TIANLONG

Human Influenza Virus B (Yamagata&Victoria) and A (H1&H3) Nucleic Acid Multiplex Detection Kit (Fluorescence PCR Method)

Σ P255H
25

Σ P655H
50

User Guide

Version 3.1

CE IVD

In-Vitro Diagnostics / For use with Real-time PCR Instruments compatible with Human Influenza Virus B (Yamagata &Victoria) and A (H1&H3) Nucleic Acid Multiplex Detection Kit (Fluorescence PCR Method)

REF P255H/P655H

Suzhou TianLong Biotechnology Co., Ltd.

Building NE-33, Nanopolis, No.99 Jinjihu Avenue,

Suzhou Industrial Park, China (Jiangsu) Pilot Free Trade Zone,

215123, Suzhou, PEOPLE'S REPUBLIC OF CHINA

EC REP **SUNGO Europe B.V.**

Fascinatio Boulevard 522, Unit 1.7, 2909VA Capelle aan den IJssel, The Netherlands

IFU_P255H/P655H_EN_May. 2024

This page is intentionally left blank

Contents

Version	3.1
Change	Symbols

Introduction

Influenza Virus (IVs) belongs to Orthomyxoviridae, a single-stranded negative-stranded RNA enveloped virus. Human IVs are divided into types A, B, and C, which are the pathogens of influenza (flu). Among them, influenza A and B viruses are more pathogenic to humans. According to the different antigens of H and N, influenza A viruses are divided into many subtypes. H can be divided into 18 subtypes and N has 11 subtypes. Influenza B virus is divided into two strains: Victoria strain and Yamagata strain. At present, the main human infections are the H1, H3 subtypes of influenza A viruses and the Victoria and Yamagata strains of influenza B viruses.
The pathogenicity of pathogens is different, and the corresponding epidemic control strategies, clinical treatment procedures and results of infections caused by different pathogens may be significantly different. Since it is not easy to distinguish different infections based on clinical symptoms alone, and there are a large number of asymptomatic influenza virus infections, it is of great significance to detect specific subtypes of influenza virus infection and carry out adequate clinical management and epidemic control procedures as early as possible. The star product for respiratory viruses is Human Influenza Virus B (Yamagata&Victoria) and A (H1&H3) Nucleic Acid Multiplex Detection Kit developed by TianLong Biotechnology is intended for the rapid and accurate detection of Influenza Virus B (Yamagata&Victoria) and A (H1&H3) RNA, assisting in the diagnosis and treatment of respiratory disease patients, as well as public healthcare management.

Intended Use

The TianLong ***Human Influenza Virus B (Yamagata&Victoria) and A (H1&H3) Nucleic Acid Multiplex Detection Kit*** is intended for the qualitative detection of Influenza Virus B (Yamagata&Victoria) and A (H1&H3) RNA by Real-time Reverse Transcription Polymerase Chain Reaction (Real-time RT-PCR) method.

The test is designed to detect Influenza Virus B (Yamagata&Victoria) and A (H1&H3) RNA in specimens such as nasopharyngeal or oropharyngeal swabs collected from individual personnel based on clinical and/or epidemiological criteria. Other sample types from the upper and lower respiratory tract (such as sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate), as well as samples collected from the environment, may serve the purpose of virus detection as well if adequate Influenza Virus B (Yamagata&Victoria) and A (H1&H3) RNA could be extracted from the samples. The potential candidate personnel to be tested could include, but are not limited to patients with symptoms of infection who are suspected of Influenza Virus B and A by healthcare providers, or persons with no symptoms from a population for screening test confirmation or exclusion of viral infection or viral carriage status.

Positive test results are indicative of the presence of Influenza Virus B (Yamagata&Victoria) and A (H1&H3) RNA; whereas clinical correlation with patient history and other diagnostic information is necessary for the determination of patient infection status. Positive results from this test do not rule out bacterial infection or co-infection with other viruses.

