



Dengue Virus Premix Reagent

User Manual

For Research Use Only

Manufacturer:

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INTENDED USE

POCKIT™ Central Dengue Virus Premix Reagent uses insulated isothermal polymerase chain reaction (iiPCR) technology (Chang *et al.*, 2012; Tsai *et al.*, 2012; Go *et al.*, 2016; Tsai *et al.*, 2018; Tsai *et al.*, 2019) to detect the specific nucleic acid sequence of dengue virus. This reagent has to be used with an iiPCR-compatible instrument, **POCKIT™ Central** Nucleic Acid Analyzer (**POCKIT™ Central**) and **POCKIT™ Central** Cartridge Set. This reagent is intended for research and *in vitro* use only.

SUMMARY AND EXPLANATION

Dengue virus (DENV), a single-stranded RNA positive-strand virus, belonging to the genus *Flavivirus* of family *Flaviviridae*, is the causative agent of classical dengue fever (DF) and the more severe dengue hemorrhage fever (DHF) and dengue shock syndrome (DSS). DENVs are divided into 4 serotypes (DENV-1, -2, -3, -4). DENV infection ranks as the most important mosquito-borne viral disease in the world. The dengue virus is typically transmitted to humans via DENV-infected mosquitos (*Aedes aegypti* and *A. albopictus*) (Rothman, 2010). The symptoms of DF may include fever, headache, muscle and joint pains, and a characteristic skin rash similar to measles. In some cases, some patients may develop into DHF and/or DSS, leading to organ damage, severe bleeding, dehydration and even death (Halstead, 2009; Gubler & Vasudevan *et al.*, 2014). The disease has significant impacts, especially for both human public health and the national economies.

PRINCIPLES OF THE PROCEDURE

The assay is based on iiPCR for qualitative detection of dengue virus. Fluorogenic probe hydrolysis chemistry is used to generate fluorescent signal when a specific sequence of dengue virus is amplified. The primers and probe target the 3' UTR of dengue virus, and do not cross-react with nucleic acids from host and other pathogens.

ANALYTICAL SENSITIVITY AND SPECIFICITY*

The detection limit of **POCKIT™ Central Dengue Virus Premix Reagent** is about 10 copies/reaction. This reagent can detect DENV-1, DENV-2, DENV-3 and DENV-4. This reagent does not detect other pathogens, such as West Nile virus, chikungunya virus, Zika virus, Japanese encephalitis virus, hepatitis C virus, influenza virus A, influenza virus B, human coronavirus, enterovirus 71, coxsackievirus A16, herpes simplex virus 1, herpes simplex virus 2, cytomegalovirus, and hepatitis B virus (Tsai *et al.*, 2018).

* Based on verification results and/or *in silico* analysis.

PRODUCT DESCRIPTION

A. Materials Provided

POCKIT™ Central Dengue Virus Premix Reagent, 24 tests/box

Component	Contents or Purpose	Amount
Premix Pack	■ Premix (lyophilized pellet) containing dNTPs, primers, probe, and enzyme for amplification. ■ Desiccating agent pack.	3 bags (8 Premix vials and 1 desiccating agent/bag)
Sticker	For labeling used.	1 piece
User Manual		1 copy

B. Materials and Equipment Required, but Not Provided

- POCKIT™ Central Nucleic Acid Analyzer
- POCKIT™ Central Cartridge Set (B) (including Extraction Cartridge and Transfer Cartridge)
- POCKIT™ Central P(+) Control Reagent
- Micropipette and filter tips
- 1.5 ml microcentrifuge tube

C. Storage and Stability

- 1) The reagent set should be stored in 2-8°C and is stable until the expiration date stated on the label.
- 2) Store Premix vials in sealed Premix Pack to avoid hydration of lyophilized components.

D. Shipping Condition

The reagent set should be shipped at room temperature.

WARNINGS AND PRECAUTIONS

- A. Check the reagent upon receiving. If any item is missing or damaged, please contact your local distributor or GeneReach immediately for replacement. Do not use any damaged items as they may lead to poor performance of the test or harms to the user.
- B. Protect reagents against heat and humidity. Prolonged exposure to humidity will affect reagent performance.
- C. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.
- D. Users should be trained with basic laboratory skill before performing the tests.
- E. Operation temperature: 15-35°C.
- F. Do not use the reagents after the expiration date.
- G. Do not open R-tube(s) after reaction to prevent any carryover contamination.
- H. Do not reuse consumables.
- I. Waste disposal should follow local regulation.
- J. Any deviation from the recommended procedures may lead to sub-optimal results. Performance of the modified protocol should be validated by the users.

OPERATION PROCEDURE

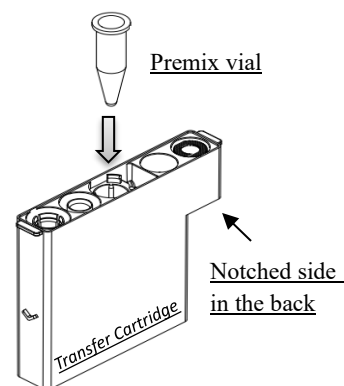
A. Sample Type

Serum.

