Instructions for Use

LyteStar™
Dengue Typing RT-PCR Kit
1.0

LyteStar™ Dengue Typing RT-PCR Kit 1.0

For detection and typing of Dengue 1, Dengue 2, Dengue 3 and Dengue 4 from human serum specimens

For use with

CFX96™ (BioRad)
CFX Opus 96 (BioRad)

IVD For *in vitro* diagnostic use

REF Product No.: 891003

96 reactions

Please refer to Storage and Shelf Life in this IFU

Ver. 03 / June 2025

AstronDX Technologies Sdn Bhd
Unit 307, Block B, Phileo Damansara 1, 9, Jalan 16/11,
46350 Petaling Jaya, Selangor, Malaysia

Content

1. Intended Use

The LyteStar™ Dengue Typing RT-PCR Kit 1.0 is intended for the simultaneous and specific detection of four serotypes of Dengue virus (DENV 1-4) RNA in human serum specimens. The LyteStar™ Dengue Typing RT-PCR Kit 1.0 is a four-target assay targeting the 5'UTR/capsid gene of DENV1 and DENV4, envelope (*E*) gene of DENV2 and protein membrane (*prM*) gene of DENV3.

The LyteStar™ Dengue Typing RT-PCR Kit 1.0 is for professional use only.

2. Kit Components

Catalog no.	891003
Master A	2 x 360 µl
Master B	4 x 300 µl
Internal Control (IC)	800 µl
Positive Control (PC)	400 µl
PCR grade water	500 µl

3. Storage and Shelf Life

- The LyteStar™ Dengue Typing RT-PCR Kit 1.0 has a shelf life of 12 months from the manufacturing date.
- Store all reagents at -15°C to -25°C upon arrival.
- Repeated thawing and freezing should be avoided, as this might affect the performance of the assay. Master B should be frozen in aliquots, if they are to be used intermittently.
- Mix Master A thoroughly by vortex mixing, and centrifuge briefly before use.
- Protect Master B from light.
- All frozen reagents should be completely thawed to room temperature before use. Immediately return unused portions to the freezer for storage.

4. Quality Control

In compliance with AstronDX Technologies' EN ISO 13485 certified Quality Management System, each lot of the LyteStar™ Dengue Typing RT-PCR Kit 1.0 is tested against pre-determined specifications to ensure consistent product quality.

5. Product Use Limitations and Precautions

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR procedures.
- Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Avoid microbial and nuclease (DNAse/RNAse) contamination of the specimen and the components of the kit.
- Always use DNAse/RNAse-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (i) specimen preparation, (ii) reaction set-up and (iii) amplification/detection activities.
- Workflow in the laboratory should proceed in unidirectional manner.
- Always wear disposable gloves in each area and change them before entering different areas.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not use components of the kit that have passed their expiration date.

- Discard sample and assay waste according to your local safety regulations.
- Wash hands thoroughly after handling specimens and test reagents.
- Do not use kits from different lots together.
- Do not use an expired kit.
- In case of damage to the packaging and leaking vials, do not use the kit (possible contamination or deterioration that can cause false interpretation).

6. Product Warranty

AstronDX Technologies guarantees the performance of the LyteStar™ Dengue Typing RT-PCR Kit 1.0 for applications as described in the manual. The user must determine the suitability of the product for the particular intended use. Should the product fail to perform satisfactorily in the described applications, please contact AstronDX Technologies Technical Support (**16. Technical Support**) for trouble-shooting.

AstronDX Technologies reserves the right to change, alter, or modify any product to enhance its performance and design.

7. Product Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles/face masks. For more information, please consult the appropriate material safety data sheets (MSDSs).

8. Introduction

Dengue viruses (DENV) are members of the genus *Flavivirus* in the family *Flaviviridae*. DENV are single stranded positive sense RNA viruses which are approximately 11kb in size. DENV is comprised of four antigenically distinct DENV serotypes (DENV1–DENV4), which co-circulate together in tropical and subtropical regions around the world. Genetic variations within the serotypes further divide the serotypes into distinct genotypes. DENV are transmitted by female mosquitoes of the species *Aedes aegypti* as the primary vector and *Aedes albopictus* as secondary vector.