Negative test results from the test do not completely preclude Influenza Virus B (Yamagata&Victoria) and A (H1&H3) RNA presence and should not be used as the sole basis for patient management decisions. Negative results must be used in combination with clinical observations, patient history, and epidemiological information for a medical decision.

The TianLong ***Human Influenza Virus B (Yamagata&Victoria) and A (H1&H3) Nucleic Acid Multiplex Detection Kit*** is intended for use by qualified clinical laboratory professionals trained in the techniques of Real-time PCR and in vitro diagnostic procedures. The TianLong ***Human Influenza Virus B (Yamagata&Victoria) and A (H1&H3) Nucleic Acid Multiplex Detection Kit*** is for use in qualified clinical labs which are in compliance with guidelines and regulations from corresponding professional organizations and government administrations.

The TianLong ***Human Influenza Virus B (Yamagata&Victoria) and A (H1&H3) Nucleic Acid Multiplex Detection Kit*** is to be used with Real-time PCR instruments with 4 or more fluorescence detection channels, which the test performance of the kit has been validated on. These Real-time PCR thermal cyclers have appropriate fluorescence reading channels for FAM, VIC (HEX), ROX (Texas Red), Cy5 channels such as Applied Biosystems™ 7500 Real-Time PCR Systems and Tianlong Gentier Real-time PCR Systems.

Principles of the Assay

The assay is designed to target the conserved regions of Influenza Virus B Yamagata/Victoria and A H1/H3 RNA genes in order to achieve the most consistent performance throughout epidemic evolution.

The specific primers and probes set are designed, and the different fluorophores labels are used for multiplex detection of Influenza Virus B Yamagata/Victoria and A H1/H3 RNA genes. Nucleic acids are extracted from designated samples with adequate methods and materials. After reverse transcription, the presented target sequences will be amplified by respective specific primers and detected by TaqMan probes. During the extension phase of each PCR cycle, the Taq polymerase cleave reporter dye off the 5' end of the probe, separating dye from the quencher, producing fluorescent signals. The increasing fluorescence signals are detected over the PCR course and data analyzed.

Reagent Kit

Reagent Kit Components

Reagents for 25/50 tests (RT-PCR reactions) are contained in one reagent kit box.

RT-PCR reagents	Volume(25T/50T)	In Tube(25T/50T)
REAG 1	425 µL/850 µL	1 tube/1 tube
REAG 2	38 µL/75 µL	1 tube/1 tube
REAG 3	38 µL/75 µL	1 tube/1 tube
Controls		
CONTROL +	40 µL/40 µL	1 tube/1 tube
CONTROL -	40 µL/40 µL	1 tube/1 tube

Note: Mix matching and use of the reagent components from different reagent lots should be avoided unless be specifically instructed to do so. The negative control could also be referred to as a "No Target Control" (NTC).

Reagent Storage, Shipment, and Handling

All reagents should be stored at the temperature between -25°C to -15°C in a non-frost-free freezer for use before the expiration date. Freeze/Thaw more than three times should be avoided during the kit usage period. The reagents should be shipped at the temperature between -25°C to 8°C.

Assay Procedures

Before Starting

- Check reagent components and supplies to ensure there are enough materials ready for planned work.
- Check to ensure equipment and instruments are ready for work.
- Follow the up-to-date instructions for use.
- Complete appropriate planning and calculations for coming testing.
- Finally, complete the testing procedures as outlined below.

Sample Requirements

The ***Human Influenza Virus B (Yamagata&Victoria) and A (H1&H3) Nucleic Acid Multiplex Detection Kit*** is designed to detect RNA from Influenza Virus B and A in specimens such as oropharyngeal swab collected from individuals based on clinical and/or epidemiological criteria. Other sample types from upper and lower respiratory tract (such as sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate), as well as samples collected from environment, may serve the purpose for virus detection normally if adequate Influenza Virus B and A RNA could be extracted from the samples.

Use the specimen collection, transportation, storage medium following reagent manufacturer instructions.

Perform nucleic acid extraction following reagent manufacturer instructions.

