

# TaqMan® Zika Virus Triplex Vector Screening Kit (ZIKV/DENV/CHIKV)

Lyophilized reagents for multiplex real-time RT-PCR detection of Zika, Dengue, and Chikungunya virus RNA (0.1-mL block)

Catalog Number A31748

Pub. No. MAN0016848 Rev. B.0

**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](http://thermofisher.com/support).

## Product description

The TaqMan® Zika Virus Triplex Vector Screening Kit (ZIKV/DENV/CHIKV) is designed to detect viral RNA, prepared from urine or serum research samples, for the following viruses:

- Zika virus with Asian lineage
- Dengue virus from 4 serotypes: DENV-1, DENV-2, DENV-3, and DENV-4
- Chikungunya virus

The kit also detects MS2 bacteriophage control, to monitor nucleic acid recovery and to serve as a process control for the RT-PCR.

The kit includes primers and TaqMan® probes for the viral and MS2 targets, and other reagents for RT-PCR, in a lyophilized format. After addition of RNA sample, the reconstituted reagents are ready for real-time RT-PCR.

## Contents and storage

**Table 1** TaqMan® Zika Virus Triplex Vector Screening Kit (ZIKV/DENV/CHIKV) (Cat. No. A31748; 96 reactions)

Contents	Amount	Storage
Lyophilized assay, Fast, 0.1-mL tube	12 × 8-tube strips	<ul style="list-style-type: none"> <li>• 18–28°C for up to 1 year<sup>[1]</sup></li> <li>• 2–8°C for long-term storage</li> <li>• Protect from moisture<sup>[2]</sup></li> </ul>
MicroAmp™ Optical 8-Cap Strips	12 × 8-cap strips	Room temperature

<sup>[1]</sup> Product is shipped at ambient temperature. See [thermofisher.com/ambientshipping](http://thermofisher.com/ambientshipping).

<sup>[2]</sup> See "Procedural guidelines" on page 2.

## Required materials

Unless otherwise indicated, all materials are available through [thermofisher.com](http://thermofisher.com). MLS: Fisher Scientific ([fisherscientific.com](http://fisherscientific.com)) or other major laboratory supplier.

Item	Source
<b>Applied Biosystems™ real-time PCR instrument and accessories, one of the following:</b>	
QuantStudio™ instrument capable of detecting at least 5 colors <sup>[1]</sup> : <ul style="list-style-type: none"> <li>• QuantStudio™ 5 Real-Time PCR System</li> <li>• QuantStudio™ 12K Flex Real-Time PCR System</li> <li>• QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System</li> </ul>	Contact your local sales office
7500 Fast Real-Time PCR Instrument 7500 Fast Precision Plate Holder, for 0.1 mL Tube Strips (A29252)	Contact your local sales office
<b>Equipment</b>	
MicroAmp™ 96-Well Base	N8010531
MicroAmp™ Cap Installing Tool	4330015
Benchtop microcentrifuge with 8-tube strip adapter, or plate centrifuge	MLS
Laboratory mixer, Vortex or equivalent	MLS
Adjustable pipettors	MLS
<b>Plastics and consumables</b>	
[Optional] MicroAmp™ Optical 8-Cap Strips <sup>[2]</sup>	4323032
[Optional] MicroAmp™ Fast 8-Tube Strip, 0.1 mL <sup>[2]</sup>	4358293
Aerosol-resistant micropipette tips	MLS
Disposable gloves	MLS
<b>Reagents</b>	
Nuclease-free Water	AM9938

<sup>[1]</sup> Precision Plate Holder is included with the instrument.

<sup>[2]</sup> Required only for the 7500 series instrument, to balance the lid pressure if less than 2 full strips are processed.

## Procedural guidelines

- Protect the lyophilized assay from moisture; ambient moisture will compromise performance very quickly. Use multiple barriers.  
For example, after the original pouch is opened:
  - Place unused strips in the original pouch with the silica desiccant pack, then seal the pouch. Use a resealable bag if the original pouch is broken.
  - Place the sealed pouch in a dry box or desiccator.
- Do not use DEPC-treated water.
- Ensure that personnel operating the real-time PCR instrument are trained.
- Ensure that the instrument is calibrated for each detector dye and passive reference dye, according to the instrument user guide. See "Dye spectral calibration plates, 96-well" on page 2.
- Ensure that the appropriate Precision Plate Holder is installed in the instrument. Follow the instrument user guide for tube placement and plate holder use.

## Guidelines for input RNA

Use high-quality RNA samples for reliable PCR results.

**Table 2** Recommended products for RNA isolation

Product	Source	Notes
MagMAX™ Pathogen RNA/DNA Kit	4462359	Use up to 25 µL of total RNA in elution buffer per PCR reaction.
PureLink™ Viral RNA/DNA Mini Kit	12280050	
ZeptoMetrix® MS2 Bacteriophage	fisherscientific.com <sup>[1]</sup> 22-156-880	Add $1 \times 10^6$ to $1 \times 10^7$ copies to each sample before you start the RNA isolation procedure. <sup>[2]</sup>

<sup>[1]</sup> This product is also available at [zeptometrix.com](http://zeptometrix.com) [Cat. No. 0810052].

<sup>[2]</sup> MS2 bacteriophage is used to monitor nucleic acid recovery and to serve as a process control for the RT-PCR.

## Set up and run the reactions

- If needed, adjust the total volume of RNA sample to 25 µL per reaction, using Nuclease-free Water.
- Remove the cap of the 8-tube strip; discard the cap.
- Add 25 µL of RNA sample to each tube, then firmly apply a new optical cap strip (provided in the kit).
- Mix by flicking the tube strip several times or by vortexing briefly, then centrifuge briefly.

- Select or create dye detectors, then assign to each tube in the layout.

Target	Reporter	Quencher
Zika	FAM™ dye	Non-fluorescent quencher (NFQ)
Dengue	VIC™ dye	
Chikungunya	ABY™ dye	
MS2	JUN™ dye	

- Load the tube strips, then run the real-time PCR instrument using the following thermal cycling conditions.
  - Run mode: Fast
  - Passive reference: MUSTANG PURPLE™ dye.

Stage	Cycles	Temperature	Time
Reverse transcription	1	50°C	20 minutes
Activation	1	95°C	2 minutes
Amplification	40	95°C	15 seconds
		60°C	1 minute

## Guidelines for data analysis

The general process for data analysis is to:

- View the amplification plots.
- Set the baseline and threshold values.
- Use the instrument software to calculate  $C_t$  values.

Expected results:

- Amplification should not be seen in no-template control (NTC) reactions.
- Amplification of the MS2 target should be seen in samples with bacteriophage control.
- Amplification of the Zika, Dengue, or Chikungunya target should be seen in samples when viral RNA is present.

## Dye spectral calibration plates, 96-well


See your instrument user guide for recommended calibration schedules and detailed calibration instructions.

Dye	Standard (0.2 mL)	Fast (0.1 mL)
ABY™	A24738	A24734
FAM™	4432327	4432389
JUN™	A24737	A24735
MUSTANG PURPLE™	4461599	4457328
VIC™	4432334	4432396

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Conditions of Sale found on Life Technologies' website at [www.thermofisher.com/us/en/home/global/terms-and-conditions.html](http://www.thermofisher.com/us/en/home/global/terms-and-conditions.html). If you have any questions, please contact Life Technologies at [www.thermofisher.com/support](http://www.thermofisher.com/support).

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Revision	Date	Description
B.0	16 April 2018	Updated the targets that have expected amplification.
A.0	30 March 2017	New document.

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