SecureDx N. fowleri Real-time PCR Kit

Product Number	**Kit Size**
SDP-NFO-100T	100 reactions

This instruction must be read carefully prior to use.
Reliability of assay results cannot be guaranteed if there is any deviation from the instructions.

1. INTENDED USE

N. fowleri (Naegleria fowleri) is a free-living amoeba commonly found in warm freshwater environments.
The SecureDx N. fowleri qPCR Kit is a molecular diagnostic reagent intended for the qualitative detection of Naegleria fowleri DNA extracted from warm freshwater and recreational water samples using a real-time PCR assay.

2. PRINCIPLE OF TESTING PROCEDURE

The principle of test is based on TaqMan® Probe Real-Time qPCR. After open the Kit box, resuspend the 2x qPCR Master mixture (1.0 mL), 4x Oligo mixture (500 μℓ), Positive control (100 μℓ), and Nuclease-free water for Negative Control (100 μℓ). Avoid generating air bubbles. Then aliquot the 2x Master mixture (10 μℓ), 4x Oligo mixture (5 μℓ), and DNA template or PC (5 μℓ) to the reaction volume of 20 μℓ into the PCR tube or plate for the chosen PCR platform. Aliquot into wells according to the number of samples to be tested, include one well for the positive control (PC) and one well for the nuclease-free water for negative control (NC). All preparation reaction mixture transfer to the Sample Processing Area. Add 5 μℓ DNA temple of the following into the appropriate wells according to plate setup with the Sample(s), Positive Control and Negative Control. After adding the samples, positive control, and negative control cover the lid immediately. Spin down briefly using a centrifuge to remove air bubbles. Transfer the mixture to amplification area. Place the tubes on the sample holder in the instrument. Setup the test panel according to the positions of the DNA samples, positive control, and negative control. Select the detection channels as following: Select FAM (*N. fowleri*), and Cy5 (Exogenous Internal Positive Control, IPC). The Product “SecureDx N. fowleri Real-time PCR Kit” is based on non-ROX option.

3. COMPONENTS OF KIT

The Product “SecureDx N. fowleri Real-time PCR Kit” is packaging for 100 tests/kit.
The insertion of the components is below

☞ 2x Master mixture (Cap label: 2x Master): 1 vial contains 1.0 mL
☞ 4x Oligo mixture (Cap label: OM): 1 vial contains 500 μℓ
☞ Positive control (Cap label: PC): 1 vial contains 100 μℓ
☞ Negative control (Cap label: NC): 1 vial contains 100 μℓ

4. REAGENT STORAGE, SHELF LIFE, AND HANDLING

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (> 10x) should be avoided, as this may reduce the sensitivity of the assay.
- All reagents should be handled on ice during preparation of mixture.
- Do not repeatedly freeze and thaw more than 10 times and avoid light when store or using the kit

5. ADDITIONALLY REQUIRED MATERIALS AND DEVICES

- Biological cabinet
- Real time PCR system
- DNA extraction kit
- Real time PCR reaction tubes/plates
- Cryo-container
- Pipettes (0.5$\mu\ell$ - 1000$\mu\ell$)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and freezer
- Tube racks
- Vortex mixer
- Desktop microcentrifuge for “Eppendorf” type tubes

6. WARNINGS AND PRECAUTION

Carefully read this instruction before starting the procedure.

- For research use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas:
  i) Isolation of the DNA and
  ii) Amplification / detection of amplification products.
- Pipettes, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Do not pipette by mouth. Do not eat, drink, smoke in laboratory.
- Avoid aerosols

7. LIMITATIONS

- It must be kept at the storage temperature until expiry date. (Storage temperature -25±5°C, expiry date 12 month after manufacturing, 30 days after opening)
- It should be kept away from light.
- Use on ice during the test.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay is not to be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors may cause false negative or invalid results.
- As with any diagnostic test, results of the N. fowleri should be interpreted in consideration of all clinical and laboratory findings.

8. SAMPLE COLLECTION, STORAGE, AND TRANSPORT

- Collected 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

9. PROCEDURE

9.1. Nucleic acid extraction

Different brand nucleic acid extraction kits are available. You may use your own extraction systems or the commercial kit based on the yield. For the viral nucleic acid extraction, please comply with the manufacturer's instructions. The recommended Extraction kit is as follows:

Nucleic acid extraction kit	Cat. No.	Manufacturer
SECURE-Prep Bacterial Genomic DNA Extraction Kit	10027	SecureDx
SECURE -Prep Tissue Genomic DNA Extraction Kit	10023T	SecureDx
SECURE -Prep Soil Genomic DNA Extraction Kit	10056	SecureDx
SECURE -Prep Total Nucleic acid Extraction Kit	10030	SecureDx

9.2. Reaction mixture and PCR conditions

Reaction mixture		PCR condition		
Components	Volume	Temp.	Time	Repeat Cycles
2x Master Mixture (MM)	10 μℓ	95°C	10 min	1
4X Oligo Mixture (4X OM)	5 μℓ	95°C	15 sec.	40
DNA Template (or PC, or NC)	5 μℓ	62°C	45 sec*	
Total reaction volume	20 μℓ	* Plate reading step		

9.3. Result interpretation

The results interpretation of investigate the amplification curve of the option with non-ROX channel.

