Masern virus, Rubella virus, and Mumps virus Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)

Reference Number

S3279E-24, S3279E-48

Product Name

Masern virus, Rubella virus, and Mumps virus Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)

Package Specification

24 tests/kit, 48 tests/kit

Intended Use

The Masern virus, Rubella virus, and Mumps virus Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from Masern virus (MeV), Rubella virus (RV), and Mumps virus (MuV) in oropharyngeal swabs, whole blood, and saliva from individuals who are suspected of MeV/RV/MuV infection. Results are for the identification of MeV/RV/MuV RNA, and should not be used as the sole basis for patient management decisions.

The Masern virus, Rubella virus, and Mumps virus Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is intended for use by professional, qualified, trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

For *in vitro* diagnostic use only. For professional use only.

Summary

The Masern virus, Rubella virus, and Mumps virus RNA are generally detected in oropharyngeal swabs, whole blood, and saliva specimens during the acute phase of infection. Positive results are indicative of the presence of Masern virus, Rubella virus, and Mumps virus RNA, clinical correlation with medical history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Negative results do not preclude Masern virus, Rubella virus, and Mumps virus infection. Negative results must be combined with clinical observations, medical history, and epidemiological information.

Test Principle

The Masern virus, Rubella virus, and Mumps virus Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is a real-time reverse transcription polymerase chain reaction (RT-qPCR) test. The MeV/RV/MuV primers and probes sets are designed to detect MeV/RV/MuV RNA from MeV/RV/MuV Nucleic Acid in oropharyngeal swabs, whole blood, and saliva from patients who are suspected of MeV/RV/MuV infection.

A specifically designed primers and probe set is targeting the human GAPDH gene as internal control, to monitor sample collection, sample handling and qPCR process to avoid false-negative results.

Components of the Diagnostic Kit

This kit contains the following components:

No.	Reagent Name	Spec. & Qty.		Main Ingredients
		24 T	48 T	
1	MeV/RV/MuV PCR Mix	624 µL/tube × 1 tube	1248 µL/tube × 1 tube	Primers, Probes, dNTPs(T), $MgCl_2$, PCR buffer
2	MeV/RV/MuV Enzyme Mix	96 µL/tube × 1 tube	192 µL/tube × 1 tube	Reverse transcriptase, Taq DNA polymerase
3	MeV/RV/MuV Positive Control	500 µL/tube × 1 tube	1000 µL/tube × 1 tube	Synthetic sequences contains targets of interest
4	MeV/RV/MuV Negative Control	500 µL/tube × 1 tube	1000 µL/tube × 1 tube	Normal Saline

Note:

1. All contents in this package are prepared and validated for the intended testing purpose. Replacement or modification of any of the package contents will affect the testing performance of the kit. Components contained within a kit are intended to be used together. Do not mix or exchange components from different kit lots.
2. Materials required but not provided: 1.5mL DNase-free and RNase-free microcentrifuge tubes, 0.2mL PCR tubes and strip, various models of pipettes and pipette tips (10µL, 200µL and 1000µL tips with filters), microcentrifuge, vortex mixer.
3. Reagent required but not provided: Ncleic Acid Extraction-Purification Kit (Reference Number: S10015E), manufactured by Sansure Biotech Inc.

Storage and Stability

1. This kit should be stored in its original box at -20 ± 5°C and protected from light. The kit is valid for 12 months.
2. Please refer to the date of manufacture and expiry date printed on the outside of the box.
3. Unopened reagents are valid and stable until the expiry date.
4. Once the reagents are opened, the maximum number of freeze/thaw cycles should not exceed three.
5. The reagents keep valid and stable before the expiry date on the outer package when transporting for up to 7 days in a sealed foam box containing coolant with the temperature lower than 20°C.

Compatible Instruments

This diagnostic kit has been validated on the following Real-Time PCR instruments:

1) Applied Biosystems 7500 System;
2) Thermofisher QuantStudio™ 5 Real-Time PCR System;
3) Roche LightCycler® 480 Instrument II;
4) MA-6000 Real-Time Quantitative Thermal Cycler;
5) SLAN®-96P Real-Time PCR System.

Specimen Requirements

1. Applicable specimen type: oropharyngeal swabs, whole blood, and saliva.
2. Collection of specimens

Collection of Oropharyngeal swab: A sterile flocking swab should be used for sampling, moderately wipe the posterior pharyngeal wall, avoid touching the tongue. Quickly place a sterile swab into the collection tube used for collection of oropharyngeal swabs. Break the sterile swab rod near the top, tighten tube cap and seal with sealing film.

