Logix Smart Zika Virus Test Kit

(ZIKV-K-003; ZIKV-PC-003; GEN-NTC-001)

Description

The Logix Smart Zika Virus Test developed by Co-Diagnostics, Inc. detects ribonucleic acid (RNA) of Zika Virus in a single step reverse transcription real-time PCR reaction. Real-time PCR detection is recommended for detection of Zika Virus during the acute stages of infection before the production of antibodies, which is typically <14 days after the mosquito bite. Zika virus testing helps prevent the transmission of virus prior to pregnancy, transfusion or transplantation, sexual relation, or the exchange of body fluids. The Logix Smart Zika Virus Test detects the virus within 40 cycles from serum or plasma samples.

- In each Logix Smart Zika Virus Test Kit supplied is a complete, ready-to-use master mix. Simply add the extracted viral RNA sample to the master mix and run!
- A human RNaseP gene marker serves as an Internal Positive Control (IPC) to monitor the quality of each reaction and is designed to also detect inadequate samples.

Components

- 1x 500 µL (100 rxns) **Logix Smart Zika Virus Master Mix (ZIKV-MM-003)**
- 1x 500 µL **Logix Smart Zika Virus Positive Control (ZIKV-PC-003)**
- 1x 500 µL **Nuclease Free Water (GEN-NTC-001)**

Materials required (not included):

- Pipettes capable of transferring 5 µL
- Ice
- Vortex
- Centrifuge
- Real-time PCR System with FAM (green) or Cal Fluor 610 (orange) dyes or equivalent and accompanying tubes/plates and caps/films

Storage:

- **Logix Smart Zika Virus Master Mix and Logix Smart Zika Virus Positive Control *must*** be stored at -20 ºC and can last up to 60 days.

WARNING! To prevent degradation of reagents in Test Kits:

1) **Immediately store Logix Smart Zika Virus Master Mix and Logix Smart Zika Virus Positive Control at or below -20°C**
2) **Always work with each Logix Smart Zika Virus Master Mix and Logix Smart Zika Virus Positive Control on ice**
3) **Return components of Logix Smart Zika Virus Master Mix and Logix Smart Zika Virus Positive Control to the -20°C freezer immediately after using**
4) **Make aliquots if necessary to avoid multiple freeze/thaw cycles**

Upon receipt of the **Logix Smart Zika Virus Master Mix and Logix Smart Zika Virus Positive Control**, check to make sure there is excess dry ice in the shipment and that the reagents are

still completely frozen. If there are any problems with the shipment please contact your distributor immediately. **Immediately store the Logix Smart Zika Virus Master Mix and Logix Smart Zika Virus Positive Control at -20°C**.

If you work in an area prone to power outages it is extremely important that you have a back-up generator for your freezer as well as a temperature data log to **ensure that the Logix Smart Zika Virus Master Mix and Logix Smart Zika Virus Positive Control remain frozen at -20°C**. Reagents must remain frozen at all times (or on ice when in use) to prevent degradation.

While working with thawed components of **Logix Smart Zika Virus Master Mix and Logix Smart Zika Virus Positive Control** it is extremely important that the **reagents are kept on ice at all times**. Return the reagents to the freezer immediately after use, and make aliquots to avoid multiple freeze/thaw cycles.

Intended Use:

The Logix Smart Zika Virus Test Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

This product is for export only and is not for sale in the United States.

Sample Extraction Recommendations
The Logix Smart Zika Virus Test Kit has been validated with serum or plasma samples collected in serum separator (red) or EDTA (purple) vacutainers and extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Cat No. 52904).

Real-time PCR Setup

- To prevent contamination, it is recommended to use Good Molecular Laboratory Practices that follow a uniflow process and separation of negative and positive materials.
- All real-time PCR master mix, positive controls, negative controls, and samples should be briefly spun down to remove residue from the lids after storage or mixing.
- Thaw all reagents and samples on ice before starting setup.
- All reagents must remain on ice for the duration of real-time PCR setup.

