

TaqMan™ Arbovirus Triplex Kit (ZIKV/DENV/CHIKV), 0.2-mL Block

Lyophilized reagents for multiplex real-time RT-PCR detection of Zika, Dengue, and Chikungunya virus RNA

Catalog Number A31746

Pub. No. MAN0016006 Rev. D00



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™ Arbovirus 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™ Arbovirus 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 3 years^[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" indicates that the material is available from fisherscientific.com or another 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 | |
| (Optional) MicroAmp™ Optical 8-Cap Strips ^[2] | 4323032 |
| (Optional) 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” 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

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

| Calibration plates | Cat. No. |
|---|----------|
| QuantStudio™ 3/5 10-Dye Spectral Calibration Kit | A26343 |
| QuantStudio™ 3/5 Spectral Calibration Plate 1 | A26331 |
| QuantStudio™ 3/5 Spectral Calibration Plate 2 | A26332 |
| 7500 Real-Time PCR Systems Spectral Calibration Kit I | 4349180 |
| ABY™ Dye Spectral Calibration Plate | A24738 |
| FAM™ Dye Spectral Calibration Plate | 4432327 |
| JUN™ Dye Spectral Calibration Plate | A24737 |
| MUSTANG PURPLE™ Dye Spectral Calibration Plate | 4461599 |
| VIC™ Dye Spectral Calibration Plate | 4432334 |

Limited product warranty

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Revision history: Pub. No. MAN0016006 D00

| Revision | Date | Description |
|----------|----------------|---|
| D00 | 25 March 2024 | <ul style="list-style-type: none">• The legal manufacturer was updated.• The shelf life was updated to 3 years.• Dye and calibration plate products were updated. |
| C.0 | 17 August 2022 | Changed the kit name from Zika Virus to Arbovirus throughout. |
| 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. |

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

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