Equipment and Instruments Required but not Provided

- Micropipette dedicated for assay setup (1-10 or 1-20 µL; 20-200 µL; 1000 µL).
- Refrigerated benchtop centrifuge with rotor for 0.5 mL/1.5 mL reaction tubes (capable of attaining 10,000 rpm)
- Benchtop vortex mixer.
- It is recommended to use a detection kit with Real-time PCR thermal cyclers with appropriate fluorescence reading channels for FAM, ROX (Texas Red), VIC (HEX), Cy5 dyes such as Applied Biosystems™ 7500 Real-Time PCR Systems and Tianlong Gentier real-time PCR Systems.

Note:
Equipment and instruments should be maintained and calibrated according to the manufacturer's recommendations.
Refer to manufacturer's manuals for operation procedures.

Nucleic Acid Extraction

TianLong ***Human Influenza Virus B (Yamagata&Victoria) and A (H1&H3) Nucleic Acid Multiplex Detection Kit*** is compatible with RNA /nucleic acids of adequate quality prepared from intended samples using common RNA/nucleic acid extraction kits/methods. The prepared RNA/nucleic acids can be used directly as sample RNA/nucleic acid material, moved forward to the Real-time RT-PCR reaction setup step.

Positive Control and Negative Control do not need to be extracted and tested directly in each Real-time RT-PCR assay Run.

If under certain circumstances prepared RNA/nucleic acids need to be frozen stored for a later time testing, storage in a freezer of -70°C or lower is recommended whenever possible for minimal nucleic acid degradation during storage.

Repeated Freeze/Thaw of prepared sample RNA/nucleic acids should be avoided whenever possible.

Real-time RT-PCR Reaction Setup

1. Thaw the following reagents on ice: ***REAG 1***, ***REAG 2*** and ***REAG 3***. Gently invert to mix each individual reagent, then briefly centrifuge (2000 rpm, 10 s) to let solutions be settled to the bottom of tubes before moving to the next step.
2. Prepare Premix solution based on the planned number of samples to be tested.
   To calculate the volume of each reagent component required for Premix preparation, it needs to cover all the samples and controls to be tested in the assigned assay Run with reasonable extra set aside for operational tolerance.
   In many cases, preparing Premix with 10-20% extra volume is a good practice.
   A demo calculation worksheet for Premix Preparation is listed below for reference:

Table 1 Calculation of Premix Preparation – A Demo Worksheet

Component Reagents	For 1 test	For 10 tests	For 40 tests	For 80 tests	For 100 tests
REAG 1	17 µL	170 µL	680 µL	1360 µL	1700 µL
REAG 2	1.5 µL	15 µL	60 µL	120 µL	150 µL
REAG 3	1.5 µL	15 µL	60 µL	120 µL	150 µL
Total volume	**20 µL**	**200 µL**	**800 µL**	**1600 µL**	**2000 µL**

3. 96-well PCR reaction plates or PCR reaction tube stripes could be used for reaction setup. Evenly aliquot 20 µL of the prepared Premix into each PCR tube. Add 5 µL of each extracted RNA/nucleic acid solution to the designated PCR tube. Add 5 µL of CONTROL + and CONTROL - to the respectively assigned tubes.
   At the end of setup, Each PCR tube shall have a total volume of 25 µL.
   Then immediately close/cover the tubes and transfer the reaction setup tube stripes/plate into a Real-time PCR cycler for amplification reactions.

Positive Control and Negative Control must be run in each assay Run.

Thermal Cycler Settings

Real-time RT-PCR cycling program:

Table 2 qPCR Cycling program

Stage	No. of cycles	Temperature	Duration
1	1	50 ℃	30 min
2	1	95 ℃	10 min
3	5	94 ℃	15 s
		50 ℃	30 s
		72 ℃	30 s
4	40	94 ℃	10 s
		58 ℃	30 s (fluorescence detection)

Assignment for Fluorescence Detection Channels:

- FAM channel for IFVB Yamagata type
- HEX/VIC channel for IFVB Victoria type
- ROX/Texas Red channel for IFVA H1 type
- Cy5 channel for IFVA H3 type

Result Analysis and Interpretation

For data analysis, a Fluorescence Threshold Setting needs to be assigned.

