

Dengue Fever (Serotype 3)

Cat. No. C02-01-1119

One-step real-time reverse transcription PCR for detection of Dengue Fever Virus (serotype 3)

Includes main components for 50 reactions



Rev. 0
May 2005
Hong Kong DNA Chips Ltd

1. KIT COMPONENTS

PLEASE READ THROUGH THE ENTIRE PROTOCOL BEFORE STARTING.

The kit contains reagents for a total of 50 reactions:

Amplification reagents

- 25 Tubes Reagent Spheres (Store at 4°C)
- 2 x 550µl Dengue 3 Sphere Diluent (Store at -20°C)
- 1 x 25µl Dengue 3 Positive Control (Store at -20°C)

Note: The real-time PCR reagent mix **DOES NOT CONTAIN ROX** as passive reference.

Storage conditions

Store Reagent Spheres at 4°C with silica gel desiccant. Store Sphere Diluent, Positive Control, and reconstituted sphere at -20°C. Thaw frozen reagents just before use. Mix reagents thoroughly (do not vortex reconstituted sphere containing enzyme).

2. PROCEDURE

Mastermix Preparation

1. Determine required number of reactions (n).
2. Number of Reagent Spheres required = 0.5 x n.
3. Add 40 µl Sphere Diluent for each Reagent Sphere.
4. Allow Reagent Sphere to reconstitute on ice.
5. Gently tap tubes to mix reagents. Do not vortex enzyme containing reagents

Set-up

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

Components	Volume per reaction
Mastermix	20 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 (FAM Filter)		
		ABI 7700	ABI 7300/7500	Other Instruments
1 Cycle	42°C 30 Minutes	☐	-	-
	95°C 10 Minutes	☐	-	-
40 Cycles	95°C 15 Seconds	☐	-	-
	58°C 60 Seconds	☐	☐	☐

(Take readings at point ☐)

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) 7700 and 7300 Sequence Detection Systems. Please contact us if you require assistance in setting your ABI 7700 for data analysis without ROX.

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 Hong Kong DNA Chips 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@dnachip.com.hk

5. WARRANTIES AND LIABILITIES

Hong Kong DNA Chips Limited warrants the products manufactured by it are free of defects in materials and workmanship when used in accordance with the applicable instructions for a period equal or shorter of one year from the date of shipment of the product(s) or the expiration date marked on the product packaging under the storage conditions recommended in the instructions and/or on the package. Application protocols published by Hong Kong DNA Chips Limited are intended to be only guidelines for the buyers of the products. Buyers are expected to validate the kit to their individual application. Hong Kong DNA Chips Limited makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.

The sole obligation of Hong Kong DNA Chips Limited with regard to the foregoing warranties shall be, at its option, to either replace or refund the purchase price of the product(s) or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Hong Kong DNA Chips Limited promptly of any such defect. Hong Kong DNA Chips Limited shall not be liable for any defect, indirect or consequential damages resulting from economic loss or property damages sustained by the buyer or any customer from the user of the product(s).

Notice

The PCR process is covered by US patents owned by F. Hoffman-La Roche, Inc. Use of the PCR process requires a license. Nothing in this publication should be construed as an authorisation or implicit license to practice PCR under any patents held by F. Hoffman-La Roche, Inc.