DENV originally evolved in sylvatic cycles in Africa, South East Asia and South Asia between mosquitoes (Aedes) and non-human primates. Spillover from these

primates to humans in Africa and South East Asia occurred between 500-1000 years ago [1]. Currently, dengue is the most rapidly spreading mosquito-borne viral disease in the world and it is estimated that over one-third of the world's population is at risk for DENV infection, resulting in almost 400 million infections annually [2, 3].

Infection with one or more of the four closely related viruses results in a range of clinical manifestations spanning asymptomatic infection, dengue fever (symptoms may include nausea and vomiting, rashes, aches and pains, typically behind the eyes, also muscle and joint pain) and severe dengue which is a medical emergency and potentially life threatening in the form of Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) due to internal bleeding (low platelets) and acute dehydration and organ failure [4]. Infection with one serotype is thought to provide long-term immunity against the same serotype; but only short-term protection for other serotypes. Thus, individuals that had a dengue infection with one serotype are still at risk for secondary infection with the remaining three serotypes and are more likely to develop severe dengue [3]. There is no specific treatment or available vaccine and patients are only treated symptomatically. The diversity amongst genotypes and serotypes of DENV is one of the major challenges in the vaccine development. Treatment for dengue patients mainly focus on alleviating symptoms (aches, pain) and keeping the patient hydrated.

As a potentially life threatening disease without a specific treatment, it is imperative to detect a dengue infection fast and accurately and avoid misdiagnosis due to similar symptoms of other vector-borne diseases. Specifically in dengue endemic areas with high incidence it is important to identify the specific dengue serotype causing the infection to stratify the patient's risk of developing severe dengue. The LyteStar™ Dengue Typing RT-PCR Kit 1.0 was developed as a four-target assay, targeting the 5'UTR/capsid gene (DENV1 and 4), *E* gene (DENV2), and *prM* gene (DENV3) for specific detection of dengue virus RNA and accurate serotyping of DENV1-4.

[1] Wang, E. *et al*. (2000). Evolutionary relationships of endemic/epidemic and sylvatic dengue viruses. *Journal of Virology*. 74, 3227–3234

[2] WHO (2023) Dengue and severe dengue. https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue. (Last updated: 13 March 2023)

[3] Centers for Disease Control and Prevention, National Center for emerging and Zoonotic Infectious Disease (NCEZID), Division of Vector-Borne Diseases (DBVD). Available online https://www.cdc.gov/dengue/about/index.html

[4] WHO (2009). Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva, World Health Organization. Available online https://apps.who.int/iris/bitstream/handle/10665/44188/9789241547871_eng.pdf

9. Product Description

The LyteStar™ Dengue Typing RT-PCR Kit 1.0 is an *in vitro* diagnostic test system, based on real-time PCR technology, for the qualitative detection and differentiation of Dengue 1, Dengue 2, Dengue 3, and Dengue 4 virus (DENV1-4) specific RNA. The LyteStar™ Dengue Typing RT-PCR Kit 1.0 consists of a single tube assay targeting four genes; the 5'UTR/capsid gene specific for DENV1 and DENV4, envelope (*E*) gene specific for DENV2 and protein membrane (*prM*) gene specific for DENV3 genome. The LyteStar™ Dengue Typing RT-PCR Kit 1.0 includes a heterologous amplification system (Internal Control) to identify possible RT-PCR inhibition and to confirm the integrity of the reagents of the kit. The Internal Control template used in the LyteStar™ Dengue Typing RT-PCR Kit 1.0 is an *in vitro* transcribed RNA of an artificial sequence with no homology to any known genomes.

Real-time RT-PCR technology utilizes reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of specific target sequences, and target-specific probes for the detection of the amplified DNA. The probes are labelled with a fluorescent reporter and quencher dyes. The probes used for specific amplification of DENV1, DENV2, DENV3 and DENV4 are labelled with the fluorophore FAM, Tex615, Tye705 and Cy5™, respectively. The probe specific for the target of the Internal Control (IC) is labelled with the fluorophore HEX. Using probes linked to distinguishable dyes enables the parallel detection of DENV1, DENV2, DENV3 and DENV4 specific RNA and the Internal Control in the corresponding detector channels of the real-time PCR instrument.