- Auto Setting: the instrument automatically sets the threshold value. Auto Setting is recommended for routine operations and data analysis.
- Manual Setting: in case the manual setting is desired under certain circumstances, the threshold value could be set just above the fluorescence baseline of the normal negative control.

Run Validity Check

All tests performed on one batch setup through the whole course of Real-time RT-PCR are considered in one Run. Only results from valid test Runs are moved forward for analysis and interpretation.

Test Run is valid when

1) In the Run/experiment there is no Ct value generated for all channels in the negative control.
2) The Ct values for all channels in the positive control are less than 30.

Results from valid test Runs could be further analyzed for reports.

If the results of the controls do not meet the validity criteria outlined in 1) and 2), the test Run is usually considered invalid. All samples involved in the test Run need to be retested for reportable results.

The analysis and interpretation of test results

FAM (IFVB Yamagata Type)	HEX/VIC (IFVB Victoria type)	CY5 (IFVA H3 type)	ROX/Texas Red (IFVA H1 type)	Result
Ct≤37	Ct≤37	Ct≤37	Ct≤37	**Positive**
No Ct value or Ct = 40	No Ct value or Ct = 40	No Ct value or Ct = 40	No Ct value or Ct = 40	**Negative**
37<Ct≤40	37<Ct≤40	37<Ct≤40	37<Ct≤40	**The sample should be retested.**

Assay Performance Characteristics

The following performance characteristics of the TianLong ***Human Influenza Virus B (Yamagata&Victoria) and A (H1&H3) Nucleic Acid Multiplex Detection Kit*** have been established as described below.

Non-clinical Studies

- Limit of detection:
  500 copies/mL
- Specificity: Cross reaction among enterovirus 71, human parainfluenza virus 1, human parainfluenza virus 2, human parainfluenza virus 3, adenovirus 3, adenovirus 7, RSV-B, and measles virus was not observed. Also the kit was used to assess potential cross-reactivity with Human adenovirus 3/7, Respiratory syncytial virus B, Human parainfluenza virus 1/2/3, Enterovirus 71, Coxsackie virus 16, Mycoplasma pneumoniae, Chlamydia pneumoniae, Rhinovirus, Human cytomegalovirus, Human partial lung virus, Human coronavirus OC43/229E, Human coronavirus NL63/HKU1, EB virus, Measles virus, Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Mycobacterium tuberculosis, Pseudomonas aeruginosa, Streptococcus saliva, Streptococcus pyogenes, Neisseria meningitidis, Bordetella pertussis, and other related pathogens, wherein the concentration of virus is above 10^5pfu/mL, and the concentration of bacteria is more than 10^6 cfu/mL. Negative results were obtained from all above-mentioned organisms.
- Precision: The assay was used to respectively detect the precise reference specimens of high and low concentrations in different time ranges 10 times, and the precision values of intra and inter Ct values were all ≤5.0%.

Quality Control

In accordance with the ISO 13485:2016 Medical devices— Quality management systems and TianLong ***Human Influenza Virus B (Yamagata&Victoria) and A (H1&H3) Nucleic Acid Multiplex Detection Kit*** Quality Control Program, each batch of the ***Human Influenza Virus B (Yamagata&Victoria) and A (H1&H3) Nucleic Acid Multiplex Detection Kit*** is tested against predetermined specifications to ensure consistent product quality.

Limits and Precautions

Limits

- All reagents in the kit are intended for in vitro diagnostic use as indicated.
  The test should be carried out by professionals adequately trained in IVD lab practices. It is the user's responsibility to verify/validate the testing system performance in their respective laboratory settings. Expired reagents should not be used.
- Strict compliance with the IFU is required for optimal results. Deviation from standard procedures during sample collection, preservation, transportation, processing, and testing could lead to false negative or false positive testing results.
- Theoretically, variations in the target sequences of Influenza Virus B and A arise from natural mutations

that could potentially influence testing performance and result in false testing results. Up to today, results from bioinformatics analysis and comprehensive laboratory studies indicate that, partially due to the emphasis on mutation tolerance concept during assay design and development, this kit could tolerate currently known Influenza Virus B and A mutations without obvious compromise on assay performance.

