

Quantiplus® ToCH Real Time Quantitative PCR Kit



QT-ToCH-25 : 25 rxns
 QT-ToCH-50 : 50 rxns



PI/QTToCH-00

Introduction & Product Description

Quantiplus® ToCH Real Time Quantitative PCR Kit is an in-vitro diagnostic kit for Quantitation of Toxoplasma, Cytomegalo Virus and Herpes Simplex Virus. The kit contains the necessary reagents. The kit contains ready to use master mix, standards, and an Internal control. IC can be added during extraction or PCR. Master mix is pipetted directly in PCR tube/ plate/strip and sample DNA is added.

Kit components

Color Coding (Caps)	Contents	Description		25 rxns (QT-ToCH-25)	50 rxns (QT-ToCH-50)
Amber	Toxo Ready Mix	Toxoplasma internal control probes, primers with amplification Mix		1 x 375µL	1 x 750µL
Amber	CMV Ready Mix	CMV internal control probes, primers with amplification Mix		1 x 375µL	1 x 750µL
Amber	HSV Ready Mix	HSV internal control probes, primers with amplification Mix		1 x 375µL	1 x 750µL
Natural	IC-B Mix	Internal Control		1 x 300 µL	1 x 600 µL
Pink	ToCHQS1	(For Toxo & HSV)	(For CMV)	3 x 50 µL	3 x 100 µL
		2 X 10 ⁴ copies/µL	2 X 10 ⁴ IU/µL		
Pink	ToCHQS2	2 X 10 ³ copies/µL	2 X 10 ³ IU/µL	3 x 50 µL	3 x 100 µL
Pink	ToCHQS3	2 X 10 ² copies/µL	2 X 10 ² IU/µL	3 x 50 µL	3 x 100 µL
Pink	ToCHQS4	2 X 10 ¹ copies/µL	2 X 10 ¹ IU/µL	3 x 50 µL	3 x 100 µL
White	MBGPW	Purified water		1 x 500 µL	1 x 500 µL

Sample Type

Toxoplasma: Plasma (K2EDTA-Blood), BAL Fluid, CSF, Biopsy

CMV: Plasma (K2EDTA-Blood), CSF and Urine

HSV: Plasma (K2EDTA-Blood), CSF and Ocular fluid.

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

Assay Procedure

DNA Extraction

- 1- Huwel Nucleic Acid Extraction Kit (HL-HNAX-100/250)
- 2- Roche High Pure Viral DNA kit (Cat. No. 11858874001)
- 3- QIAamp Viral DNA Mini Kit (Cat. No. 51104)

qPCR Protocol

For Toxoplasma/ CMV and HSV

Components	Volume per reaction (µL) (For final vol. of 26 µL)
Toxo Ready Mix/CMV Ready Mix/ HSV Ready Mix	15.0
IC-B Mix (if not added at extraction step)	1.0
Extracted DNA/ ToCHQS1- ToCHQS4/ MBGPW	10.0

Quick Reference Guide

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 HEX/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).

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

The values for unknown samples would appear in the result column in *copies/µL* or *IU/µL* in FAM Channel. Samples showing no amplification in FAM channel should show amplification in IC channel, and then only results should be considered. The negative control should not show any value in the result column.

Interpretation

Interpret the values for unknown samples, only if the Slope of Standards is between -3.1 to -3.6 and PCR efficiency is between 90%-110% (0.9 - 1.1) and there should be no amplification in negative control.

Result and Interpretation

S.No	FAM (TOXO/CMV/HSV)	HEX/VIC (IC)	Interpretation	Conclusion
1	√	√	DNA within the Quantitation range	Proceed for further Analysis
2	√	-		
3	-	√		
4	-	-	Possible inhibition of PCR	Make sure reaction setup and PCR conditions are followed correctly Recheck quality and quantity of DNA

Note: All the Target channels (**FAM**, **HEX/VIC**) to be analyzed individually as described below

Channels to be analyzed		Parameters
(FAM)	(HEX/VIC)	
√		Analyze for Toxoplasma
√		Analyze for Cytomegalovirus
√		Analyze for Herpes simplex virus
	√	Analyze for IC-B

Assay Characteristics

Analytical Sensitivity of qPCR assay for Toxoplasma is 50 copies/mL

Analytical Sensitivity of qPCR assay for CMV is 50 IU/mL and conversion factor for CMV is 1 IU/µL = 2.60 copies/ µL

Analytical Sensitivity of qPCR assay is 1 copy/µL and 50 copies/mL when above mentioned DNA extraction kits are used

Specificity

The analytical specificity of the Quantiplus® ToCH Real Time Quantitative PCR Kit for Toxoplasma, CMV, HSV 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
Sulthanpur villiage, Ameenpur Mandal,
Sangareddy Dist, TS-502319