

Protocol for Real-Time RT-PCR of Multiplex Dengue Serotyping with P&P

For RUO (Research Use Only)

Caution: The vials containing the primers and probe mix (P&P) should be stored after reception at -20°C in the dark. Stable at least 6 months in the described conditions.

NB: Probe: FAM-QSY, VIC-QSY, Cy5-QSY, ROX-QSY

Single-Reaction, Multiplex, Real-Time RT-PCR for the Detection, Quantitation, and Serotyping of Dengue Viruses. Jesse J. Waggoner, Janaki Abeynayake, Malaya K. Sahoo, Lionel Gresh, Yolanda Tellez, Karla Gonzalez, Gabriela Ballesteros, Anna M. Pierro, Paolo Gaibani, Frances P. Guo, Vittorio Sambri, Angel Balmaseda, Kumudu Karunaratne, Eva Harris, Benjamin A. Pinsky. PLOS Neglected Tropical Diseases | www.plosntds.org April 2013 | Volume 7 | Issue 4 | e2116

Reagents with which the assay has been validated at UVE (Unité des Virus Emergents, Marseille, France):

- SuperScript™ III Platinum™ One-Step qRT-PCR Kit, ThermoFischer ref 11732-088
- Water molecular grade
- Dengue 1, Dengue 2, Dengue 3 and Dengue 4 strains extracted with EZ1 Advanced XL (Qiagen)

1. Kit contents :

Nom	Number of assays/vial
QC_Lyoph-P&P multiplex DenV Serotypes	18+2

Table 1. List of products in the kit

1. Rehydration of Lyoph-P&P before use (Table 1)

- Write the date on the vial before opening. - Lyoph-P&P is resuspended as described:
 - Add 140 µl of Water molecular grade
 - Homogenization by 10 to 20 times pipetting up and down in the glass vial a 70 µL-volume
 - Rehydrated P&P must be incubated at room temperature for 10 min after which
 - A second step of 10 times multiple pipetting must be done.

WARNING: These steps are critical to ensure adequate homogenization

Number of tests/vial	20
H2O (µL)	140

Table 2. Lyoph-P&P regeneration; rehydrated Lyoph-P&P can be stored at 4 °C for up to 7 days

*** this volume is adapted to the SuperScript™ III Platinum™ One-Step qRT-PCR Kit and can vary depending upon the RT-PCR kit**

2. Preparation of the reaction mix

MasterMix	25µL Single rxn, µL
2X Reaction mix*	12,5
Rehydrated Primers and probe P&P**	7
SSIII/Taq Enzyme Mix*	0,5
Total	20
Template RNA	5

*, ThermoFischer / Invitrogen: SuperscriptIII One-Step RT-PCR system with Platinum Taq DNA Polymerase

** , as indicated in Table 2.

3. Cycling program and RT-PCR reaction

1 : 52°C – 15min

2 : 94°C – 2min

3 : 94°C - 15sec

4 : 55°C - 20sec – Plate read

5 : 60°C - 20sec

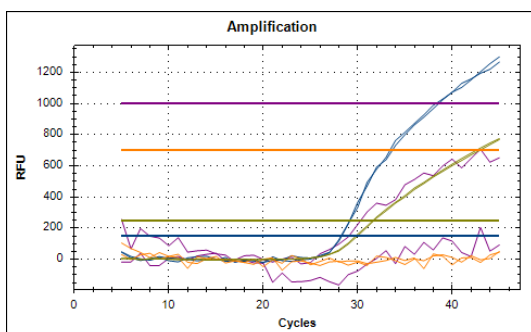
6 : 68°C - 20sec

7 : GOTO3, 45 more time

Dengue 1 strain

Mean Ct : 28.27

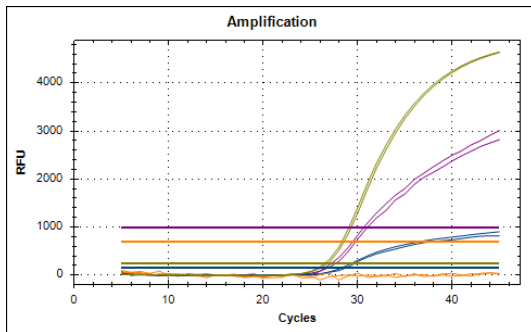
Result obtain with Multiplex Dengue Serotyping assay and 5µL of Dengue 1 strain



Dengue 2 strain

Mean Ct : 26.60

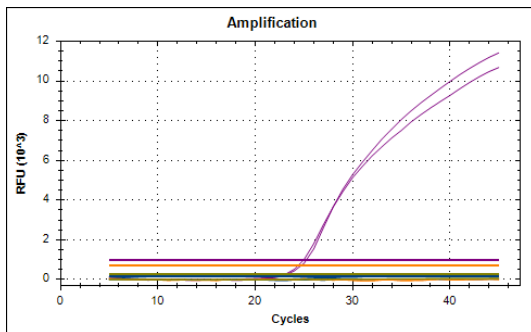
Result obtain with Multiplex Dengue Serotyping assay and 5µL of Dengue 2 strain



Dengue 3 strain

Mean Ct : 30.58

Result obtain with Multiplex Dengue Serotyping assay and 5µL of Dengue 3 strain



Dengue 4 strain

Mean Ct : 30.58

Result obtain with Multiplex Dengue Serotyping assay and 5µL of Dengue 4 strain

