


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

Lyophilized reagents for multiplex real-time RT-PCR detection of Zika, Dengue, and Chikungunya virus RNA (0.2-mL block)

Catalog Number A31746

Pub. No. MAN0016006 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.

Product description

The TaqMan® Zika Virus Triplex 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 human endogenous control PPIA (Cyclophilin A), 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 PPIA 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 Kit (ZIKV/DENV/CHIKV) [Cat. No. A31746; 96 reactions]

Contents	Amount	Storage
Lyophilized assay, Standard, 0.2-mL tube	12 × 8-tube strips	<ul style="list-style-type: none"> • 18–28°C for up to 1 year^[1] • 2–8°C for long-term storage • Protect from moisture^[2]
MicroAmp™ Optical 8-Cap Strips	12 × 8-cap strips	Room temperature

^[1] Product is shipped at ambient temperature. See thermofisher.com/ambientshipping.

^[2] See “Procedural guidelines” on page 2.

Required materials

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

Item	Source
Applied Biosystems™ real-time PCR instrument and accessories, one of the following:	
QuantStudio™ instrument capable of detecting at least 5 colors ^[1] : <ul style="list-style-type: none"> • QuantStudio™ 5 Real-Time PCR System • QuantStudio™ 12K Flex Real-Time PCR System • QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System 	Contact your local sales office
7500 Real-Time PCR Instrument Precision Plate Holder for 0.2 mL Tubes and Strips (4367033)	Contact your local sales office
Equipment	
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
Plastics and consumables	
<i>(Optional)</i> MicroAmp™ Optical 8-Cap Strips ^[2]	4323032
<i>(Optional)</i> MicroAmp™ 8-Tube Strip, 0.2 mL ^[2]	N8010580
Aerosol-resistant micropipette tips	MLS
Disposable gloves	MLS
Reagents	
Nuclease-free Water	AM9938

^[1] Precision Plate Holder is included with the instrument.

^[2] 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 RNA isolation kits

Kit	Cat. No.	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	

Set up and run the reactions

- If necessary, 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	
PPIA	JUN™ dye	

- Load the tube strips and 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 PPIA target should be seen in samples with human RNA present.
- 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

Limited product warranty

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Revision history: Pub. No. MAN0016006

Revision	Date	Description
B.0	16 April 2018	<ul style="list-style-type: none">Updated the targets that have expected amplification.Update license information.
A.0	29 August 2016	New document.

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