

Quantiplus® Cryptococcus Detection Kit

(Real Time Qualitative PCR Kit)


QL-CRP-25 : 25 rxns

PI/QLCRP-00

Introduction & Product Description

Cryptococcosis is an infection caused by fungi that belong to the genus *Cryptococcus*. There are over 30 different species of *Cryptococcus*, but two species *Cryptococcus neoformans* and *Cryptococcus gattii* – cause nearly all cryptococcal infections in humans. Cryptococcal disease once rare, has become a major fungal infection in immunocompromised, mainly human immunodeficiency virus (HIV)-infected patients. Quantiplus® Cryptococcus Detection Kit is an *in-vitro* diagnostic kit for detection for specific region of Cryptococcosis pathogen in Body fluids (CSF, Pleural Fluid, Ascitic Fluid and Synovial fluid) Sputum.

Kit components

| Color Coding (Caps) | Contents | Description | 25 rxns (QL-CRP-25) |
|---------------------|------------------|---|---------------------|
| Amber | Crypto Ready Mix | Crypto, internal control probes, primers with amplification Mix | 1 x 375 µL |
| Natural | Huwel IC-B Mix | Internal Control | 1 X 300 µL |
| Red | Crypto PC | Crypto Positive Control | 1 x 50 µL |
| White | Huwel PW | Purified water | 1 x 500 µL |

Sample Type

Plasma (K₂EDTA-Blood), CSF and Urine.

Heparinized Blood must not be used as they inhibit the PCR reaction

Assay Procedure

DNA Extraction

- 1- Huwel Nucleic Acid Extraction Kit (HL-HNAX-100)
- 2- Roche High Pure Viral DNA kit (Cat. No. 11858874001)
- 3- QIAamp Viral DNA Mini Kit (Cat. No. 51104)

qPCR Protocol

| Components | Volume per reaction (µL) (For final vol. of 26 µL) |
|--|---|
| Crypto Ready Mix | 15.0 |
| Huwel IC-B Mix (if not added at extraction step) | 1.0 |
| Extracted DNA/Crypto PC/Huwel PW | 10.0 |

Cycling conditions

| Steps | No. of cycles | Temperature (°C) | Time |
|--------------------------|---------------|------------------|---------|
| 1 (Initial denaturation) | 1 | 95 | 15 min. |
| 2 (PCR cycling-1) | 40 | 95 | 15 sec. |
| | | 60 | 60 sec. |

*Plate Read/Data Acquisition in **FAM** and **Yakima Yellow/VIC** channel

- Set the reaction volume as 25 µL (the final volume is 26 µL but selecting 25 µl doesn't make any difference to the final result/sensitivity).

Quick Reference Guide

Data Analysis

Setting the threshold for the qPCR Data analysis

The threshold should be set either automatically (by the machine itself)/ or manually just above the background signal of the negative controls and negative samples by referring to $R_n/Cycle$ amplification plot.

Result and Interpretation

| S.No | FAM (CRYPTO) | Yakima Yellow / VIC (IC) | Fluorophore | | Conclusion |
|------|-----------------|-----------------------------|----------------------------|---|------------|
| | | | ← | ↓ | |
| | | | Interpretation | | |
| 1 | √ | √ | Crypto DNA detected | Proceed for further Analysis | |
| 2 | √ | - | Crypto DNA detected | | |
| 3 | - | √ | Crypto DNA not detected | | |
| 4 | - | - | Possible inhibition of PCR | Make sure reaction setup and PCR conditions are followed correctly Recheck quality and quantity of DNA | |

Assay Characteristics

The analytical sensitivity of Quantiplus® Cryptococcus Detection Kit is *5 copies* per reaction.

Specificity

The analytical specificity of the Quantiplus® Cryptococcus Detection Kit for Cryptococcus was assured by primer design and stringent PCR conditions. Blast search was done to avoid any homology on primers with other organisms.



HLSS Manufacturing Pvt Ltd
Plot No's M14, M15, M16, TSIC Medical device park
Sultanpur villiage, Ameenpur Mandal,
Sangareddy Dist, TS-502319