Collection of Whole blood: Collect 2~4mL of blood samples into vacuum blood collection tube containing EDTA anticoagulant.

Collection of Saliva: Collect saliva into a disposable aseptic sampling cup with screw cap into sampling cup, then tighten tube cap and seal with sealing film.

3. Storage and delivery of specimens:

Specimens to be tested can be immediately processed, specimens to be tested within 24 hours can be stored at 4°C. Specimens that cannot be detected within 24 hours should be stored at -70°C or below (in the absence of -70°C storage conditions, specimens to be tested can be stored at -20°C for 10 days, nucleic acid can be stored at -20±5°C for 15 days). Multiple freeze/thaw cycles should be avoided. Specimens should be transported in a sealed frozen container with ice or in a sealed foam box with ice packs.

Test Method

Please process according to the following steps for Applied Biosystems 7500 System, Thermofisher QuantStudio™ 5 Real-Time PCR System, Roche LightCycler® 480 Instrument II, MA-6000 Real-Time Quantitative Thermal Cycler, SLAN®-96P Real-Time PCR System:

1. Preparation of reagent (performed at "reagent preparation region")

1.1 Take out each component from the diagnostic kit and place them at room temperature. Equilibrating all reagents to room temperature, vortex follow by a short spin to avoid liquid remain on the lid to cross-contaminate the test.

1.2 Prepare the MeV/RV/MuV PCR Master Mix according to following table. The volume required is based on the total number of specimens, plus a MeV/RV/MuV Positive Control and a MeV/RV/MuV Negative Control. Mix thoroughly then centrifuge it for later use. The remaining reagent must be stored at -20°C immediately.

	1 sample	24 samples	48 samples
MeV/RV/MuV PCR Mix (µL)	26	624	1248
MeV/RV/MuV Enzyme Mix (µL)	4	96	192

Note: The above configuration is for reference only.

2. Processing and loading of specimens (performed at "specimen processing region")

2.1 Use Ncleic Acid Extraction-Purification Kit (Reference Number: S10015E) manufactured by Sansure Biotech Inc. to extract the nucleic acid according to corresponding manual.

2.2 Pipette 20 µL of each of the processed samples, MeV/RV/MuV Positive Control and MeV/RV/MuV Negative Control into the corresponding PCR tubes, add 30 µL PCR Master Mix to each tube, and cover the tube lid, centrifuge at 2000 rpm for 10 seconds, and place into the Real-Time PCR instruments.

3. PCR Amplification (Refer to user manual of each instrument to adjust the settings.)

3.1 Place PCR tubes into the specimen wells of the amplification equipment. Set up the MeV/RV/MuV Negative Control, MeV/RV/MuV Positive Control and specimens to be tested in order and input specimen name.

3.2 Select PCR test channel:

1) Select FAM channel (Reporter: FAM, Quencher: None) to test MeV, 2) Select ROX channel (Reporter: ROX, Quencher: None) to test RV, 3) Select CY5 channel (Reporter: CY5, Quencher: None) to test MuV, 4) Select HEX/VIC channel (Reporter: HEX/VIC, Quencher: None) to test Internal Control, 5)Set passive reference: none. Set sample volume: 50.

3.3 Set cycle parameters

	Steps	Temperature	Time	Cycles.
1	Reverse transcription	50°C	30 min	1
2	cDNA pre-denaturation	95°C	1 min	1
3	Denaturation	95°C	15 sec	45
	Annealing, extension and fluorescence collection	60°C	30 sec*	
4	Device cooling(optional)	25°C	10 sec	1

When the settings are completed, save the settings and carry out the reaction procedure. (*Note: the time of step 3 of ABI7500 version 1.5 is set to 31 seconds or 32 seconds)

4. Result Analysis (Refer to user manual of instrument to adjust the settings.)

Results will be saved automatically when reactions are completed. Analyze amplification curve of target of detection and internal control. Adjust Start, End and Threshold values of Baseline of the graph according to analysis result (Users can adjust the values according to the actual situation. Start value can be set between 3-15, and End value between 5-20. Adjust the amplification curve of negative control to be flat or below threshold). Click "Analyze" to implement the analysis, make sure each parameter satisfies the requirements given in "5. Quality Control". Go to "Plate" window to record qualitative results.

5. Quality Control

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. The Ct cutoff value of this kit is set as 40 and the end user is required to review fluorescent curves before final interpretation.

