ParoReal® Kit *Acanthamoeba* T4

Kit version 1.1

For *in vitro* diagnostic use only

ParoReal® Kit *Acanthamoeba* T4			
Order no.	**Reactions**	**Pathogen**	**Internal positive control**
DHUP00153	50	FAM channel	Cy5 channel

Kit contents:

- Assay for detection of *Acanthamoeba* genotype T4 and of internal DNA positive control (IPC)
- IPC-Target DNA (control of DNA extraction and of PCR amplification)
- DNA reaction mix (contains a highly purified Taq Polymerase for rapid hot-start PCR, dNTPs with dUTP and Uracil-N glycosylase (UNG) to eliminate amplicon carryover, ROX™ dye (passive reference) and buffer components – additives optimized to handle PCR inhibitors)
- DNA positive control for *Acanthamoeba*
- Nuclease-free water

Pathogen information: The genus *Acanthamoeba* is primarily a free-living protozoan in natural habitats, but also causative agent of human and animal disease. *Acanthamoeba* serves as host for a variety of pathogenic bacteria such as *Mycobacterium avium*. Infestation with *Acanthamoeba* is associated with potentially sight-threatening contact lens-related keratitis, serious infections of other organs and fatal granulomatous amoebic encephalitis. More than 20 species of *Acanthamoeba* are known, which can be classified into three morphologic groups (Group I, II and III) and 22 genotypes (T1-T22). Some species ((*A. castellanii* (T4), *A. lugdunensis* (T4), *A. polyphaga* (T4), *A. rhysodes* (T4), *A. quina* (T4), *A. palestinensis* (T2), *A. griffinii* (T3), *A. lenticulata* (T5), *A. astronyxis* (T7), *A. culbertsoni* (T10), *A. hatchetti* (T11), *A. healyii* (T12), *A. byersi* (T18), *A. divionensis*) have been recently associated with human disease. Genotype T4 has been considered the most important genotype in both ocular and CNS infections. According to literature, T4 genotype is the most prevalent *Acanthamoeba* genotype causing keratitis worldwide (approx. 86%) (Diehl et al., 2021).

Intended purpose: ParoReal® Kit *Acanthamoeba* T4 is a non-automated CE-certified IVD real-time PCR test for the qualitative detection of DNA (18S rRNA gene) of *Acanthamoeba* species of genotype T4 *(A. castellani, A. lugdunensis, A. mauritaniensis, A. polyphaga, A. rhysodes, A. royreba).* T4 genotype is the most prevalent (approx. 86%) *Acanthamoeba* genotype causing keratitis worldwide. The test does not detect other *Acanthamoeba* genotypes (T3, T15, T11, and T5) also causing keratitis.
Proper specimens are DNA extracts isolated from human clinical specimens associated with keratitis (ocular swabs, corneal biopsies, ocular punctates, corneal scrapings) as well as contact lenses and contact lens solution.
This test is suitable for patients of all ages with suspected infection with *Acanthamoeba* genotype T4 (causative agent of *Acanthamoeba* keratitis, AK) and is intended as an aid in the diagnosis of infection with this pathogen in combination with patient history and additional clinical information.
The test is intended for professional use and is limited to qualified personnel instructed in the procedures of real-time PCR and *in vitro* diagnostic procedures.
A probe-specific amplification-curve in the FAM channel indicates the amplification of *Acanthamoeba* specific DNA. An internal DNA positive control (IPC) is detected in Cy5 and serves as a control for DNA extraction and possible real-time PCR inhibition. The target for the DNA IPC (artificial target DNA) is added during sample extraction.

PCR-platforms: This test has been validated with the ABI® 7500 Fast instrument (fast cycle parameters are not supported, Thermo Fisher Scientific) and was also tested with a LightCycler® 480 II (Roche Diagnostics), QuantStudio™ 7 real-time PCR system (Thermo Fisher Scientific), Mic instrument (bio molecular systems), CFX Opus Real-time PCR System (Bio-Rad), CFX Dx (Bio-Rad) and T-COR 8 (Tetracore).
It is also compatible with other real-time PCR instruments which detect and differentiate fluorescence in FAM and Cy5 channel (e.g., QuantStudio™ 7 real-time PCR system (Thermo Fisher Scientific), qTOWER^{3}G (Analytik Jena), cobas z 480 Analyzer (Roche)).

