Primerdesign

R01020

genesig®PLEX Insect-borne

Kit version: 1

Target regions

Zika virus (ZIKV) - Polyprotein gene
Chikungunya virus (CHIKV) - NSP
Dengue virus (DENV) - 3'UTR
Tick-borne encephalitis virus (TBEV) - NS3
Yellow fever virus (YFV) - 5'UTR
West Nile virus (WNV) - 5'UTR into Poly gene

genesig®PLEX kit

100 tests

For general laboratory and research use only

GENESIG
Kits by Primerdesign

Product description

The genesig®PLEX qPCR detection kit detects Zika virus, Chikungunya virus, Dengue virus, Tick-borne encephalitis virus, Yellow fever virus and West Nile virus.

Zika virus (ZIKV):

Causes Zika fever, which often presents with mild symptoms like fever, rash, joint pain, and conjunctivitis. Severe cases can lead to birth defects (e.g. microcephaly) if pregnant women are infected.

Chikungunya virus (CHIKV):

Causes chikungunya fever, characterized by high fever, severe joint pain (often chronic), rash, and fatigue.

Dengue virus (DENV):

Causes dengue fever, which can range from mild flu-like symptoms to severe dengue hemorrhagic fever or dengue shock syndrome, with bleeding, plasma leakage, and potentially death.

Yellow fever virus (YFV):

Causes yellow fever, with symptoms including fever, jaundice (yellowing skin/eyes), chills, muscle aches, and in severe cases, bleeding, shock, and organ failure.

Tick-borne encephalitis virus (TBEV):

Causes tick-borne encephalitis, a neurological disease with symptoms like fever, headache, neck stiffness, and potentially meningitis or encephalitis.

West Nile virus (WNV):

Causes West Nile fever, a flu-like illness, but can lead to encephalitis or meningitis in severe cases, especially in vulnerable populations.

Specificity

The genesig®PLEX Insect-borne kit is designed for the in vitro detection of Zika virus (ZIKV), Chikungunya virus (CHIKV), Dengue virus (DENV), Tick-borne encephalitis virus (TBEV), Yellow fever virus (YFV) and West Nile virus (WNV). The assays within this kit are predicted to detect over 95% of sequences available on the NCBI database at the time of design.

The TBEV assay in this kit is predicted to cross react with Greek goat encephalitis virus, Spanish sheep encephalitis virus, Spanish goat encephalitis virus, Louping ill virus, Langat virus and Alpine chamois encephalitis virus. These would give a signal in the Cy5 channel of tube two.

The dynamics of genetic variation means that new sequence information may become available after the initial design. Primerdesign periodically reviews the detection profiles of our kits and when required releases new versions.

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

Kit contents

- **Insect borne Tube 1 (T1) primer/probe mix (100 reactions BROWN))**
  FAM, VIC, ROX and Cy5 labelled (see table below)

Target	Fluorophore
Zika virus	FAM
Internal extraction control	VIC
Chikungunya virus	ROX
Dengue virus	Cy5

- **Insect borne Tube 2 (T2) primer/probe mix (100 reactions BROWN)**
  FAM, VIC, ROX and Cy5 labelled (see table below)

Target	Fluorophore
West Nile virus	FAM
Internal extraction control	VIC
Yellow fever virus	ROX
Tick-borne encephalitis virus	Cy5

- **Insect borne Tube 1 (T1) positive control template (RED)**
- **Insect borne Tube 2 (T2) positive control template (RED)**
- **genesig® Easy RNA internal extraction control (BLUE)**
- **4x Lyophilised oasig®PLUS OneStep Lyophilised qPCR Master Mix (GOLD)**
- **4x oasig™ resuspension buffer (BLUE)**
  for resuspension of the lyophilised master mix
- **2x Template preparation buffer (YELLOW)**
  for resuspension of the positive control templates and internal extraction control RNA
- **RNase/DNase free water (WHITE)**
  for resuspension of the primer/probe mix

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

Must be able to read fluorescence through FAM, HEX/VIC, ROX and Cy5 channels.

Extraction kit

This kit is recommended for use with genesig EASY DNA/RNA Extraction kit or exsig®Mag. However, it is designed to work well with all processes that yield high quality RNA and DNA with minimal PCR inhibitors.