The oligonucleotides included in the four assays were designed, modified, and based on the sequences/targets published in the articles listed below:

Target	**Publication**
DENV1	Kim and Hwang (2020)
DENV2 and DENV4	Mun *et al*., (2019)
DENV3	Johnson *et al*., (2005)
Internal Control	Deer *et al*., (2010)

The test consists of three processes in a single tube assay:

- Reverse transciption of target and Internal Control RNA to cDNA
- PCR amplification of target and Internal Control cDNA
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The LyteStar™ Dengue Typing RT-PCR Kit 1.0 consists of:

- Two Master reagents (Master A and Master B)
- The template of the Internal Control (IC)
- The template of the Positive Control (PC)
- PCR grade water (for setting up of “No Template Control”, NTC)

Master A and Master B reagents contain all components (buffer, enzymes, primers and probes) to allow PCR mediated reverse transcription, amplification and target detection of the 5’UTR/capsid gene of DENV1 specific RNA and of DENV4 specific RNA, envelope (*E*) gene of DENV2 specific RNA, protein membrane (*prM*) gene of DENV3 specific RNA, and Internal Control in one reaction setup.

The Positive Control (PC) contains *in vitro* transcripts of synthesized target genes of DENV1-4.

The Internal Control used in the LyteStar™ Dengue Typing RT-PCR Kit 1.0 is an *in vitro* transcribed RNA of an artificial sequence with no homology to any known genomes.

The LyteStar™ Dengue Typing RT-PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:

- CFX96™ (BioRad)
- CFX Opus 96 (BioRad)

10. Material and Devices required but Not Provided

- Appropriate real-time PCR instrument
- Appropriate nucleic acid extraction system or kit
- 1.5 ml microcentrifuge tubes (with safe-lock or screw cap)
- Microcentrifuge (with speed ≥ 13,000 rpm)
- Pipettes, adjustable (range: 10 µl, 100 µl, 200 µl, 1000 µl)

- Pipette tips (with aerosol barriers)
- Disposable gloves (powder-free)
- Heating block for lysis of specimens during extraction
- Vortex mixer
- Appropriate 96-well reaction plates or reaction tubes with corresponding (optical) closing material **(Clear tubes are recommended. Do not use white tubes)**

11. Specimen Storage

- Suitable specimens include serum, plasma, and whole blood. If the patients were suspected with central nervous system manifestations such as encephalopathy and aseptic meningitis, testing cerebrospinal fluid is recommended.

- Follow specimen transport and storage conditions outlined in the following guidelines:

  - World Health Organization (2000). Guidelines for the collection of clinical specimens during field investigation of outbreaks. https://apps.who.int/iris/handle/10665/66348

  - World Health Organization (2011). Comprehensive guidelines for prevention and control of dengue and dengue hemorrhagic fever. Revised and expanded edition. https://apps.who.int/iris/handle/10665/204894

  - Centers for Disease Control and Prevention (2020). Dengue - Testing Guidance. https://www.cdc.gov/dengue/healthcare-providers/testing/testing-guidance.html

12. Instructions for Use

12.1. Sample Preparation

Extracted RNA is the starting material for the LyteStar™ Dengue Typing RT-PCR Kit 1.0. The quality of the extracted RNA has a profound impact on the performance of the whole test system. It has to be ensured that the nucleic acid extraction system used is compatible with real-time PCR technology.

The following nucleic acid extraction kits / systems are suitable for use with the LyteStar™ Dengue Typing RT-PCR Kit 1.0:

- SpinStar™ Viral Nucleic Acid Kit (AstronDX Technologies)
- MagCore® Plus II Automated Nucleic Acid Extractor (RBC Bioscience)
- QIAamp® MiniElute Virus Spin Kit (Qiagen)
- QIAamp® Viral RNA Mini Kit (Qiagen)
- QIAsymphony® (Qiagen)

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with LyteStar™ Dengue Typing RT-PCR Kit 1.0 has to be validated by the user.

If using a spin column-based sample preparation procedure including washing buffers containing ethanol, an additional centrifugation step for 10 min at approximately 17000 × g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid is highly recommended.

NOTE

Ethanol is a strong inhibitor in real-time PCR. If your sample preparation system is using washing buffers containing ethanol, you need to make sure to eliminate any traces of ethanol prior to elution of the nucleic acid.

12.2. Master Mix Setup

1. All reagents and samples should be thawed completely, mixed (by gentle vortex mixing) and centrifuged briefly before use. Prepare a marginal excess (additional 0.5 reaction) of the required Master Mix volume.

2. The LyteStar™ Dengue Typing RT-PCR Kit 1.0 contains a heterologous Internal Control (IC), which can either be used as (i) a PCR inhibition control or as (ii) a control of the sample preparation procedure (nucleic acid extraction) and PCR inhibition control.

