Dengue virus (DENV) universal MGB TaqMan 2017 ("DU5" assay) Version 3

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Abstract

This protocol was designed and developed at this laboratory.

The assay targets the capsid peptide coding region of DENV 1-4 and is desigend as a qualitative screening test for human cases of DENV infection.

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Before start

- If using a different brand or model of real-time thermocycler, check the concentration of ROX is adequate.
- Method assumes the user is familar with the thermocycler and software used to run the protocol.

Materials

SensiFAST™ Probe Lo-ROX Kit BIO-84002 by Bioline

Protocol

Oligonucleotide sequences

Step 1.

Name	Sequence (5'-3')
DU5-F1	GAA**Y**AACCAACG**R**AA**R**AAG**R**CG
DU5-F2	ATGAACCAACG**R**AA**R**AAGGTGG
DU5-R13	GAGAATCTCTTCGCCAACTGTG
DU5-R2	TGAGAATCTCTT**Y**GTCA**R**CTG**Y**TG
DU5-R4	GAGAATCTCTTCACCAACCCTTG
DU5-MGB2017	6FAM – AATATGCTGAAACGCG - MGBNFQ

Reagents

Step 2.

REAGENTS

SuperScript™ III Platinum™ One-Step qRT-PCR Kit 11732088 by Life Technologies

Reaction set-up

Step 3.

The assay has been used on both a Rotor-Gene 6000 and a Rotor-Gene Q real-time thermocycler

Prepare sufficient mix for the number of reactions.

Include a suitable 'dead volume' as necessary if using a robotic dispenser.

MIX PREPARATION

Reagent	Vol. (µl) x1	Final reaction concentration
Nuclease-free water	4.26	N/A
DU5-F1	0.09	900nM
DU5-F2	0.09	900nM
DU5-R13	0.03	300nM
DU5-R2	0.03	300nM
DU5-R4	0.03	300nM
DU5-DU5-F1	0.03	150nM
2X Reaction Mix[1]	10	1X
ROX reference dye (25µM)	0.04	50nM
SuperScript™ III/Platinum™ Taq Mix[1]	0.4	1X
TOTAL	15	N/A

[1]Superscript™III Platinum™ One-step qRT-PCR kit; [2]See Guidelines

Dispense 15µL to each reaction well.

Add 5µL of template (extracted RNA, controls or NTC [nuclease-free water]).

Total reaction volume is 20µL

Amplification

Step 4.

50°C	5min	
95°C	2min	
95°C	3s	40X
60°C	30s*	

*Florescence acquisition step

Result analysis

Step 5.

The definition used for a satisfactory positive result from a real-time fluorogenic PCR should include each of the following:

1. A **sigmoidal curve** – the trace travels horizontally, curves upward, continues in an exponential rise and followed by a curve towards a horizontal plateau phase

2. A **suitable level of fluorescence** intensity as measured in comparison to a positive control (y-axis)

3. A **defined threshold (C_T) value** which the fluorescent curve has clearly exceeded (Fig.1 arrow), which sits early in the log-linear phase and is <40 cycles

A flat or non-sigmoidal curve or a curve that crosses the threshold with a C_T >40 cycles is considered a negative result. NTCs should not produce a curve

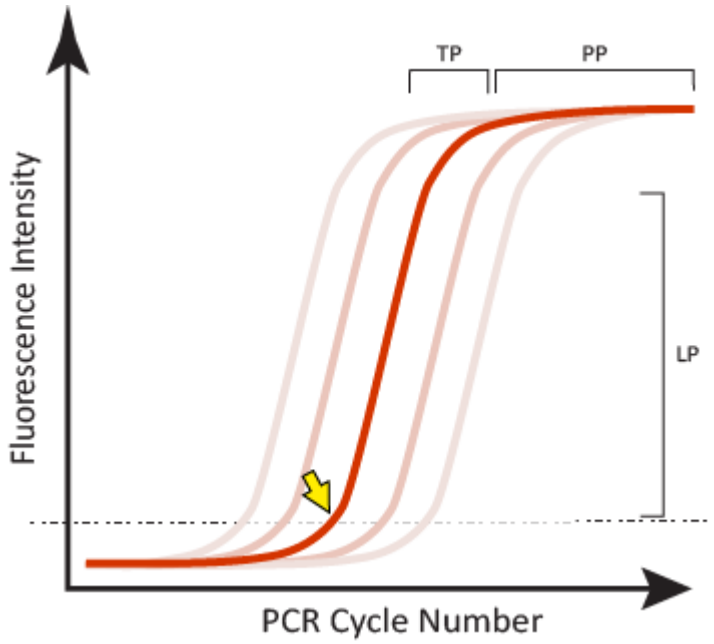

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary) threshold level as shown in this normalized linear scale depiction. LP-log-linear phase of signal generated during the exponential part of the PCR amplification; TP-a slowing of the amplification and accompanying fluorescence signal marks the transition phase; PP-the plateau phase is reached when there is little or no increase in fluorescent signal despite continued cycling.