Pipettors and filter tips

Vortex and centrifuge

1.5ml microtubes

qPCR plates or 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 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 kits have very high priming efficiencies of >90% with the exception of Dengue. Due to design limitations from the requirement to detect multiple subtypes, the Dengue target displays efficiencies of >80%. All targets can detect between $1x10^6$ 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, Primer Design Ltd genesig® detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained in violation of the general GLP guidelines and the manufacturer's recommendations, the right to claim under guarantee is expired.

Trademarks

genesig® is a registered trademark of Primer Design Ltd.

oasig® is a trademark of Primer Design Ltd.

exsig®Mag is a trademark of Primer Design Ltd.

Principles of the test

Real-time PCR
Individual primer and probes designed for each pathogen have been combined into a single reaction and these can be detected through the different fluorescent channels as described in the kit contents.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the target DNA. Fluorogenic probes are 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 two positive control tubes, each tube contains the templates for all three targets detected by the corresponding primer/probe tube. The kit positive control will therefore give a positive signal in FAM, ROX, and Cy5 channels. Each time the kit is used, at least one positive control reaction for each primer/probe tube must be included in the run. A positive result indicates that the primers and probes for detecting each virus are working properly in that particular run. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does 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 validate any positive findings 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. It is also known as a No Template Control or NTC.

Internal RNA extraction control
When performing RNA extraction, it is often advantageous to have an exogenous source of RNA template that is spiked into the lysis buffer. This control RNA is then co-purified with the sample RNA and can be detected as a positive control for the extraction process. Successful co-purification and qPCR for the control RNA also indicates that PCR inhibitors are not present at a high concentration.

Within the Insect borne primer/probe mixes are primers and probes to detect the exogenous RNA using qPCR. The PCR primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control cDNA does not interfere with detection of the target cDNA even when present at low copy number. The Internal control is detected through the VIC channel in both tubes and gives a Cq value of

28+/-3 depending on the level of sample dilution.

Resuspension protocol

To minimise the risk of contamination with foreign RNA, 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/probe mix or template is in the base of the tube and is not lost upon opening the tube.

2. **Resuspend the primer/probe mix in the RNase/DNase free water supplied, according to the table below:**
   To ensure complete resuspension allow primer/probe mixes to rehydrate for 10 minutes at room temperature. Vortex each tube thoroughly, followed by pipetting up and down 10 times. Failure to mix well can produce poor kit performance.

Component – resuspend in water	Volume
Pre-PCR pack	
Insect borne T1 primer/probe mix (**BROWN**)	110µl
Insect borne T2 primer/probe mix (**BROWN**)	110µl

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

Component – resuspend in template preparation buffer	Volume
Pre-PCR heat-sealed foil	
genesig® RNA internal extraction control (**BLUE**)	600µl
Post-PCR heat-sealed foil	
Insect borne T1 Positive control template (**RED**)*	500µl
Insect borne T2 Positive control template (**RED**)*	500µl

*** 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.**

4. **Resuspend the lyophilised OneStep Master Mix in oasig resuspension buffer, according to the table below:**

Component – resuspend in oasig resuspension buffer	Volume
oasig®PLUS OneStep Lyophilised qPCR Master Mix (**GOLD**)	525µl

RNA extraction

The internal extraction control RNA can be added to either lysis/extraction buffer or to the sample once it has been resuspended in lysis buffer.

DO NOT add the internal extraction control RNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.

1. **Add 4µl of the Internal extraction control RNA (BLUE) to each sample in RNA lysis/extraction buffer per sample.**

2. **Complete RNA extraction according to the manufacturer's recommended protocols.**

OneStep RT-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 prepared, proceed immediately to the OneStep amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

1. **Prepare 2 reaction mixes, one for each primer/probe mix according to the table below:** Include sufficient reactions for all samples, positive and negative controls.

   Insect borne Tube 1 reaction mix:

Component	Volume
oasig®PLUS OneStep Lyophilised qPCR Master Mix (**GOLD**)	10µl
Insect borne T1 primer/probe mix (**BROWN**)	1µl
RNase/DNase free water (**WHITE**)	4µl
Final volume	**15µl**

   Insect borne Tube 2 reaction mix:

Component	Volume
oasig®PLUS OneStep Lyophilised qPCR Master Mix (**GOLD**)	10µl
Insect borne T2 primer/probe mix (**BROWN**)	1µl
RNase/DNase free water (**WHITE**)	4µl
Final volume	**15µl**

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

3. **Pipette 5µl of RNA sample 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.

