RealHelix™ Pathogenic Amoeba Detection kit

Kit Contents

RealHelix™ *Pathogenic Amoeba* Detection kit	
Cat. No.	**AMQP50 (50 rxns)**
2x qPCR premix	0.5 ml
4x Oligo mix	0.25 ml
Positive control 1 (10^6 copies/5 ㎕)	0.5 ml
Positive control 2 (10^6 copies/5 ㎕)	0.5 ml
Negative control	0.5 ml
Instruction for Use	1ea

Positive control	Target	Fluorescent dye
Positive control 1	*Naegleria fowleri*	FAM
Positive control 2	*Acanthamoeba spp.*	HEX

Description

Naegleria fowleri (commonly referred to as the "brain-eating amoeba" or "brain-eating ameba"), is a free-living microscopic ameba, (single-celled living organism). It can cause a rare and devastating infection of the brain called primary amebic meningoencephalitis (PAM). *Naegleria fowleri* usually infects people when contaminated water enters the body through the nose. Once the ameba enters the nose, it travels to the brain where it causes PAM, which is usually fatal.

Acanthamoeba is a microscopic, free-living ameba, or amoeba (single-celled living organism), that can cause rare, but severe infections of the eye, skin, and central nervous system. The *Acanthamoeba* can be spread to the eyes through contact lens use, cuts, or skin wounds or by being inhaled into the lungs.

NanoHelix Co., Ltd.
43-15, Techno 5-ro, Yuseong-Gu, Daejeon, 34014, South Korea. TEL : 82-42-867-9055, FAX : 82-42-867-9057
E-mail : info@nanohelix.net < **www.nanohelix.net** **www.nanohelix.co.kr/KOR** >

Pathogenic Amoeba Detection kit

RealHelix™ *Pathogenic Amoeba* Detection kit is a Taqman probe-based real-time PCR assay for the simultaneous detection of *Naegleria fowleri* and *Acanthamoeba spp.* in clinical samples. 2X qPCR premix contains hot-start PCR enzyme, dNTPs, buffers, Mg^{2+} and stabilizing agent. The hot-start PCR enzymes provides high specific amplification of target DNA and minimizes the side products such as primer dimers. Based on the Taqman® probe detection principle, the 5′-reporter dye and 3′-quencher dual-labelled oligonucleotide (Taqman® probe) hybridizes on a specific region within the amplified fragment. Target pathogen amplification is detected using FAM and HEX channel.

Quality control assay data

Functional analysis

RealHelix™ *Pathogenic Amoeba* Detection kit was evaluated by real-time PCR using the 10-fold serial-diluted positive control DNA and a set of target-specific primer with MIC real-time PCR (Bio molecular systems, Australia), Rotor-Gene Q instrument (QIAGEN, Germany), and Bio-Rad CFX96 (Bio-Rad, USA).

Quality authorized by Yountaek Go

Application

- Detection of target DNA sample by real-time PCR

Storage and Handling

The components of the RealHelix™ Pathogenic Amoeba Detection kit should be stored at -20℃. Repeated thawing and freezing should be avoided, as this may reduce the sensitivity.

Additional required materials

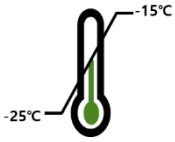
- Pipettes
- Disposable gloves
- Sterile pipette tip
- Microcentrifuge tubes

Instrument compatibility

- MIC real-time PCR (Bio molecular systems)
- CFX96 Real-Time PCR Detection System (Bio-Rad)
- Rotor-Gene Q (QIAGEN)

NanoHelix Co., Ltd.
43-15, Techno 5-ro, Yuseong-Gu, Daejeon, 34014, South Korea. TEL : 82-42-867-9055, FAX : 82-42-867-9057
E-mail : info@nanohelix.net < **www.nanohelix.net** **www.nanohelix.co.kr/KOR** >

Storage



-20±5℃

Shelf life

6 months

※ 4x Oligo mix should be stored in the dark.

Protocol

1) Prepare a reaction mixture according to the table below.

Components	Volume (㎕)
Template DNA	1~5
2X qPCR Premix	10
4X oligo mix	5
DNase free water	Up to 20 ㎕

※ Fluorescent dye

Target	Fluorescent dye	Quencher
Naegleria fowleri	FAM	BHQ1
Acanthamoeba spp.	HEX	BHQ1

2) For the positive control PCR, use 5 ㎕ of the PC instead of Template DNA

3) For the negative control PCR, use 5 ㎕ of the DNase free water instead of template DNA.