- Test results should be used in combination with clinical and epidemic information for medical decisions.

Laboratory Precautions

Laboratories using the assay should be ISO 15189 qualified and/or in compliance with local regulations.

Use extreme caution to prevent:

- **RNase contamination that may cause degradation of the template RNA.**
- **Amplicon contamination that may result in false positive test results.**

The following are recommended for desirable test performance:

- Use DNase/RNase-free disposable pipette tips, tubes, and supplies as appropriate.
- A standard PCR Lab Suite under workflow and air pressure control would be desirable for testing use. If not available, separated/segregated working areas could be used with precaution for contamination control:
  1) Reagent preparation area: preparing the reagents for amplification,
  2) sample preparation area: extraction and separation of the RNA/nucleic acids from samples and controls,
  3) Amplification area: amplification and detection of the nucleic acid target.
- Perform regular decontamination practice and cleaning of working areas, equipment, and instruments. Commercially available cleaning products containing sodium hypochlorite, 75% alcohol, and ultraviolet light could be applied for the purpose of cleaning and decontamination.
- Nucleic acid samples should be stored at -70℃or lower for long term storage.
- Equipment such as micropipette needs to be calibrated per the manufacturer's recommendation.
- The Real-time PCR instrument needs calibration per manufacturer's schedule.
- The handling and management of samples and lab wastes should be in compliance with relevant guidelines recommended by professional organizations and regulations imposed by authorities.

Symbols

The following table describes the symbols that may appear on the labeling or in this document.

	Catalog number
	Batch code
	Contains reagents sufficient for <N> tests
	Use-by date
	Date of manufacture
	Caution
	Temperature limit
	In vitro diagnostic medical device
	Manufacturer
	Conformed with EU standard
	Authorized representative in the European Community

Consult instructions for use

Keep away from sunlight

Fragile handle with care

CONT	Content of the Kit
REAG 1	P255H/P655H Rx Sol (IFVA/B Genotype Reaction Solution)
REAG 2	P255H/P655H EM (IFVA/B Genotype Enzyme Mix)
REAG 3	P255H/P655H P&P (FVA/B Genotype Primer and Probe Mix)
CONTROL +	P255H/P655H PC (FVA/B Genotype Positive Control)
CONTROL -	P255H/P655H NC (FVA/B Genotype Negative Control)

Recycling symbol PAP21: non corrugated cardboard

References

1. A multiplex reverse transcription-PCR assay for the detection of influenza A virus and differentiation of the H1, H3, H5 and H9 subtypes. Wu et al. Journal of Virological Methods, 188(1-2), 47 – 50. doi: 10.1016/j.jviromet.2012.11.032
2. Ten influenza seasons in France: distribution and timing of influenza A and B circulation, 2003 – 2013. Mosnier et al. BMC Infectious Diseases, 2015, 15(1), 357–. doi:10.1186/s12879-015-1056-z
3. Processing of Sputum Specimens for Nucleic Acid Extraction https://www.cdc.gov/coronavirus/2019-ncov/downloads/processing-sputum-specimens.pdf
4. Interim Clinical Guidance for Management of Patients with Confirmed Coronavirus Disease (COVID-19) https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-guidance-management-patients.html
5. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline-second edition. Clinical & Laboratory Standard Institute (CLSI): EP12-A2, 2008
6. Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline; Clinical & Laboratory Standard Institute (CLSI): EP17A

Contact Information

For technical assistance and more information, please contact with our Technical Support Center at +86-29-82682132 (Tel), +86-512-62956337 (Fax), inquiry@medtl.com (Mail) or contact your local distributor.

For up-to-date licensing information or product-specific disclaimers, please see the respective User Guide. TianLong User Guides are available at www.medtl.net or requested from TianLong Technical Services or the local distributor.