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

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

Catalog Number A31747

Pub. No. MAN0016038 **Rev.** C.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. A31747; 96 reactions)

Contents	Amount	Storage
Lyophilized assay, Fast, 0.1-mL tube	12 × 8-tube strips	• 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/ambientshippping**.

[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]: • 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 Fast Real-Time PCR Instrument 7500 Fast Precision Plate Holder, for 0.1 mL Tube Strips (A29252)	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	
(Optional) MicroAmp™ Optical 8-Cap Strips[2]	4323032
(Optional) MicroAmp™ Fast 8-Tube Strip, 0.1 mL[2]	4358293
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

1. If necessary, adjust the total volume of RNA sample to 25 µL per reaction, using nuclease-free water.
2. Remove the cap of the 8-tube strip; discard the cap.
3. Add 25 µL of RNA sample to each tube, then firmly apply a new optical cap strip (provided in the kit).
4. Mix by flicking the tube strip several times or by vortexing briefly, then centrifuge briefly.
5. 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	
Chikingunya	ABY™ dye	
PPIA	JUN™ dye	

6. 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:

1. View the amplification plots.
2. Set the baseline and threshold values.
3. 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

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **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**.

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The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0016038

Revision	Date	Description
C.0	16 April 2018	Updated the targets that have expected amplification.
B.0	13 June 2017	Change product name; update instrumentation; update licensing.
A.0	29 August 2016	New document.