipment to be supplied by the user
- Real-Time PCR Instrument
- Genomic DNA extraction kit (REF. W71060)
- Pipettors and Tips
- Vortex and centrifuge
- Thin walled PCR reaction tubes or plates

Sample Material
- Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors. For preparation of genomic DNA from various sample enrichments, refer to the corresponding product package inserts of a suitable sample preparation kit.

Warnings and Precaution
- Carefully read this instruction before starting the procedure.
- For research purpose only. For in Vitro Use Only
- Do not use any reagent after the expiration date
- Do not use together with reagents of other products
- Follow the instructions
- Store all kit components at -20 °C
- Always wear gloves and a mask when handling biohazardous agents
- DO NOT repeatedly freeze/thaw Kit components
- Always use sterile, filtered pipette tips
- All positive controls should be added in a physically separate location from where the premix is reconstituted
- Briefly vortex and spin-down all Kit components after thawing to ensure optimum results
- Take care in handling of specimen to minimize risk of infection.

Sample Collection, Storage and Transportation
- Collect samples in sterile tubes.
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.

Quality Control
The WizDia-Q™ Blastomyces dermatitidis qPCR Kit is function tested using the CFX-96 Real-time PCR System (Bio-Rad).

Protocol
Please read through the entire procedure before starting.

1. DNA Preparation
- The following isolation kits are recommended:
  ⇒ WizPrep™ gDNA Mini Kit (Cell/Tissue) ; REF. W71606
- Please carry out the DNA extraction according to the instructions.

2. Prepare the raction mixture

Component	Volume
qPCR Reaction Mix	10 µl
B. dermatitidis Detection Mix	5 µl
DNA Template	5 µl
Total	20 µl

3. Real-time PCR program set-up
- Prepare appropriate qPCR tubes and label. Additional qPCR tubes for positive control & negative control.

Step	Temp.	Time	Cycle
UDG treatment	50 °C	2 min.	1
Initial Denaturation	95 °C	5 min.	1
Denaturation	95 °C	10 sec	40
Annealing/detection (Data collection)	60 °C	40 sec	

4. Fluorescence probe setting

Target	Fluoresence
Blastomyces dermatitidis	FAM
Internal Control	Cy5

5. Interpretation
The amplification of the *Blastomyces dermatitidis*-specific DNA region is analyzed in the fluorescence channel suitable for FAM labeled probes detection. The specific amplification of the Internal Control is analyzed in the fluorescence channel suitable for Cy5 labeled probes.

FAM	Cy5	Interpretation
Positive (Ct < 38)	Positive or Negative	Positive for *B. dermatitidis*
Negative	Positive	Negative for *B. dermatitidis*
Negative	Negative	Invalid

Note: A prerequisite for the unambiguous discrimination of *Blastomyces dermatitidis* and the Internal Control DNA in this multi-color experiment is a suitable calibration of the PCR instrument for channels FAM and Cy5. Please refer to the operation manual of your real-time PCR cycler for further information.

Ordering Information

Product	Cat No.	Package
WizDia-Q™ Blastomyces dermatitidis qPCR Kit	WQ0114	100 Test
WizPrep™ gDNA Mini Kit (Cell/Tissue)	W71060-100	100 Prep
	W71060-300	300 Prep

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Troubleshooting Guide

Observation	Possible Reason	Recommendation
No signal increase is observed, even with positive controls	• Incorrect detection channel has been chosen.	• Set Channel settings to FAM and Cy5
	• Pipetting errors	• Check for correct reaction setup. Repeat the PCR run.
	• No data acquisition programmed.	• Check the cycle programs
No signal increase in channel Cy5 is observed	• Inhibitory effects of the sample material (e.g., caused by insufficient purification). • Inappropriate storage of kit components.	• Use the recommended RNA preparation kit to purify template RNA. • Dilute samples or pipet a lower amount of sample RNA • Store the kit at -20 °C, protected from light and moisture
Fluorescence intensity is too low	• Low initial amount of target RNA.	• Increase the amount of sample RNA. • Exchange all critical solutions.
Negative control samples are positive.	• Carry-over contamination.	• Repeat the complete experiment with fresh aliquots of all reagents. • Always handle samples, kit components and consumables in accordance with commonly accepted practices to prevent carry-over contamination. • Add positive controls after sample and negative control reaction vessels have been sealed.
Fluorescence intensity varies	• Insufficient centrifugation of the PCR strips. Resuspend PCR mix is still in the upper part of the vessel.	• Centrifuge PCR strips.
	• Outer surface of the vessel or the seal is dirty (e.g., by direct skin contact).	• Always wear gloves when handling the vessels and seal

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