

Dengue Fever Multiplex (Serotype 1-4)

Cat. No. C02-01-1121

One-step real-time reverse transcription PCR for detection of Dengue Fever Multiplex for Serotype 1-4

Includes main components for 50 reactions



Rev. 2

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HAI KANG LIFE CORPORATION LIMITED

PLEASE READ THROUGH THE ENTIRE PROTOCOL BEFORE STARTING.

1. KIT COMPONENTS

Amplification Reagents

- 2 x 500 µl Dengue Fever Mastermix (store at -20°C)
- 2 x 25 µl Positive Control (store at -20°C)
- 1 x 25 µl Taq Polymerase (store at -20°C)

Storage Conditions

Store Mastermix, Positive Control and Taq polymerase at -20°C. Thaw frozen reagents just before use. Mix reagents thoroughly (do not vortex Mastermix containing enzyme).

Note:

Please aliquot the Mastermix into appropriate volume according to your test frequency, in order to minimize repeated freeze and thaw cycles. Frequent thawing and freezing may inactivate some kit components.

2. PROCEDURES

Set-up

- In addition to the RNA obtained from the test samples, each experiment requires a positive and negative (water) control.
- Set up real-time PCR components according to the table below:

| Components | Volume per reaction |
|----------------|---------------------|
| Mastermix | 19.5 µl |
| Taq Polymerase | 0.5 µl |
| Sample RNA | 5 µl |
| Total Volume | 25 µl |

Note:

- Use real time PCR tubes and consumable recommended for your real-time PCR equipment.
- Keep RNA samples on ice throughout experiment.
- 5 µl of provided Positive Control can be used to monitor the success of amplification.
- It is advisable to run samples in duplicate to ensure reliability of results.
- Spiking Control: 1 µl of Positive Control can be spiked into test sample to check whether the test sample contains PCR inhibitory substances.

Cycling conditions:

| Data Collection Points | | | |
|------------------------|------|------------|---|
| 1 Cycle | 45°C | 30 Minutes | - |
| | 95°C | 10 Minutes | - |
| 5 Cycles | 95°C | 10 Seconds | - |
| | 45°C | 30 Seconds | - |
| | 72°C | 1 Minute | - |
| 40 Cycles | 95°C | 15 Seconds | - |
| | 58°C | 1 Minute | □ |

(Take readings at point □)

Filter settings:

- Serotype 1: FAM
- Serotype 2: JOE
- Serotype 3: NED
- Serotype 4: Cy5

3. DATA ANALYSIS AND INTERPRETATION

Detailed explanations of the basic and advanced operating procedures should be provided with your real-time PCR equipment. This kit is optimized using the Applied Biosystems (ABI) 7500 Real-Time PCR System.

Spiking Control

Negative real-time PCR result may be due to a few scenarios: 1. absence of detected sequence in the sample; 2. presence of detected sequence below limit of detection; 3. presence of PCR inhibitory substances. The purpose of spiking control is to verify whether the test sample contains substances, which may affect PCR reactions. If Ct equals 40 (or similar to Ct given by negative control) when the test sample is spiked with the positive control, the test sample is highly likely to contain PCR inhibitory substances, and the result should NOT be taken as negative. Repeated extraction and real-time PCR of the sample will be required.

If you require more detailed analysis information, please contact Hai Kang Life Corporation Limited for technical assistance.

4. TECHNICAL ASSISTANCE

Our technical staff will provide technical assistance you may need in using this kit. Simply call +(852) 2111 2123 during office hours:

Monday – Friday: 9:00am to 5:30pm
Saturday: 9:00am to 1:00pm

A recorded message (in English, Cantonese or Putonghua) may be left outside office hours.

Alternatively, you may contact our technical staff by fax or email.

Fax: +(852) 2111 9762

Email: technical@haikanglife.com

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