ELITechGroup MDx LLC

Zika ELITe MGB® Kit U.S. used with ELITe InGenius®

M800552

This document is a simplified version of the official instructions for use. Please refer to the complete document before use: www.elitechgroup.com/north-america/

The assay performed on the ELITe InGenius instrument is authorized for use under FDA Emergency Use Authorization (EUA).

A. Intended use

The Zika ELITe MGB® Kit U.S. is a real-time RT-PCR test intended for the qualitative detection of RNA from the Zika virus in serum and EDTA plasma from individuals meeting CDC Zika virus clinical criteria and/or CDC Zika virus epidemiological criteria.

B. Amplified sequence

	Gene	Fluorophore
Target	Zika NS3 protein	FAM
Internal Control	Genomic RNA of MS2 phage	AP525 (VIC)

C. Validated matrix

- **Plasma** EDTA
- **Serum**

D. Kit content

The Zika ELITe MGB® Kit U.S. contains all the PCR reagents and associated controls.

20x Zika PreMix	PCR MasterMix	RT EnzymeMix	PCR Grade Water
PreMix Primer, probe, oligo-nucleotides mixture 1 x 98 µL	MasterMix Mixture of Reagents 1 x 972 µL	RT Reverse transcriptase 1 x 20 µL	Water DNase and RNase-free water 1 x 208 µL

Zika - Positive Control	Negative Control	MS2 RNA Internal Control
Synthetic Zika RNA 2 x 800 µL	DNase and RNase-free water 1 x 1600 µL	IC 4 x 108 µL

› Reactions per kit: 48 › Storage Temperature: -20°C › Freeze-thaw cycles per tube: 5 › Maximum shelf-life: 24 months

E. Material required not provided in the kit

- **ELITe InGenius Instrument:** INT030-K
- **ELITe InGenius SP200 Extraction Cassette:** INT032SP200
- **ELITe InGenius PCR Cassette:** INT035PCR
- **ELITe InGenius SP200 Consumable Set:** INT032CS
- **ELITe InGenius Waste Box:** F2102-000
- **Filter Tips 300 Axygen:** TR-350-LRS
- **Sarstedt 2.0 mL tube:** 72.694.005
- **Sarstedt 0.5 mL tube:** 72.730.005

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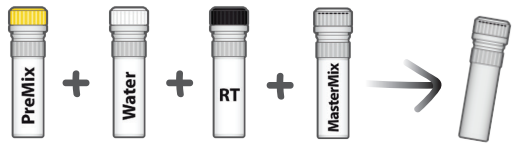
F. Reagent Preparation

Monoreagent

- Thaw Zika PreMix, PCR MasterMix, PCR Grade Water, vortex, centrifuge
- Gently shake, centrifuge RT EnzymeMix. RT EnzymeMix must be kept at -20°C
- Prepare 2.0 mL tube for the Monoreagent
- Calculate required volume of the components
- Add Zika PreMix, PCR MasterMix, PCR Grade Water
- Complete monoreagent by adding RT EnzymeMix
- Vortex, centrifuge and keep on ice until ready for ELITe InGenius transfer
- Stability on board: 6 hours



Zika Monoreagent

Number of Reactions	20x Zika PreMix Vol. (µL)	PCR MasterMix Vol. (µL)	PCR Grade Water Vol. (µL)	RT EnzymeMix Vol. (µL)
1	3.2	31.5	6.7	0.63
2	4.8	48.0	10.2	0.96
3	6.5	64.5	13.8	1.29
4	8.1	81.5	17.3	1.62
5	9.8	97.5	20.8	1.95
6	11.4	114.0	24.3	2.28
7	13.1	130.5	27.8	2.61
8	14.7	147.0	31.4	2.94
9	16.4	163.5	34.9	3.27
10	18.0	180.0	38.4	3.60
11	19.7	196.5	41.9	3.93
12	21.3	213.0	45.4	4.26

Internal Control

- Thaw MS2 RNA Internal Control, vortex, centrifuge
- Prepare 0.5 mL tube for the Internal Control
- Calculate required volume of the components
- Add nuclease-free water and MS2 RNA Internal Control template
- Vortex, centrifuge and keep on ice until ready for ELITe InGenius transfer