In the designated negative control setup area:

1. Thaw **Logix Smart Zika Virus Master Mix** (ZIKV-MM-003) on ice (for minimum time possible).
2. Vortex and centrifuge Logix Smart Zika Virus Master Mix for a max 3 seconds.
3. Put the **Logix Smart Zika Virus Master Mix** on ice.
   ***All steps involving Logix Smart Zika Virus Master Mix should be performed on ice or on a frozen tray**
4. Put PCR tubes on ice.
5. Aliquot 5 uL of **Logix Smart Zika Virus Master Mix** into desired wells.
6. Add 5 uL **Nuclease Free Water (GEN-NTC-001)** to the appropriate well(s).

In the designated positive control area (preferably separated from the master mix setup area to avoid contamination)

7. Thaw extracted/purified RNA on ice (if frozen).
8. Vortex and centrifuge purified RNA for a few seconds.
9. Add 5 uL of purified RNA sample to each well using a new tip between each sample.
10. Thaw **Logix Smart Zika Virus Positive Control (ZIKV-PC-003)** on ice (for minimum time possible).
11. Vortex and centrifuge **Zika Virus PC** for a few seconds.
12. Add 5 µL of **Zika Virus PC** to appropriate well(s).
13. Place caps on the tubes according to the real-time system being used.
14. Put plate/tubes in real-time PCR machine and start the run.

Thermal Cycling Setup

Program the thermal cycler to the following conditions for a total reaction volume of 10µL:

Temperature	Time	Cycles	Capture
55˚C	10 minutes		N/A
95˚C	20 seconds		N/A
95˚C	3 seconds	45	N/A
55˚C	32 seconds		FAM (Green) and Cal Fluor Red 610CF610 (Orange)

Interpretation of Results

1. When the run is finished, ensure that the run file is saved.
2. Check to see that the controls passed.

Analyzing Controls

Positive Controls:

Highlight the positive control reaction well. Each positive control should show two amplification curves similar to that seen below:

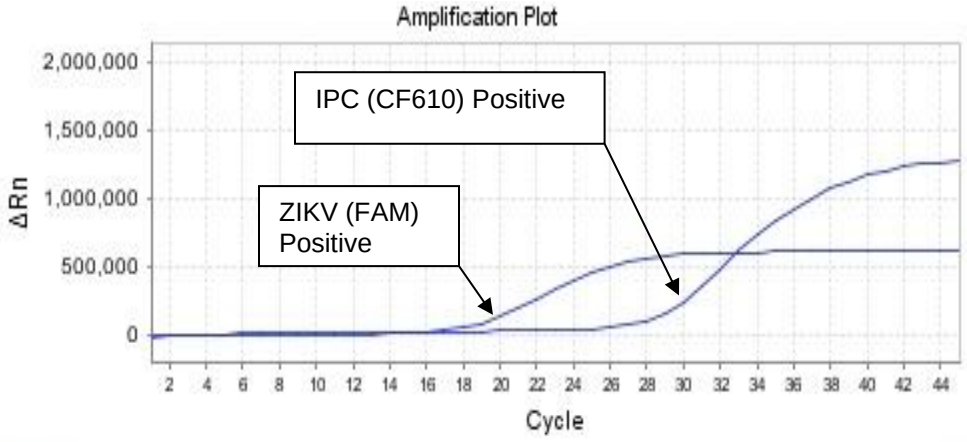

Figure 1. Positive Control Amplification

Range for Positive Control Ct Values*	
ZIKV (FAM)	20-26
IPC (CF610)	16-19

Table 1. Positive Control Ranges for Cycle Threshold Values
ZIKV (FAM): Zika Virus Marker
IPC (CF610): RnaseP Internal Positive Control Marker
*Ct values may vary by ± 2 cycles based on instrument differences.

If the Ct values do not match the graph, then the threshold or the baseline must be manually changed until the values represent the growth curves. The threshold must be set as close to the start of the growth curve and above the background noise. The baseline must be set before the first amplification curve. Check the instrument user manual for instructions for adjusting the threshold or baseline.

If the positive control does not show amplification, then the tests are invalid. Loss of amplification for a positive control is indicative of primer degradation which may result from reagents being at temperatures above -20˚C for more than one hour or being used past the expiration date. Pipetting error may also account for lack of positive control amplification by pipetting control into the wrong well, missing a well, or pipetting an inadequate amount of reagent into a reaction well.