   (i) If the IC is used as a PCR inhibition control, but not as a control for the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions	1	12
Master A	7.5 µl	90 µl
Master B	12.5 µl	150 µl
IC	1.0 µl	12 µl
Volume Master Mix	**21 µl**	**252 µl**

(ii) If the IC is used as a control for the sample preparation procedure and as a PCR inhibition control, the IC has to be added during the nucleic acid extraction procedure.

No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture.

The volume of the IC which has to be added depends always and only on the elution volume. It represents **10% of the elution volume**. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC must be added into the specimen/lysis buffer mixture.

If the IC was added during the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions	1	12
Master A	7.5 µl	90 µl
Master B	12.5 µl	150 µl
Volume Master Mix	**20 µl**	**240 µl**

NOTE

⚠ ***Never add the Internal Control directly to the specimen.***

12.3. Reaction Setup

1. Pipette 20 µl Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.

2. Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Positive Control; or water as No Template Control, NTC).

3. Make sure at least one Positive Control and one NTC are used per run.

4. Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.

5. Close the 96-well reaction plate with an appropriate optical adhesive film and the reaction tubes with appropriate caps.

6. Centrifuge the 96-well reaction plate at 1,000 x g (~3,000 rpm) for 30s.

Reaction Setup	
Master Mix	20 µl
Sample or Control	10 µl
Total Volume	**30 µl**

13. Programming the Real-Time PCR Instrument

13.1 Settings

• Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default

13.2 Fluorescent Detectors (Dyes)

• Define the following fluorescent detectors:

Detection	Detector Name	Reporter	Quencher
DENV1 (5'UTR/capsid gene) specific RNA	DENV1	FAM	IBFQ
DENV2 (*E* gene) specific RNA	DENV2	Tex615	IBRQ
DENV3 (*prM* gene) specific RNA	DENV3	Quasar705	BHQ1
DENV4 (5'UTR/capsid gene) specific RNA	DENV4	Cy5	IBRQ
Internal Control	IC	HEX	IBFQ

13.3 Temperature Profile and Dye Acquisition

• Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature	Time
Reverse-transcription	Hold	1	-	50 °C	10:00 min
Denaturation	Hold	1	-	95 °C	2:00 min
Amplification	Cycling	45	-	95 °C	5 sec
			√	60 °C	30 sec

√ Signal acquisition: activate FAM, Tex615, Quasar705, Cy5 and HEX channels in all runs.

14. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the manual of the respective instrument. For detailed instructions regarding data analysis of the LyteStar™ Dengue Typing RT-PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (**16.Technical Support**).

14.1. Validity of Diagnostic Test Runs

14.1.1 Valid Diagnostic Test Runs (Qualitative)

For a **valid** diagnostic test run (qualitative), the following control conditions must be met:

Control ID	FAM / Tex615 / Quasar705 / Cy5 Detection Channel	HEX Detection Channel
Positive Control	POSITIVE	POSITIVE
Negative Control	NEGATIVE	POSITIVE

14.1.2 Target CT values of PC and IC

	Positive Control *(DENV1)*	Positive Control *(DENV2)*	Positive Control *(DENV3)*	Positive Control *(DENV4)*	Internal Control
Target CT value	< 35 cycles	< 35 cycles	< 35 cycles	< 35 cycles	≤ 40 cycles*

*Required for unknown samples that do not amplify in FAM, Tex615, Quasar705, and Cy5 channels

Note: The above CT target values are exclusively given for **monitoring the integrity of the product and validated assay conditions** and should be achieved **ONLY for the provided Positive Control (PC) and Internal Control (IC)** when used as per the instructions given under section 12.3. Reaction set up. **The target CT values for PC MUST NOT be misinterpreted as the diagnostic cut-off values for clinical samples.**

14.1.3 Invalid Diagnostic Test Runs (Qualitative)

A **qualitative** diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, **repeat testing by using the remaining purified nucleic acids** or start from the original samples again.

