Primerdesign™ Ltd

Dengue Virus

subtypes 1, 2, 3 and 4 (Multiplex kit)

genesig® advanced kit

100 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Contents

Introduction to Dengue Virus

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are caused by one of four closely related, but antigenically distinct, virus serotypes (Dengue-1, Dengue-2, Dengue-3, and Dengue-4), of the genus Flavivirus. Infection with one of these serotypes provides immunity to only that serotype for life, so persons living in a dengue-endemic area can have more than one dengue infection during their lifetime. DF and DHF are primarily diseases of tropical and sub-tropical areas, and the four different dengue serotypes are maintained in a cycle that involves humans and the Aedes mosquito. However, *Aedes aegypti*, a domestic, day-biting mosquito that prefers to feed on humans, is the most common Aedes species. Infections produce a spectrum of clinical illness ranging from a nonspecific viral syndrome to severe and fatal hemorrhagic disease. Important risk factors for DHF include the strain of the infecting virus, as well as the age, and especially the prior dengue infection history of the patient.

Specificity

The Primerdesign genesig kit for Dengue subtypes genomes is designed for the in vitro differentiation of Dengue subtypes. The kit is designed to have the broadest detection profile possible whilst remaining specific to the individual Dengue subtypes.

The primers and probe sequences in this kit have 100% homology with a broad range of clinically relevant reference sequences based on a comprehensive bioinformatics analysis. They therefore have a very broad quantification profile.

If you require further information, or have a specific question about the detection profile of this kit then please send an email to enquiry@primerdesign.co.uk and our bioinformatics team will answer your question.

Kit contents

- **Dengue subtype specific primer/probe mix (100 reactions BROWN)**
  FAM, VIC, ROX and CY5 labelled (see table below)

Subtype	Fluorophore
Dengue 1	FAM
Dengue 2	VIC
Dengue 3	ROX
Dengue 4	CY5

- **Dengue positive control template, for all 4 subtypes (RED)**
- **Endogenous control primer/probe mix (150 reactions BROWN)**
  FAM labelled
- **Lyophilised oasig™ OneStep Master Mix**
- **Lyophilised oasig™ OneStep Master Mix resuspension buffer (BLUE)**
- **RNase/DNase free water (WHITE)**
  for resuspension of primer/probe mixes
- **Template preparation buffer (YELLOW)**
  for resuspension of positive control template

Reagents and equipment to be supplied by the user

- **Real-Time PCR Instrument**
- **RNA extraction kit**
  This kit is recommended for use with genesig® EASY DNA/RNA Extraction kit. However, it is designed to work well with all processes that yield high quality RNA with minimal PCR inhibitors.
- **Pipettors and tips**
- **Vortex and centrifuge**
- **Thin walled 1.5ml PCR reaction tubes**

Kit storage and stability

This kit is stable at room temperature but should be stored at -20ºC on arrival. Once the lyophilised components have been resuspended they should not be exposed to temperatures above -20ºC for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.
Primerdesign does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and RNA/DNA integrity. Always run at least one negative control with the samples. To prepare a negative-control, replace the template RNA sample with RNase/DNase free water.

Dynamic range of test

Under optimal PCR conditions genesig Dengue subtype detection kits have very high priming efficiencies of >95% and can detect between $1X10^8$ and $1X10^2$ copies of target template.

Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Primerdesign genesig detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired.
PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applera Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U.S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

Trademarks

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The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG. BI, ABI PRISM® GeneAmp® and MicroAmp® are registered trademarks of the Applera Genomics (Applied Biosystems Corporation). BIOMEK® is a registered trademark of Beckman Instruments, Inc.; iCycler™ is a registered trademark of Bio-Rad Laboratories, Rotor-Gene is a trademark of Corbett Research. LightCycler™ is a registered trademark of the Idaho Technology Inc. GeneAmp®, TaqMan® and AmpliTaqGold® are registered trademarks of Roche Molecular Systems, Inc., The purchase of the Primerdesign ™ reagents cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Principles of the test

Real-time PCR

Four Dengue subtype specific primer and probe mixes are provided in a single tube, and this can be detected through the four different channels as described in the kit contents. The primer and probe mixes provided exploit the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridise to the Dengue cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labelled with a 5`-dye and a 3`-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

Positive control

The kit contains one positive control which acts as a control for all 4 Dengue subtypes. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the target gene worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive controls do not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling these components in a Post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Negative control

To confirm the absence of contamination, a negative control reaction should be included every time the kit is used. For this reaction, the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run.

Endogenous control

To confirm extraction of a valid biological template, a primer/probe mix is included to detect an endogenous gene. Detection of the endogenous control is through the FAM channel and it is NOT therefore possible to perform a multiplex with the pathogen primers. A poor endogenous control signal may indicate that the sample did not contain sufficient biological material.

Resuspension protocol

To minimise the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. Pulse-spin each tube in a centrifuge before opening.
This will ensure lyophilised primer and probe mix is in the base of the tube and is not spilt upon opening the tube.

2. Resuspend the primer/probe mixes in the RNase/DNase free water supplied, according to the table below:
To ensure complete resuspension, vortex each tube thoroughly.