4) Add each component, mix gently and collect by brief centrifugation.

5) Standard DNA

Prepare a 10-fold serial dilution of the positive control (10^6 copies/5㎕).

(10^5-10^4-10^3 copies/5㎕)

6) Start the PCR reaction using following program

Step	DNA Denaturation	45 cycles	
		Denaturation	Annealing/Extension
Temperature	95℃	95℃	60℃
Time	15 min	15 sec	45 sec **(plate read)**

NanoHelix Co., Ltd.

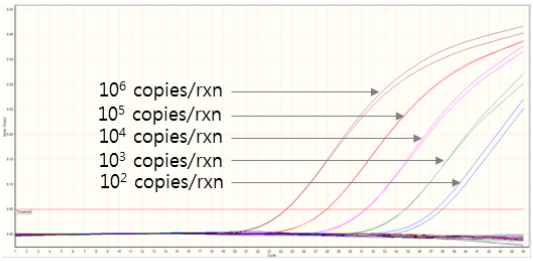
43-15, Techno 5-ro, Yuseong-Gu, Daejeon, 34014, South Korea. TEL : 82-42-867-9055, FAX : 82-42-867-9057

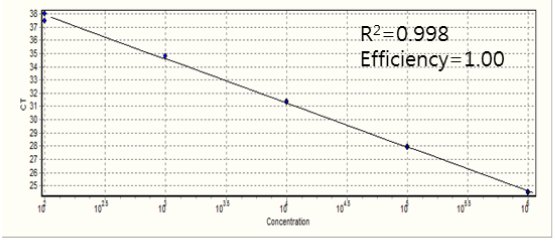
E-mail : info@nanohelix.net < **www.nanohelix.net** **www.nanohelix.co.kr/KOR** >

Experimental Data

RealHelix™ Pathogenic Amoeba Detection kit was evaluated by real-time PCR using the 10-fold serial-diluted positive control DNA and a set of pathogen specific primer with CFX96 Real-Time PCR Detection System (Bio-Rad, USA).

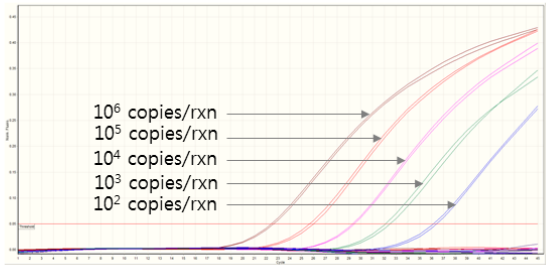
Positive control 1
(*Naegleria fowleri* target, FAM channel)

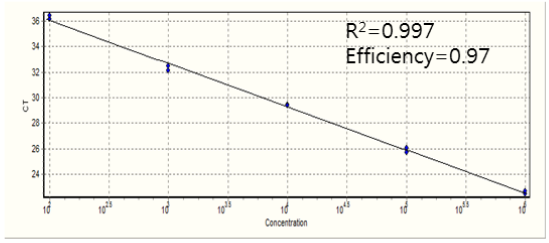




	copies/rxn							
	1.E+06	1.E+05	1.E+04	1.E+03	1.E+02	1.E+01	1.E+00	NTC
Ct (1)	24.47	27.99	31.40	34.78	37.42	N/A	N/A	N/A
Ct (2)	24.51	27.92	31.32	34.77	38.00	N/A	N/A	N/A
average	24.49	27.96	31.36	34.78	37.71			

Positive control 2
(*Acanthameoba spp.* target, HEX channel)





	copies/rxn							
	1.E+06	1.E+05	1.E+04	1.E+03	1.E+02	1.E+01	1.E+00	NTC
Ct (1)	22.44	26.03	29.47	32.10	36.15	N/A	N/A	N/A
Ct (2)	22.63	25.69	29.33	32.51	36.43	N/A	N/A	N/A
average	22.54	25.86	29.40	32.31	36.29			

Results

	Quantification cycle	Determination
Positive control	Ct 22-40	positive
Specimen	Ct <40	positive

Products

Cat. No.	Products	Size
AMQP50	RealHelix™ *Pathogenic Amoeba* Detection kit	50 rxns

NanoHelix Co., Ltd.
43-15, Techno 5-ro, Yuseong-Gu, Daejeon, 34014, South Korea. TEL : 82-42-867-9055, FAX : 82-42-867-9057
E-mail : info@nanohelix.net < **www.nanohelix.net** **www.nanohelix.co.kr/KOR** >