14.2 Interpretation of Results

FAM *DENV1*	Tex615 *DENV2*	Quasar 705 *DENV3*	Cy5 *DENV4*	HEX *Internal Control*	Result Interpretation
+	+	+	+	+*	Dengue 1, Dengue 2, Dengue 3, and Dengue 4 specific RNA detected. *Positive for Dengue 1, Dengue 2, Dengue 3, Dengue 4*
-	+	+	+	+*	Dengue 2, Dengue 3, and Dengue 4 specific RNA detected. *Positive for Dengue 2, Dengue 3, Dengue 4*
+	-	+	+	+*	Dengue 1, Dengue 3, and Dengue 4 specific RNA detected. *Positive for Dengue 1, Dengue 3, Dengue 4*
+	+	-	+	+*	Dengue 1, Dengue 2, and Dengue 4 specific RNA detected. *Positive for Dengue 1, Dengue 2, and Dengue 4*
+	+	+	-	+*	Dengue 1, Dengue 2, and Dengue 3 specific RNA detected. *Positive for Dengue 1, Dengue 2, and Dengue 3*
+	+	-	-	+*	Dengue 1 and Dengue 2 specific RNA detected. *Positive for Dengue 1 and Dengue 2*
+	-	+	-	+*	Dengue 1 and Dengue 3 specific RNA detected. *Positive for Dengue 1 and Dengue 3*

+	-	-	+	+*	Dengue 1 and Dengue 4 specific RNA detected. *Positive for Dengue 1 and Dengue 4*
-	+	+	-	+*	Dengue 2, Dengue 3 specific RNA detected. *Positive for Dengue 2 and Dengue 3*
-	+	-	+	+*	Dengue 2 and Dengue 4 specific RNA detected. *Positive for Dengue 2 and Dengue 4*
-	-	+	+	+*	Dengue 3, Dengue 4 specific RNA detected. *Positive for Dengue 3 and Dengue 4*
+	-	-	-	+*	Dengue 1 specific RNA detected. *Positive for Dengue 1*
-	+	-	-	+*	Dengue 2 specific RNA detected. *Positive for Dengue 2*
-	-	+	-	+*	Dengue 3 specific RNA detected. *Positive for Dengue 3*
-	-	-	+	+*	Dengue 4 specific RNA detected. *Positive for Dengue 4*
-	-	-	-	+	Dengue 1, Dengue 2, Dengue 3, and Dengue 4 specific RNA not detected. The samples do not contain detectable amounts of Dengue 1, Dengue 2, Dengue 3, and Dengue 4 specific RNA.
-	-	-	-	-	PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

Note: For Dengue 1 5'UTR/capsid gene (FAM channel), Dengue 2 *E* gene (Tex615 channel) Dengue 3 *prM* gene (Quasar705 channel) and Dengue 4 5'UTR/capsid gene (Cy5 channel) "+" refers to amplification curve detected at CT ≤ 45 cycles. "-" refers to no amplification / no CT obtained.

* Detection of the Internal Control in the HEX channel is not required for positive results in the FAM/Tex615/Quasar705/Cy5 detection channels. A high Dengue viral load in the sample can lead to reduced or absent Internal Control signals.

14.2.1 Baseline Settings for Cycler Software

After the run is completed, normalize fluorescence signals in all channels by selecting "Apply Fluorescence Drift Correction" from the Baseline Setting menu.

14.2.2 Threshold Settings for Cycler Software

Cycler	Threshold				
	FAM DENV1 Channel	Tex615 DENV2 Channel	Quasar705 DENV3 Channel	Cy5 DENV4 Channel	HEX IC Channel
CFX96™	100 RFU	100 RFU	100 RFU	100 RFU	100 RFU
CFX Opus 96	100 RFU	100 RFU	100 RFU	100 RFU	100 RFU

14.2.3 CT Cut-Off Values for Clinical Samples

	FAM DENV 1 Channel	TEX615 DENV 2 Channel	Quasar705 DENV 3 Channel	Cy5 DENV 4 Channel
CT Cut-Off Value	< 45 cycles	< 45 cycles	< 45 cycles	< 45 cycles

15. Performance Evaluation

The analytical performance evaluation of the LyteStar™ Dengue Typing RT-PCR Kit 1.0 was accomplished using quantified DENV1, DENV2, DENV3 and DENV4 specific RNA.

15.1 Analytical Sensitivity

The analytical sensitivity (limit of detection: LoD) of LyteStar™ Dengue Typing RT-PCR Kit 1.0 is defined as the concentration of DENV1, DENV2, DENV3, and/or DENV4 RNA molecules that can be detected with a positivity rate of ≥ 95%. The analytical sensitivity was determined by analyzing DENV1-4 genomic RNA of known concentration.