Component – to resuspend in water	Volume
Pre-PCR pack	
Dengue primer/probe mixes (**BROWN**)	110 µl
Endogenous control primer/probe mix (**BROWN**)	165 µl

3. Resuspend the positive control template in the template preparation buffer supplied, according to the table below:
To ensure complete resuspension, vortex each tube thoroughly.

Component – to resuspend in template preparation buffer	Volume
Post-PCR heat-sealed foil	
Dengue positive control template (**RED**)*	500 µl

*** This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.**

One-step qPCR detection protocol

For optimum performance and sensitivity

All pipetting steps and experimental plate set up should be performed on ice. After the plate is poured proceed immediately to the one-step amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artefacts that reduce the sensitivity of detection.

1. **For each RNA sample prepare a reaction mix according to the table below:**
   Include sufficient reactions for positive and negative controls.

Component	Volume
oasig OneStep Master Mix	10 µl
Dengue primer/probe mix (**BROWN**)	1 µl
RNase/DNase Free water (WHITE)	4 µl
Final volume	**15 µl**

2. **For each RNA sample prepare an endogenous control reaction according to the table below (optional):**
   This control reaction will provide crucial information regarding the quality of the biological sample.

Component	Volume
oasig OneStep Master Mix	10 µl
Endogenous control primer/probe mix (**BROWN**)	1 µl
RNase/DNase Free water (WHITE)	4 µl
Final volume	**15 µl**

3. **Pipette 15µl of these mixes into each well according to your qPCR experimental plate set up.**

4. **Pipette 5µl of RNA template into each well according to your experimental plate set up.**
   For negative control wells use 5µl of RNase/DNase free water. The final volume in each well is 20µl.

5. **Pipette 5µl of positive control template into the positive control wells.**

One-step qPCR amplification protocol

If using a machine that uses ROX as a passive reference, then the passive reference must be turned off or set to “none” for no passive reference

Amplification conditions using oasig OneStep Master Mix

	Step	Time	Temp
	Reverse transcription	10 mins	55°C
	Enzyme activation	2 mins	95°C
X 50 cycles	Denaturation	10 secs	95°C
	DATA COLLECTION*	60 secs	60°C

* Fluorogenic data should be collected during this step through the FAM, VIC, Cy5 and ROX channels.

Interpretation of results

Different dengue subtypes are detected in different channels according to the table below. The full data analysis must be performed for each channel to obtain individual results for each Dengue subtype.

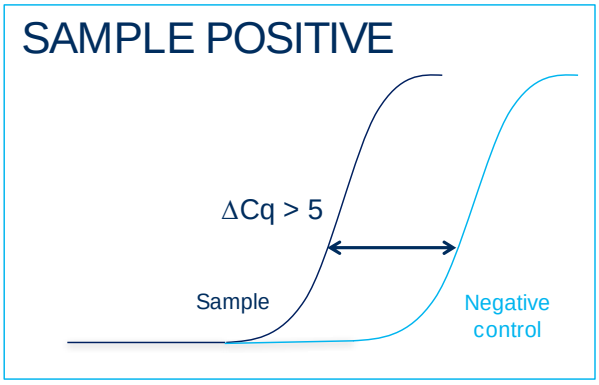
Subtype	Fluorophore
Dengue 1	FAM
Dengue 2	VIC
Dengue 3	ROX
Dengue 4	CY5

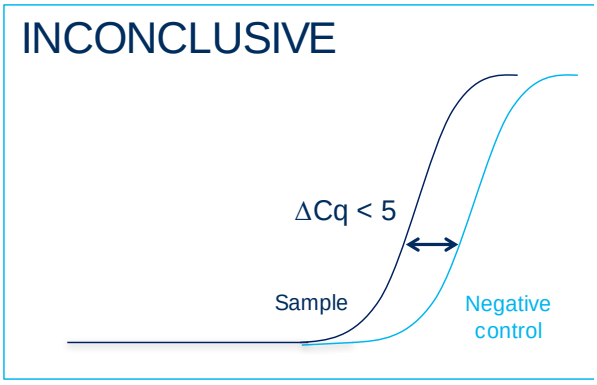
Target	Positive control	Negative control	Interpretation
+	**+**	**-**	**POSITIVE RESULT**
-	**+**	**-**	**NEGATIVE RESULT**

+ / -	**+**	**≤35**	**EXPERIMENT FAILED** Due to test contamination
+ / -	**+**	**>35**	*****
-	**+**	**-**	**SAMPLE PREPARATION FAILED**
+ / -	**-**	**+ / -**	**EXPERIMENT FAILED**

Positive control template (**RED**) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

*Where the test sample is positive, and the negative control is positive with a Cq>35, the sample must be reinterpreted based on the relative strength of the two results:





If the sample amplifies > 5 Cq earlier than the negative control then the sample should be reinterpreted (via the table above) with the negative control verified as negative.

If the sample amplifies < 5 Cq earlier than the negative control then the positive sample result is invalidated and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.

Endogenous control

The signal obtained from the endogenous control primer and probe set will vary according to the amount of biological material present in a given sample. An early signal indicates the presence of a good yield of biological material. A late signal suggests that little biological material is present in the sample.