Ready-to-Use Internal Control

Number of Reactions	MS2 RNA Internal Control Vol. (µL)	Nuclease-Free Water Vol. (µL)
1	20.0	20.0
2	29.0	29.0
3	36.0	36.0
4	43.0	43.0
5	50.0	50.0
6	57.0	57.0
7	64.0	64.0
8	71.0	71.0
9	78.0	78.0
10	85.0	85.0
11	92.0	92.0
12	99.0	99.0

G. ELITe InGenius protocol

- Sample volume 200 µL
- MS2 Internal Control volume 10 µL
- Total eluate volume 50 µL
- PCR eluate input volume 10 µL
- Monoreagent Mix volume 20 µL
- Frequency of positive control every day
- Frequency of negative control every run

H. Performance

Matrix	Limit of Detection	Positive Percent Agreement	Negative Percent Agreement
Plasma	270 cp/mL	**94.7%** 89/94*	**90.9%** 70/77*

**confirmed samples/ tested samples*

I. Reference material tested

Panel name	Provider	Qualitative Results
QCMD 2016: ZIKA16	Qnostics	Concordance 100% (10/10)*

**confirmed samples/ tested*

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J. Procedure

The user is guided step-by-step by the ELITe InGenius software to prepare the run. All the steps: extraction, amplification and result interpretation are automatically performed.

Before Run

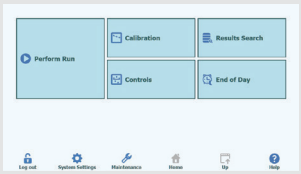
1. Switch on ELITe InGenius
Enter username and password
Select the mode "Closed"

2. Thaw the PCR reagents: Zika PreMix, PCR MasterMix, RT EnzymeMix, PCR Grade Water
Thaw the controls: positive, negative and internal

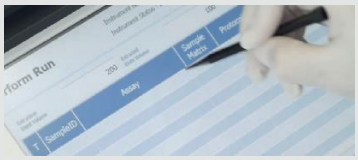
3. Prepare the monoreagent and the Internal Control (refer to Section F: Reagent Preparation)

Complete run: Extraction + PCR

1. Select "Perform Run" on the touch screen

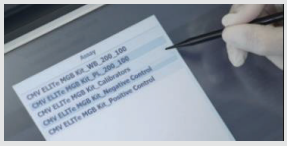


2. Verify the extraction volumes:
Input: "200 µL", eluate: "50 µL"



3. Select the protocols: **Zika ELITe MGB Positive Control** for track 1 and **Zika ELITe MGB Negative Control** for track 2.

4. Select
Zika ELITe MGB_Sample_PL_200_50
for the samples in tracks 3 to 12



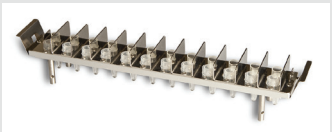
5. Select the sample position:
"Sonication tube"

6. Scan the sample barcodes with the barcode reader or type the sample ID

7. Load the monoreagent and Internal Control into the inventory block

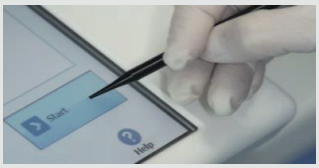
8. Load the positive and negative control tubes in tracks 1 and 2 of the elution rack. Load empty elution tubes in other positions

9. Transfer 200 µL of each sample in a sonication tube in tracks 3 to 12

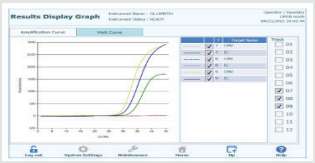


10. Load PCR cassette, Extraction cartridge, Elution tube, Tip cassette, and sonication tube racks

11. Close the door
Start the run



12. View, approve and store the controls and the patient sample results



Note: The position of samples and controls on the tracks are only indicative. They can be placed indifferently in any tracks.

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Zika ELITe MGB® Kit U.S.

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by authorized laboratories;
- This test has been authorized only for the detection of RNA from Zika virus and diagnosis of Zika virus infection, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of the emergency use of in vitro diagnostic tests for detection of Zika virus and/or diagnosis of Zika virus infection under section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

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