A dilution series of the AmpliRun® Dengue 1, 2, 3 and 4 Virus RNA control was prepared by using 1× TE buffer as diluent. Dilutions of DENV1-4 RNA were tested with LyteStar™ Dengue Typing RT-PCR Kit 1.0. Results were analyzed by Probit analysis (Table 1, Table 2, Table 3, and Table 4).

The analytical sensitivity of the LyteStar™ Dengue Typing RT-PCR Kit 1.0 in combination with the CFX Opus 96 (BioRad) platform was determined at 2.01 copies/µl for Dengue 1 5'UTR/capsid gene target, 2.88 copies/µl for Dengue 2 *E* gene target, 1.56 copies/µl for Dengue 3 *prM* gene target, and 2.67 copies/µl for Dengue 4 5'UTR/capsid gene target (p≤ 0.05).

Table 1. PCR results used for the calculation of the analytical sensitivity of Dengue 1 5'UTR/capsid gene target for the LyteStar™ Dengue Typing RT-PCR Kit 1.0 in combination with the CFX Opus 96 platform (BioRad).

Concentration (copies/µl)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
20.0	12	12	100
6.3	12	12	100
2.0	12	12	100
0.63	12	7	58.3
0.20	12	4	33.3
0.063	12	1	8.3
0.020	12	0	0
0.0063	12	0	0

Table 2. PCR results used for the calculation of the analytical sensitivity of Dengue 2 *E* gene target for the LyteStar™ Dengue Typing RT-PCR Kit 1.0 in combination with the CFX Opus 96 platform (BioRad).

Concentration (copies/µl)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
20.0	12	12	100
6.3	12	12	100
2.0	12	4	33.3
0.63	12	0	0
0.20	12	0	0
0.063	12	0	0
0.020	12	0	0
0.0063	12	0	0

Table 3. PCR results used for the calculation of the analytical sensitivity of Dengue 3 *prM* gene target for the LyteStar™ Dengue Typing RT-PCR Kit 1.0 in combination with the CFX Opus 96 platform (BioRad).

Concentration (copies/µl)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
20.0	12	12	100
6.3	12	12	100
2.0	12	12	100
0.63	12	7	58.3
0.20	12	2	16.7
0.063	12	0	0
0.020	12	0	0
0.0063	12	0	0

Table 4. PCR results used for the calculation of the analytical sensitivity of Dengue 4 5'UTR/capsid gene target for the LyteStar™ Dengue Typing RT-PCR Kit 1.0 in combination with the CFX Opus 96 platform (BioRad).

Concentration (copies/µl)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
20.0	12	12	100
6.3	12	12	100
2.0	12	5	41.7
0.63	12	0	0
0.20	12	0	0
0.063	12	0	0
0.020	12	0	0
0.0063	12	0	0

15.2 Analytical Specificity

The analytical specificity of the LyteStar™ Dengue Typing RT-PCR Kit 1.0 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that the applied primer/probes in LyteStar™ Dengue Typing RT-PCR Kit 1.0 specifically detect Dengue 1, Dengue 2, Dengue 3, and Dengue 4 virus.

The specificity of the LyteStar™ Dengue Typing RT-PCR Kit 1.0 was evaluated by testing genomic RNA/DNA extracted from other pathogens likely to be present in the same sample material as Dengue virus, or that cause similar symptoms to these viruses (Table 5).

Table 5. Microorganisms tested to demonstrate the analytical specificity of the LyteStar™ Dengue Typing RT-PCR 1.0.

LyteStar™ Dengue Typing RT-PCR Kit 1.0					
Organisms	***DENV1 (FAM channel)***	***DENV2 (Tex615 channel)***	***DENV3 (Quasar 705 channel)***	***DENV4 (Cy5 channel)***	***Internal Control (HEX channel)***
West Nile Virus	negative	negative	negative	negative	valid
Ross River Virus	negative	negative	negative	negative	valid
Japanese Encephalitis Virus	negative	negative	negative	negative	valid
Yellow Fever Virus	negative	negative	negative	negative	valid
Chikungunya Virus	negative	negative	negative	negative	valid
Zika virus (Asia lineage)	negative	negative	negative	negative	valid
Zika virus (Africa lineage)	negative	negative	negative	negative	valid
Epstein Barr Virus	negative	negative	negative	negative	valid
Parvovirus B19	negative	negative	negative	negative	valid
Rickettsia conorii	negative	negative	negative	negative	valid
Rickettsia monacensis	negative	negative	negative	negative	valid
Chlamydophila pneumonia	negative	negative	negative	negative	valid
Borrelia burgdorferi	negative	negative	negative	negative	valid
Streptococcus pyogenes	negative	negative	negative	negative	valid
Streptococcus pneumonia	negative	negative	negative	negative	valid
Orientia tsutsugamushi	negative	negative	negative	negative	valid
Enterovirus 71	negative	negative	negative	negative	valid
Human herpesvirus 5	negative	negative	negative	negative	valid
Dengue 1	positive	negative	negative	negative	valid
Dengue 2	negative	positive	negative	negative	valid
Dengue 3	negative	negative	positive	negative	valid
Dengue 4	negative	negative	negative	positive	valid