4. **Pipette 5µl of positive control template into each well according to your plate set up.**
   The Tube 1 positive control contains template for Zika Virus, Chikungunya virus, and Dengue virus and Tube 2 positive control contains templates for West Nile virus, Yellow Fever virus, and Tick-borne Encephalitis virus. The final volume in each well is 20µl.

OneStep RT-qPCR amplification protocol

Amplification conditions using lyophilised OneStep Master Mix

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

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

Interpretation of results

Positive control
Each positive control tube contains the all the templates for the targets detected by the corresponding primer/probe tube, and should produce positive amplification plots in the FAM, ROX, and Cy5 channels. There is no Internal control template within the positive control so the VIC channel should give no signal (flat amplification plot). The positive control signals indicate that the kit is working correctly to detect each virus.

No template control (NTC)
The NTC should give a flat line (flat amplification plots) through all channels. Signals in the NTC indicate cross contamination during plate set up.

Internal RNA extraction control
The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of RNA added to the PCR reaction and the individual machine settings. Cq values of 28±3 are within the normal range. When amplifying a target sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

Sample data
Presence of the viruses are detected in the channels indicated in the kit contents section. Positive signals indicate positive tests for those viruses. It may be possible for samples to contain multiple viruses, therefore positive results in the FAM, ROX, and Cy5 channels may be present.

Summary of data interpretation

Insect borne Tube 1 data interpretation

Target (FAM/ROX/Cy5)	Internal extraction control (VIC)	Positive Control	Negative Control	Interpretation
FAM +	**+ / -**	**+**	**-**	**ZIKA VIRUS POSITIVE RESULT**
ROX +	**+ / -**	**+**	**-**	**CHIKUNGUNYA VIRUS POSITIVE RESULT**
Cy5 +	**+ / -**	**+**	**-**	**DENGUE VIRUS POSITIVE RESULT**
-	**+**	**+**	**-**	**NEGATIVE RESULT**

Insect borne Tube 2 data interpretation

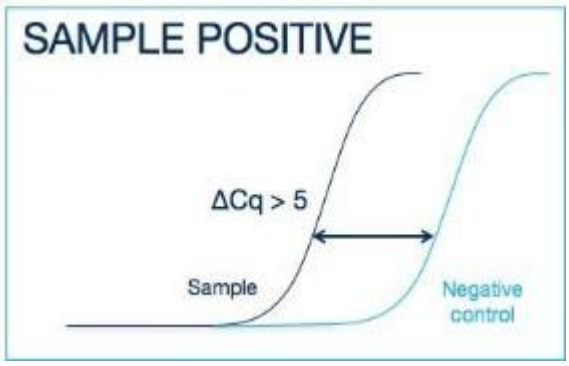
Target (FAM/ROX/Cy5)	Internal extraction control (VIC)	Positive Control	Negative Control	Interpretation
FAM +	**+ / -**	**+**	**-**	**WEST NILE VIRUS POSITIVE RESULT**
ROX +	**+ / -**	**+**	**-**	**YELLOW FEVER VIRUS POSITIVE RESULT**
Cy5 +	**+ / -**	**+**	**-**	**TICK-BORNE ENCEPHALITIS VIRUS POSITIVE RESULT**
-	**+**	**+**	**-**	**NEGATIVE RESULT**

Interpretation applicable to both tubes

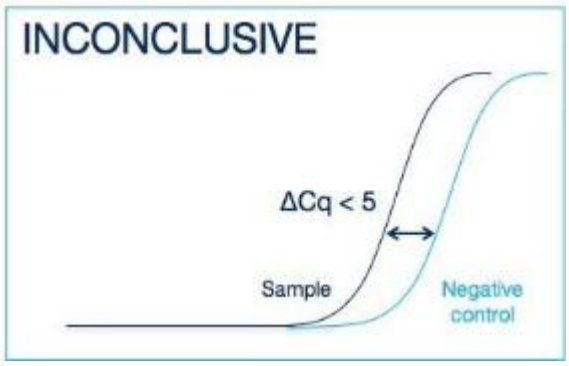
Target (FAM/ROX/Cy5)	Internal extraction control (VIC)	Positive Control	Negative Control	Interpretation
+ / -	**+ / -**	**+**	**≤35**	**EXPERIMENT FAILED Due to test contamination**
+ / -	**+ / -**	**+**	**>35**	*****
-	**-**	**+**	**-**	**SAMPLE PREPARATION FAILED**
+ / -	**+ / -**	**-**	**+ / -**	**EXPERIMENT 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 signal 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.