If Ct ≤ 36, it indicates that the detection is valid, and users can continue the subsequent analysis:

If a typical S-type amplification curve is detected by the FAM (*N. fowleri*) channel, with Ct ≤ 36, it indicates that *N. fowleri* is positive.

If a typical S-type amplification curve of the Exogenous Internal Positive Control (IPC) is detected failed or Ct ≥ 31 by the Cy5 channel, it indicates that there is an inhibitory reaction from the interfering substances. Users have to repeat the experiment. For every reaction of positive samples and negative sample, IPC will be amplified. If the IPC is not amplifying, the test result of the sample is invalid. The cause should be found and eliminated. Users should redo sampling and repeat the experiment. (If the retest result is still invalid, please contact the manufacturer.)

10. RESULT ANALYSIS

All the results are based on Ct values that automatically calculated by software.

10.1. Fluorophore and cut-off value

Target	Fluorophore	Cut-off of Ct value
N. fowleri	FAM	≤36
IPC	Cy5	≤30

* Refer to the appropriate threshold line for each instrument

10.2. Interpretation of sample results

N. fowleri (FAM)	IPC (Cy5)	Result
+	+	Valid, N. fowleri detection
-	+	Valid, N. fowleri not detection
-/+	-	Invalid, re-test

10.3. Results trouble shooting

Problems	Probable cause	Recommendation
Cannot see any signal in all channel including positive control	Wrong operation of instrument	Please check Real-time PCR condition and run the assay under correct setting
	Incorrect preparation of mixture	Please check all components and repeat assay
	Not available storage condition	Repeat the assay using fresh reagents
False positive at the negative control	Carry-over contamination	Discard all the components of assay. Repeat the assay using new components
Not acceptable positive control	Degradation of positive control	Aliquot when thaw positive control. Avoid repeated freezing and thawing
	Incorrect preparation	Please confirm the protocol and repeat assay.
No appearance or high Ct value of IPC	High concentration of sample	Retest after diluting the DNA using nuclease free water

10.4. Test instruments

Equipment type	Equipment
Rotor type	Rotor-Gene Q or equivalent
Plate type	CFX96 Touch, CFX96 Opus, ABI 7500 standard, ABI 7500fast, QuantStudio 5 or equivalent

11. PERFORMANCE EVALUATION

11.1. Limit of Detection (Analytical Sensitivity)

LOD is determined as copy number of diluents that showed 100% detection up to 20 copies of *N. fowleri.*

11.2. Analytical Specificity with interfering substances

The SecureDx N. fowleri Real-time PCR Kit was affected by the interfering substance using a standard substance as the minimum detection limit concentration. One lot was used, and 3 repetitions were performed on one machine. As a result, it was confirmed that there was no effect on the test results.

11.3. Cross-reactivity

The cross-reaction test results are performing with 11 organisms. It was confirmed that positive samples are detected and where negative samples not detected. The internal positive control (IPC), which can confirm the inhibition of the PCR reaction, was detected in all reaction solutions that remarked effectiveness of the test could be confirmed. The test was confirmed 3 times repeated using one lot in an equipment.

No.	Specimens
1	*Naegleria gruberi*
2	*Naegleria australiensis*
3	*Naegleria italica*
4	*Naegleria lovaniensis*
5	*Acanthamoeba castellanii*
6	*Acanthamoeba polyphaga*
7	*Balamuthia mandrillaris*
8	*Vermamoeba (Hartmannella) vermiformis*
9	*Pseudomonas aeruginosa*
10	*Legionella pneumophila*
11	*Escherichia coli*

11.4. Test of Repeatability

Repeatability test with the SecureDx N. fowleri Real-time PCR Kit, the concentration of each sample is 3 repeats (3X LoD, 1X LoD, 0.5X LoD), and two expert researchers used one lot with same experiment in twice per experiment (am/pm) for 20 days. As a result of the experiment, 100% of all samples were detected in moderate positive (3X LoD) and 100% in low positive (1X LoD). At the below concentrations the minimum detection limit (0.5X LoD), 54% of the N. fowleri were successfully detected. As a result, the repeatability of the SecureDx N. fowleri Real-time PCR Kit was confirmed within 5% of CV.

11.5. Test of Reproducibility

As a result of the reproducibility test experiment, 100% of all samples were detected in sensible positive (3X LoD),

and 100% was detected in near to the ground positive (1X LoD). Consequence of the results, 0.5X LoD was detected 53.6% of the N. fowleri, respectively. The reproducibility of the SecureDx N. fowleri Real-time PCR Kit was confirmed within 5% of CV.

12. QUALITY CONTROL

• In compliance with ISO 13485-Certified Quality Management System, each lot of Real-time PCR Kit has been tested against predetermined specifications to ensure consistent product quality.

• Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures.

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