The LyteStar™ Dengue Typing RT-PCR Kit 1.0 did not cross-react with any pathogen or genotype/subtype other than its own target.

15.3 Diagnostic Sensitivity and Specificity

The clinical performance of the LyteStar™ Dengue Typing RT-PCR Kit 1.0, in regards to diagnostic sensitivity and specificity, was evaluated through a retrospective study at a public laboratory. The diagnostic sensitivity and specificity of the LyteStar™ Dengue Typing RT-PCR Kit 1.0 was compared to the clinical site's reference method.

In comparison to the reference test, all samples tested with 100% concordance. The LyteStar™ Dengue Typing RT-PCR Kit 1.0 achieved 100% diagnostic sensitivity and 100% diagnostic specificity for the detection of Dengue 1, Dengue 2, Dengue 3, and Dengue 4.

		Reference Method			
		DENV1 (n=8)	DENV2 (n=8)	DENV3 (n=5)	DENV4 (n=7)
		Positive			
LyteStar™ Dengue Typing RT-PCR Kit 1.0	Detected	8	8	5	7
	Not Detected	0	0	0	0
	Total	**8**	**8**	**5**	**7**
Overall Concordance		100 % 8 / 8 Sensitivity	100 % 8 / 8 Sensitivity	100 % 5 / 5 Sensitivity	100.0 % 7 / 7 Sensitivity
		Reference Method			
		DENV1 (n=10)	DENV2 (n=10)	DENV3 (n=10)	DENV4 (n=10)
		Negative			
LyteStar™ Dengue Typing RT-PCR Kit 1.0	Detected	0	0	0	0
	Not Detected	10	10	10	10
	Total	**10**	**10**	**10**	**10**
Overall Concordance		100 % 10 / 10 Specificity	100 % 10 / 10 Specificity	100 % 10 / 10 Specificity	100 % 10 / 10 Specificity

To calculate the diagnostic sensitivity of LyteStar™ Dengue Typing RT-PCR Kit 1.0:

$$\text{Diagnostic Sensitivity [\%]} = \frac{\text{“correct positive”}}{\text{(“correct positive” + “false positive”)}} \times 100$$

To calculate the diagnostic specificity of LyteStar™ Dengue Typing RT-PCR Kit 1.0:

$$\text{Diagnostic Specificity [\%]} = \frac{\text{“correct negative”}}{\text{(“correct negative” + “false negative”)}} \times 100$$

16. Technical Support

For customer support, please contact our Technical Support:

e-mail: **techsupport@astrondx.com**
phone: **+603 7931 6760**

17. Appendix

Explanation of Symbols

Symbol	Explanation
	*In vitro* diagnostic medical device
	Product Number
	Batch Code
	Manufacturer
	Date of Manufacture
	Contains sufficient for “n” tests/rxns
	Temperature limitation
	Version
	Use-By Date

18. Ordering Information

Products	Packing (reactions)	Order No.
LyteStar™ Dengue Typing RT-PCR Kit 1.0	96	891003
SpinStar™ Viral Nucleic Acid Extraction Kit 1.0	100	811803
MagCore® Viral Nucleic Acid Extraction Kit, High Sensitivity (200µl/400µl), CART CODE 203	96	MVN400-06

NOTES

NOTES

NOTES

AstronDX Technologies Sdn Bhd [200901035495 (878612-H)]
Unit 307, Block B, Phileo Damansara 1,
9, Jalan 16/11, 46350 Petaling Jaya,
Selangor, Malaysia

phone +603 7931 6760
fax +603 7931 5352
email info@astrondx.com

www.astrondx.com