Negative Controls:
Next highlight the negative control. The results of the negative control should show results similar to those seen below:

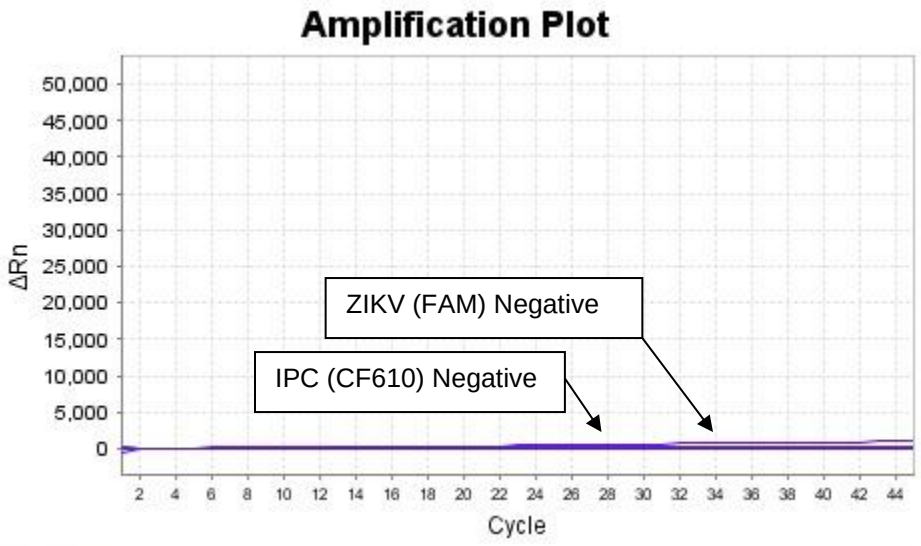

Figure 2. Negative Control Amplification

Occasionally, ubiquitous binding will cause amplification of RNaseP as seen in the figure below:

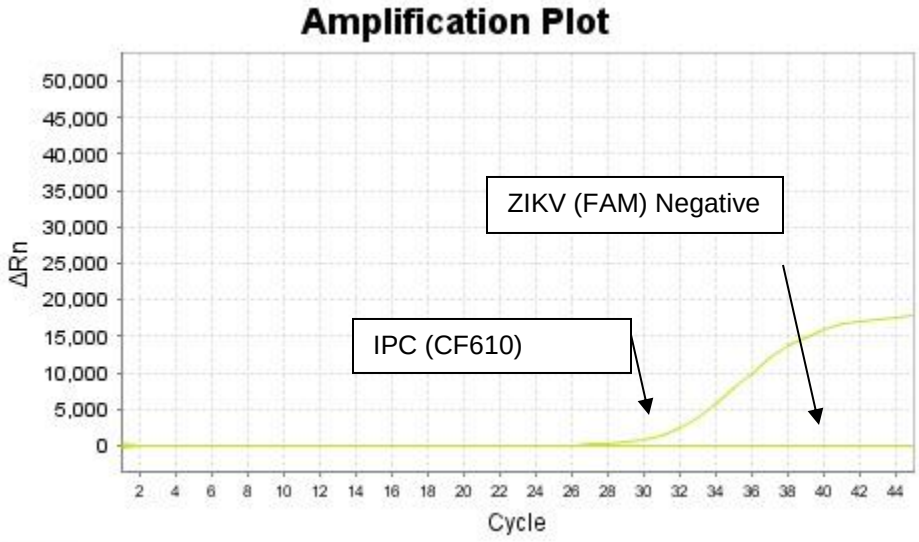

Figure 3. Ubiquitous Binding of Negative Control

If the negative control shows any amplification of ZIKV <40 cycles the results are invalid and the entire experiment must be repeated. Amplification of ZIKV in a negative control indicates contamination in one or more of the reagents or pipetting error. Amplification of ZIKV >40 cycles is outside the detectable range and is considered negative.