- **Note: Please note that poor sample quality may influence test results.**
- **Sample pretreatment**
 - **For serum sample:** Add 200 µl sample into an Extraction Cartridge.

B. Transfer Cartridge Preparation

- 1) Prepare one Premix and one Transfer Cartridge for each sample. (Premix vials are in Premix Pack. Transfer Cartridges are in **POCKIT™ Central Cartridge Set**.)
 - **Note: Do not remove the sealing film of the Premix.**
 - **Note: Cut any excessive film and plastics outside the rim to avoid overlapping with other wells in the Transfer Cartridge (Step 4).**
- 2) Label the sample ID and Premix ID on the side of Transfer Cartridge.
- 3) Remove Transfer Cartridge Cap, turn the notched side of the cartridge away from you.
- 4) Snap the Premix vial into well #3 of the Transfer Cartridge.
 - **Note: When the pellet is not found at the bottom of the vial, spin vial briefly to bring it down.**
 - **Note: Check if all accessories are included in the cartridge (Refer to **POCKIT™ Central Cartridge Set manual**).**

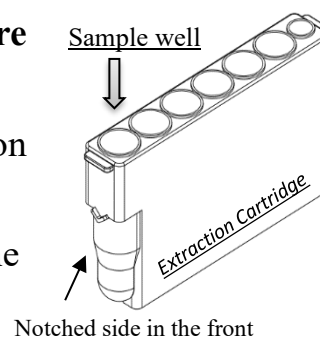


C. Extraction Cartridge Preparation

- 1) Prepare one Extraction Cartridge (B) for each sample. (Extraction Cartridges are in the **POCKIT™ Central Cartridge Set (B).**)
- 2) Remove the Extraction Cartridge from the aluminum pack.

■ **Note: Make sure all the contents are collected at the bottom.**

- 3) Label the sample ID on the side of Extraction Cartridge.
- 4) Slowly peel off the aluminum film, turn the notched side toward you.



Warning: Make sure to remove any residual sealing film from the cartridge surface.

- 5) Load 200 µl homogenized sample (Section A) to the sample well of the Extraction Cartridge.

D. Load and Run

■ **Note: Refer to POCKIT™ Central user manual for programming and setup instructions.**

- 1) Enter Extraction lot number of the Extraction Cartridge (from Section C) using onscreen keyboard. Place the loaded Extraction Cartridge to the selected slot.

■ **Note: Make sure the indicator light of the slot is off after loading the cartridge.**

- 2) Enter Reagent lot number of the Premix Reagent (from Section B) using onscreen keyboard. Place the loaded Transfer Cartridge to the selected slot.

■ **Note: Please make sure channel “520 nm” is selected.**

■ **Note: Make sure the indicator light of the slot is off after loading the cartridge.**

- 3) Press confirm to proceed to **Run Overview**.
- 4) Check if all the information is correct, press **Run** to start the run.

■ **Note:**

1. Please make sure all the consumables are in the corresponding position (Please refer to the user manual of POCKIT™ Central Cartridge Set). If not, please contact your local distributor of GeneReach for assistance.
2. If special disposal handling are required for Steel Tips due to local regulation, soad used Steel Tips with bleach prior to disposal.
3. Used consumables and waste products should be discarded following local regulation once reaction is finished. Cap the Transfer Cartridges prior to disposal.

DATA INTERPRETATION

An example of results shown on the monitor.

Reaction Overview				Operator:SCOTT		Report No:202011241019	
	Sample ID	Extraction Lot No.	Reagent Lot No.	Target	Target Result	IC Result	
A	TEST1	1104206			+		
B	TEST2	1104206			-		
C	TEST3	1104206			?		
D	TEST4	1104206			!		
E	TEST5	1104206			-		
F	TEST6	1104206			-		
G	Inactive	Inactive	Inactive	Inactive	Inact...	Inact...	
H	Inactive	Inactive	Inactive	Inactive	Inact...	Inact...	
UV Time: 14:24				Save to USB		End	

Target Result	Interpretation
+	Positive. Dengue virus specific nucleic acids detected.
—	The nucleic acids of dengue virus are not detected.
?	Repeat reaction with freshly prepared sample.
!	Warning.
*	Abnormal, signals outside detection range.

- **Note: When you see a " ! " or " * ", please check if R-tube is in its designated well in the Transfer Cartridge (Please refer to the user manual of POCKIT™ Central Cartridge Set). Otherwise, contact local distributors or GeneReach for assistance.**

TROUBLESHOOTING

Problems	Possible causes	Solutions
False Positive	1) Reuse of any consumables.	■ Any consumables are for single-use only. Reusing these accessories would cause cross-contamination. ■ Used consumables should be collected and discarded immediately according to local regulation. Do not place the wastes close to the working area to prevent cross-contamination.
	2) Contaminated micropipette.	■ Use filter tips.
	3) Contaminated working area.	■ Consult with a GeneReach technical support representative on how to clean up working area.

REFERENCE

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