MeV/RV/MuV Positive Control				MeV/RV/MuV Negative Control				Results	Actions
MeV (FAM)	RV (ROX)	MuV (CY5)	IC (HEX/VIC)	MeV (FAM)	RV (ROX)	MuV (CY5)	IC (HEX/VIC)		
+	+	+	+	-	-	-	-	valid	Continue to result interpretation
Any one of them shows negative				Not required				invalid	Failed, re-run
Not required				Any one of them shows positive					Contaminated, re-run

Note: Result of (-): Ct value >40 or No Ct; Result of (+): Ct value ≤35.

If there is contamination for the re-run, please perform decontamination procedures.

Reference Range

Through the research on reference values, the Ct reference value of target genes and internal control are determined to be 40.

Explanation of Detection Result

1. For the FAM channel, specimens which are detected with Ct value ≤40, and internal control detected Ct value ≤40, the report is positive for the MeV. For specimens which are detected with Ct value >40 or No Ct, and internal control detected Ct value ≤40, the report is negative for MeV.
2. For the ROX channel, specimens which are detected with Ct value ≤40, and internal control detected Ct value ≤40, the report is positive for the RV. For specimens which are detected with Ct value >40 or No Ct, and internal control detected Ct value ≤40, the report is negative for RV.
3. For the CY5 channel, specimens which are detected with Ct value ≤40, and internal control detected Ct value ≤40, the report is positive for the MuV. For specimens which are detected with Ct value >40 or No Ct, and internal control detected Ct value ≤40 the report is negative for MuV.
4. For the FAM, ROX, and CY5 channels, specimens which are detected with Ct value >40 or No Ct, and the internal control detected with Ct value > 40 or No Ct, then

the specimen's detection result is invalid. An investigation should be performed to find out reasons and then retest the specimens. (If repeated tests still produce invalid results, please contact Sansure Biotech Inc.).

Limitations of Detection Method

1. False negative results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology.
2. Mutation in the target sequence of MeV/RV/MuV may lead to false negative results.
3. Improper reagent storage may lead to false negative results.
4. Use of this assay is limited to personnel who are trained in the procedure.
5. Test results of the diagnostic kit can only be used as an aid in clinical diagnosis. Symptoms and physical signs, medical history, other laboratory examinations and therapeutic reactions of the patients should be comprehensively considered for the clinical diagnosis and treatment.
6. Unverified interfering substances or PCR inhibitors may lead to false negative or invalid results.

Product Performance Index

1. Specificity

The Masern virus, Rubella virus, and Mumps virus Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is no cross-reactions with Poliovirus, Japanese encephalitis virus , Hepatitis C virus, Forest encephalitis virus, West Nile virus, Yellow fever virus.

2. Limit of detection

The limit of detection of this kit is 500 copies/mL.

3. Precision

The coefficient of variation (CV%) of Ct value of the within-run precision is ≤5%.

4. Possible interfering substances in specimens

Hemoglobin (≤2mg/dL), Total bilirubin (≤28mg/dL), Triglyceride (≤3g/dL), Total IgG (≤40mg/mL) have no significant influence on the detection; Acyclovir (≤1.2mg/L), Ribavirin (≤300μg/mL), Dipyridamole (≤2μg/ mL) have no significant interference with the detection results of the kit.

Precautions

1. For *in vitro* diagnostic use only (IVD).
2. Follow standard precautions. All specimens and positive controls should be considered potentially infectious and handled accordingly.
3. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
4. Please read the package insert carefully prior to operation. The Masern virus, Rubella virus, and Mumps virus Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is only for emergency use as an *in vitro* diagnostic (IVD) test. Each step of operation, from specimen collection, storage and transportation, and laboratory testing, should be strictly conducted in line with relevant biosafety regulations and molecular laboratory management.
5. Separated laboratory rooms, dedicated to perform predefined procedures of the assay, are required. a) 1st Room: Preparation Room-Prepare testing reagent b) 2nd Room: Specimen Processing-Process the specimen and controls c) 3rd: Amplification Room-PCR conducted.
6. All specimens for detection should be handled as if infectious. Wear laboratory coats, protective disposable gloves and change the gloves often to avoid cross-contamination between samples. Handling of specimens and waste must meet relevant requirements outlined in local, state and national regulations.

Bibliography

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Symbols

Symbols	Meanings	Symbols	Meanings
IVD	*In Vitro* Diagnostic Medical Device		Date of Manufacture
	Use By		Consult Instructions for Use
	Temperature Limitation		Manufacturer
LOT	Lot Number	REF	Reference Number
Σ	Number of Tests	EC REP	Authorized representative in the European Community
	Any warnings and /or precautions to take	CE	This product fulfills the requirements of the European Directive 98/79/EC for *in vitro* diagnostic medical devices.

Basic information

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