Analyzing Samples

Once the controls have passed, the unknown samples can be interpreted based on three possible outcomes (figures may vary based on machine used and quantity of MM and sample):

1. Positive (figures 4 and 5)
2. Negative (figure 6)
3. Negative Due To Inadequate Nuclear Material (figure 7)

A **Positive** result will show an amplification curve or cycle threshold value for ZIKV at or below 40 cycles. Amplification curves greater than 40 cycles for ZIKV are outside of the detection limits for the assay. A positive sample will have the following curves:

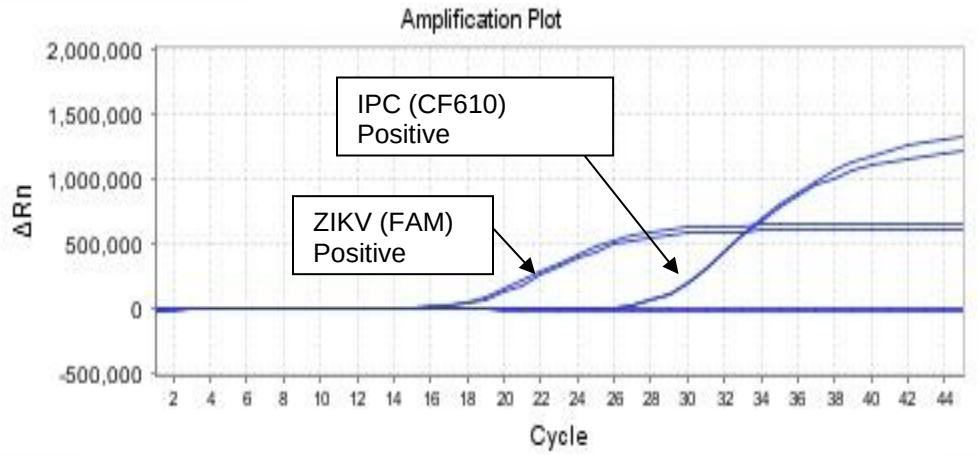

Figure 4. ZIKV positive sample: The presence of a curve for ZIKV (FAM) indicates a positive result. The amplification of the IPC (CF610) shows that the extraction was successful.

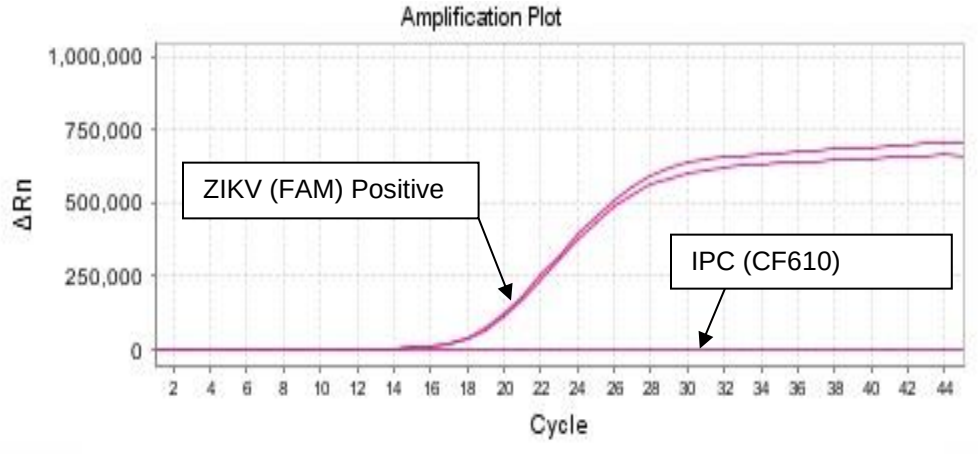

Figure 5. ZIKV positive sample: The presence of a curve for ZIKV indicates a positive result even when the RNase P (IPC) marker is negative. This will occur when the concentration of ZIKV is greater than the concentration of RNaseP or when using cell lysates or extremely pure/sterile samples.

