

Protocol for pan-Dengue virus (DENV) Real-time RT-PCR with Lyoph-P&P DENV all Duo on Panther Fusion (HOLOGIC)

Vial content

Lyoph P&P DENV all Duo contains lyophilized primers and probe (P&P) for detection of pan-dengue virus (DENV), with no serotyping.

Caution

Vials containing primers and probe mix (Lyoph P&P) must be stored after receipt at -20°C in the dark. Stable for at least 6 months under the described conditions.

Design

RT-qPCR systems included in Lyoph P&P DENV all Duo:

- Early diagnosis of dengue in travelers: Comparison of a novel real-time RT-PCR, NS1 antigen detection and serology. Huhtamo et al., J Clin Virol, 2010. [DOI: 10.1016/j.jcv.2009.11.001](https://doi.org/10.1016/j.jcv.2009.11.001)
- Development and validation of real-time one-step reverse transcription-PCR for the detection and typing of dengue viruses. Leparc-Goffart et al., J Clin Virol, 2010. [DOI : 10.1016/j.jcv.2009.02.010](https://doi.org/10.1016/j.jcv.2009.02.010)

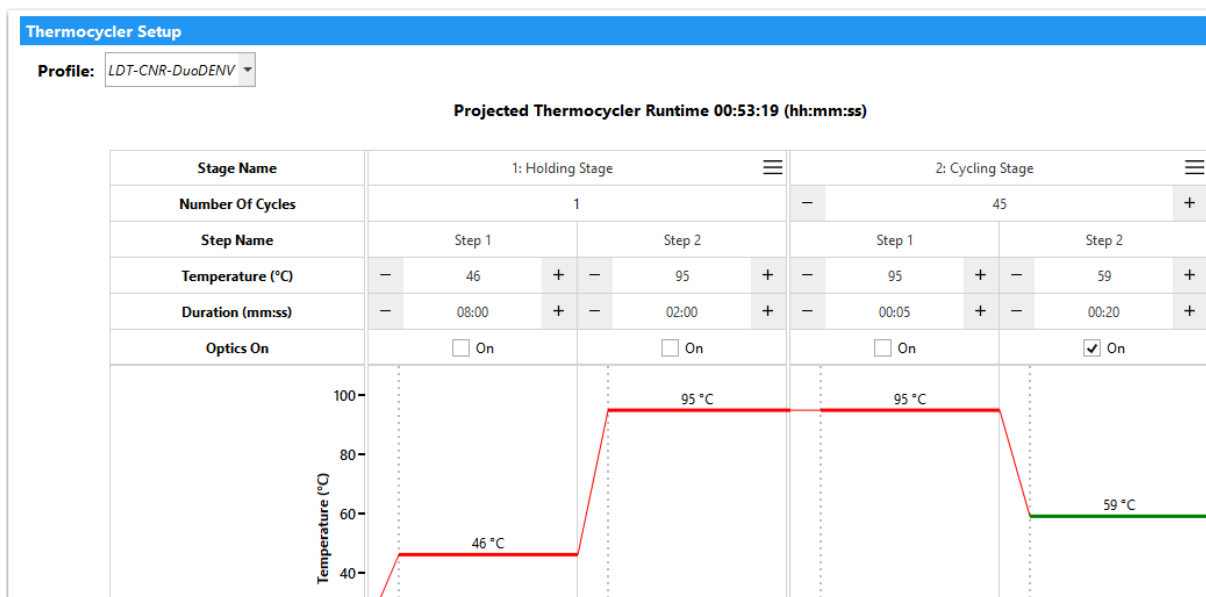
Material

- Lyoph-P&P DENV all Duo
- Potassium Chloride (KCl), ref HOLOGIC PRD-04927
- Magnesium Chloride (MgCl₂), ref HOLOGIC PRD-04926
- Tris buffer, ref HOLOGIC PRD-04935
- Internal control (IC) ARN primers, ref HOLOGIC PRD-04307
- Internal control (IC) ARN probe, ref HOLOGIC PRD-04309
- PPR skirted 2ml tubes, ref HOLOGIC PRD-04311
- Caps for PPR 2ml tubes, ref HOLOGIC PRD-04312
- PPR Aptima Oil, ref HOLOGIC PRD-04304
- Specimen Lysis Tubes, ref HOLOGIC PRD-04339
- Whole Blood diluent tubes, ref HOLOGIC PRD-06783
- Open access cartridges, RNA/DNA enzyme, ref HOLOGIC PRD-04303
- Water

1. Protocol details (myAccess)

On myAccess software, create DENV all Duo protocol:

- Extraction protocol: High (level sensing)-Viral
- Results processing: Qualitative
- Enzyme cartridge: RNA/DNA enzyme
- Reagent kit: FCR-S
- Targets setup: FAM (for DENV all), Quasar 705 (for IC)
- Thermocycler setup:



- Positivity criteria: Ct threshold 1000 (for DENV all), 500 (for IC).