Performance data: The LoD95% (smallest number of copies of target DNA which can be detected in 95% of cases) is 0.5 genome copies/reaction. The 18S rRNA gene is a multicopy gene and is present up to 22 times in the haploid genome of *Acanthamoeba*.This kit is specific for 18S rDNA gene of *Acanthamoeba* species of genotype T4 *(A. castellani, A. lugdunensis, A. mauritaniensis, A. polyphaga, A. rhysodes, A. royreba).* Clinical validation was performed with 168 clinical samples (Table 1).

Table 1 Results of clinical validation

	Value	95% CI
Sensitivity	88.06%	77.82% to 94.70%
Specificity	100.00%	96.41% to 100.00%
NPV	92.66%	86.82% to 96.03%
PPV	100.00%	
Prevalence	39.88%	32.42% to 47.71%

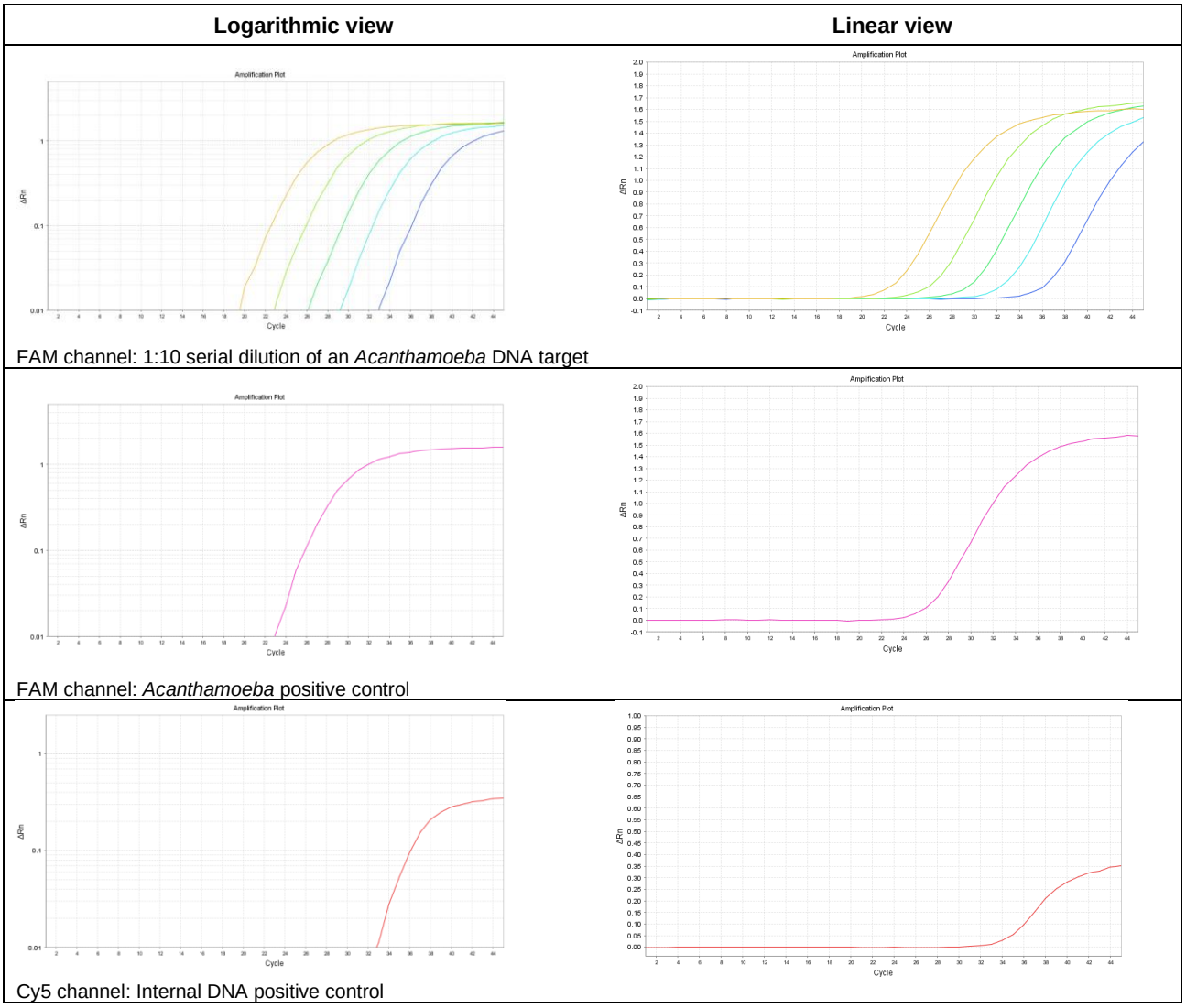

Figure 1 Performance of ParoReal® Kit *Acanthamoeba* T4