A **Negative** result will show no amplification for ZIKV; however, occasionally amplification greater than 40 cycles occurs in ZIKV or RNaseP channels. Any amplification curves greater than 40 cycles for ZIKV are outside of the detection limits for the assay. A negative sample result will have the following curve:

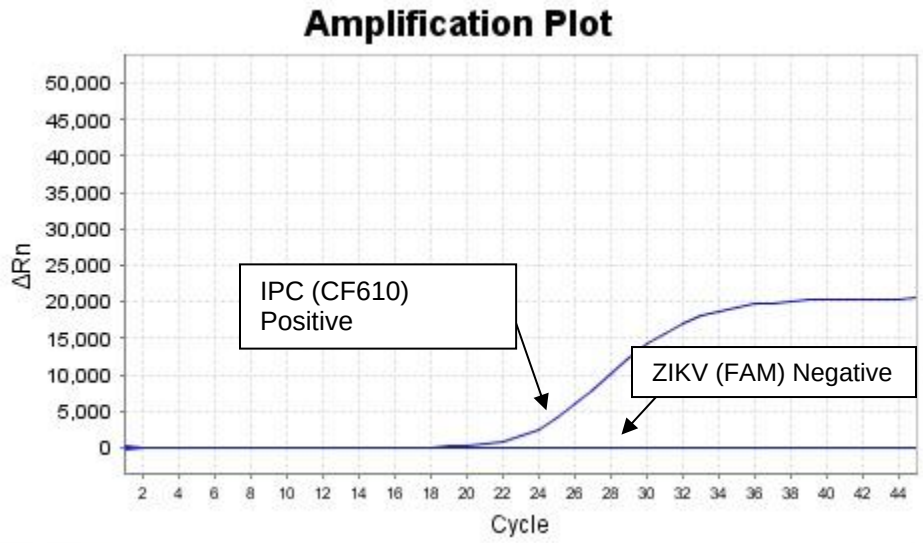

Figure 6. ZIKV negative sample: The absence of a curve for ZIKV indicates a negative result ONLY when the RNase P (IPC) marker is positive.

A **Negative Due To Inadequate Nuclear Material** result will have the following curve:

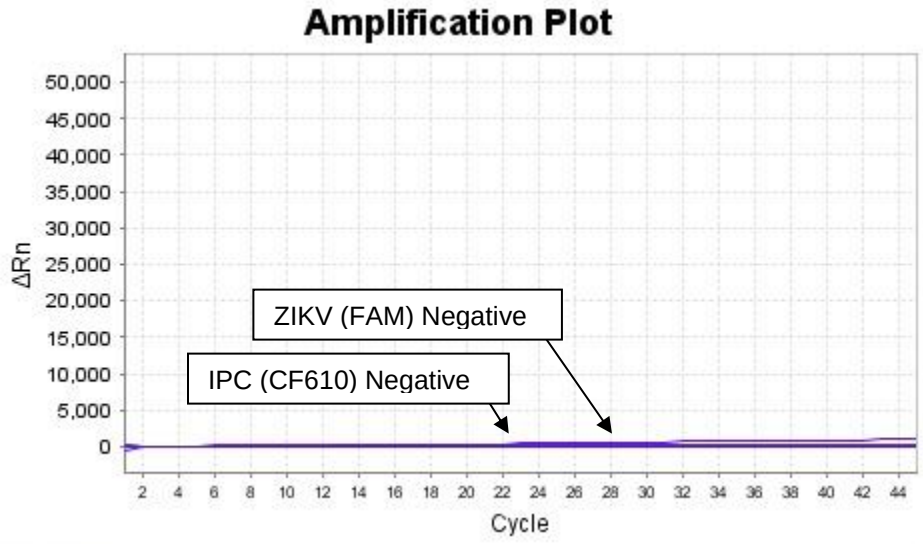

Figure 7. Negative Due To Inadequate Nuclear Material: If the RNase P (IPC) control marker is also negative, then the result is negative due to inadequate nuclear material. This can be the result of human error in sample prep, sample degradation, or an inadequate sample. The test may be repeated with a new sample or called negative due to inadequate nuclear material.
Note: Samples obtained from culture or sterile/pure sites (*e.g.* CSF, urine, cell lysates, etc) may not contain RnaseP. In such case, the two negative markers indicate a true negative result for zika virus.

For Information or Questions:

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