2. Rehydration of Lyoph-P&P before use

- Write the date on the vial before opening.
- Rehydrate Lyoph-P&P as follows:
 - Add water according to Table 1 (10µL/test)
 - Pipet up and down the volume in the glass vial 10 to 20 times to ensure adequate homogenization
 - Incubate rehydrated P&P at room temperature for 10 minutes
- Perform a second pipetting step after incubation.
- Rehydrated Lyoph-P&P can be stored at 4°C for up to 7 days.

Table 1. Lyoph-P&P regeneration according to the vial format

	Number of tests/vial				
	8+1	16+2	24+2	48+3	96+5
H ₂ O (µL)	90	180	260	510	1010

3. Preparation of reaction mix (PPR)

The final volume of the reaction mix (PPR) depends on the format of the PPR tubes (number of tests/PPR tube) used on Panther Fusion (Table 2). The choice of the format of the PPR tubes (from 6rx up to 40rx/PPR tube) depends on the frequency of the tests and the number of samples to test, considering that PPRs are stable on board for 14 days. If a test is used very

sporadically and to test very few samples, it is recommended to prepare PPRs in a small format (6rx, 12rx or 18rx) to avoid having to discard, 14 days later, PPR tubes that are not finished yet.

Table 2. PPR tubes format suitable for Panther Fusion

Number of tests/PPR tube	Reaction mix (PPR) volume/ PPR tube (µL)	Oil volume/PPR tube
40	1200	400
36	1100	400
30	1000	400
24	850	350
18	700	300
12	550	250
6	400	250

Once the PPR tube format has been chosen, prepare PPR as follows (Table 3):

Table 3. Preparation of PPR tubes according to the format (number of tests/PPR tube)

	Volume (µL)						
	1 PPR tube of 6 tests	1 PPR tube of 12 tests	1 PPR tube of 18 tests	1 PPR tube of 24 tests	1 PPR tube of 30 tests	1 PPR tube of 36 tests	1 PPR tube of 40 tests
KCl	25,0	34,4	43,8	53,1	62,5	68,8	75,0
MgCl ₂	1,0	1,4	1,8	2,1	2,5	2,8	3,0
Tris	5,0	6,9	8,8	10,6	12,5	13,8	15,0
Rehydrated Lyoph-P&P	60,0	120,0	180,0	240,0	300,0	360,0	400,0
IC primers	10,0	13,8	17,5	21,3	25,0	27,5	30,0
IC probe	10,0	13,8	17,5	21,3	25,0	27,5	30,0
Water	289,0	359,9	430,8	501,6	572,5	599,8	647,0
Reaction mix (PPR) volume/tube (µL) *	400,0	550,0	700,0	850,0	1000,0	1100,0	1200,0
PPR Oil volume/tube *	250,0	250,0	300,0	350,0	400,0	400,0	400,0

* Depends on the PPR tube format (see Table 2)

It is recommended to prepare a large number of PPR tubes (skirted 2mL tubes), without oil, stored at -20°C, ready-to-use. When necessary, thaw a new aliquot, vortex and then add the PPR oil gently to the surface of the PPR reaction mix. Before loading the PPR tube into Panther Fusion, mark the date on the tube; PPRs are stable on board for 14 days and it's not necessary to unload them from the machine after PCR run.

4. Samples preparation

DENV all Duo protocol has been validated on serum, plasma, whole blood, urine and pharyngeal swab.

- For serum, plasma, urine and pharyngeal swab: add 600µL of sample in a Specimen Lysis Tube (blue tube, containing 710µL of lysis buffer). ATTENTION: do not vortex blue tubes.
- For whole blood: add 600µL of sample in a Whole Blood diluent tube (grey tube, containing 1,5mL of lysis buffer). ATTENTION: grey tubes must be vortexed to ensure homogenization.

Remove the cap from the lysis tube, place the tubes in the rack and load it into the Panther; assign DENV all Duo protocol to the sample.