RevoDx Measles morbillivirus / Rubella virus Multiplex qPCR Kit

Instruction for Use

Qualitative detection of Measles morbillivirus and Rubella virus RNA

For in vitro diagnostic use

For professional use only

Product numbers:
IP202606-50 – 50 tests
IP202606-100 – 100 tests

CE IVD

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Product Components

	Component Name	50 Tests	100 Tests
1	MR RM-1	700 µl	1400 µl
2	MR RM-2	50 µl	100 µl
	MR Internal Control	150 µl	300 µl
3	MR Positive Control	100 µl	100 µl
4	MR Negative Control	100 µl	100 µl

Transport, Storage and Stability

The kits may be shipped at +2°C to +8°C. All components of RevoDx Measles morbillivirus / Rubella virus Multiplex qPCR Kit should be stored at -25°C to -15°C. Storage at higher temperatures should be avoided. If properly stored, all kit components are stable until the expiration date printed on the product label. MR RM 1 vial should not be freeze-thawed more than 3 times; as this may reduce the sensitivity. Otherwise, divide them into conveniently sized aliquots, and store at -25°C to -15°C.

Intended Use

RevoDx Measles morbillivirus / Rubella virus Multiplex qPCR Kit is a real-time PCR test intended for the qualitative detection of Measles morbillivirus and Rubella virus RNA.
Negative results do not preclude Measles morbillivirus and Rubella virus infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.
RevoDx Measles morbillivirus/Rubella virus Multiplex qPCR Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Product Use Restrictions

- For prescription use only
- RevoDx Measles morbillivirus/Rubella virus Multiplex qPCR Kit is for in vitro diagnostic use only.
- Potential mutations in the target regions of the Measles morbillivirus and Rubella virus genome covered by the oligos in the kit may lead to false negative test results.
- This kit has been validated for use with nasopharyngeal specimens (such as human oropharyngeal (OP) swab, nasopharyngeal (NP) swab or combined nasopharyngeal (NP) and oropharyngeal (OP) swab or Nasal aspirates or Throat washes) and urine specimens.
- PCR inhibitors in eluates may lead to false negative or invalid test results.
- Reliable results depend on proper specimen collection, transport, storage and handling methods.
- It is intended for professional use by properly trained personnel.
- Follow the instructions in product manual for optimum PCR results.
- Do not use a kit after its expiration date. Kit components from different lots should not be mixed.

Product Description

RevoDx Measles morbillivirus / Rubella virus Multiplex qPCR Kit is a fluorogenic probe-based PCR assay in which, situated between two PCR primers, there is an internal oligonucleotide probe with a fluorescent label attached at the 5'-end and a quenching molecule that suppresses the fluorescent reporter at the 3'-end. During DNA replication in the PCR process, the internal oligonucleotide hybridizes to the template and is digested by the 5'-3' endonuclease activity of the Thermus aquaticus (Taq) DNA polymerase as the PCR primer is extended. The internal oligonucleotide is digested only if DNA replication occurs, separating the fluorescent and quencher molecules. PCR products are detected within minutes by monitoring the increase in fluorescence that occurs exponentially with successive PCR amplification cycles. The parameter Ct (threshold cycle) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. RevoDx Measles morbillivirus / Rubella virus Multiplex qPCR Kit utilizes an internal control, which controls for target isolation and amplification.

General Description

Measles morbillivirus belongs to the family Paramyxoviridae, under the genus Morbillivirus. It is a highly contagious virus that causes measles, a disease characterized by fever, cough, conjunctivitis, and a distinctive rash. Measles virus is a single-stranded, negative-sense RNA virus. The virus is transmitted via respiratory droplets from coughs and sneezes of infected individuals. After entering the host, the virus infects epithelial cells in the respiratory tract, then spreads to the lymphatic system and eventually disseminates throughout the body. The characteristic rash is a result of the immune response to infected endothelial cells in capillaries.
Measles remains a significant public health concern, particularly in regions with low vaccination coverage. The high contagiousness of the virus makes early diagnosis and containment critical during outbreaks.
Rubella virus, also known as German measles virus, is the causative agent of rubella, a contagious viral infection. Rubella virus is a single-stranded RNA virus of the genus Rubivirus, belonging to the family Togaviridae. Its genome is approximately 9,762 nucleotides long and encodes for structural and non-structural proteins.
Rubella virus is primarily transmitted through respiratory droplets when an infected person coughs or sneezes. In pregnant women, the virus can be transmitted to the fetus via the placenta, leading to congenital rubella syndrome (CRS). Early diagnosis, appropriate management, and public health measures are essential components of rubella control and prevention efforts.

Safety Information

- Clinical specimens should be treated as potentially infectious; they should be handled in Bio-safety Level 1 or Bio-safety Level 2 area, depending on the infective agents.
- All resulting waste should be considered potentially infectious. They should be handled and discarded according to local safety regulations.
- Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water.
- Avoid producing spills or aerosol.
- Never pipette solutions by mouth
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands after handling samples and test reagents.
- All MSDS information is available upon request
- When working, always wear a protective lab coat, disposable gloves and protective goggles.
- Before and after procedure, disinfect all work surfaces thoroughly with a freshly prepared solution of 10% bleach or antiviral agents.
- Make sure everything is DNase/RNase-free when handling this system.
- Handle all materials according to Good Laboratory Practices in order to prevent cross-contamination.
- Use only calibrated pipettes, always change pipette tips between liquid transfers (aerosol-barrier pipette tips recommended)
- Keep the kit away from any source of contaminating nucleic acids, especially amplified nucleic acid.
- The operations should ideally be done in three separate areas. (i.e. for DNA/RNA purification, PCR setup, amplification) to prevent contamination.
- All equipment and consumables for a particular operation should be kept in the area where that operation is done and should not be moved between separated areas. Gloves should be removed and disposed of before leaving one area to proceed to the next. Lab coats should be specific to each area and never be worn outside the area.
- The work should flow in one direction, beginning in the extraction area followed by the chosen downstream application areas.

Performance Data

Analytical Sensitivity To determine the limit of detections (LoD), a dilution series of a secondary Measles morbillivirus and Rubella virus standard was prepared to give the final concentrations of 1000, 200, 40, 8 and 1.6 copies/ml. Viral RNA was purified using RevoDx Pathogen DNA/RNA Purification Kit. Each dilution was tested in 24 replicates. The Limit of Detection (LoD) value was found 86 copies/mL.

Diagnostic Specificity 115 Measles morbillivirus and Rubella virus RNA negative clinical specimens from individual donors were tested to determine the diagnostic specificity of RevoDx Measles morbillivirus/Rubella virus Multiplex qPCR Kit. None of the tested samples gave positive test result for target. Diagnostic specificity of RevoDx Measles morbillivirus / Rubella virus Multiplex qPCR Kit is ≥ 99 %.

Cross Reactivity The in silico analysis of the RevoDx Measles morbillivirus / Rubella virus Multiplex qPCR Kit primers and probes against the sequences of 29 pathogens showed the kit would be specific to the target Measles morbillivirus and Rubella virus genes and not cross-react with these pathogens. The 15 pathogens listed below were wet tested with the RevoDx Measles morbillivirus / Rubella virus Multiplex qPCR Kit for cross-reactivity. No false positive results were observed.

The results from the cross-reactivity, both in silico and wet testing, are summarized below.

***In silico* Cross Reactivity Analysis**

Organism	Target oligos
Hepatitis C virus	No homology
Human Cytomegalovirus (HCMV)	No homology
Hepatitis B virus	No homology
SARS-CoV-2	No homology
Human coronavirus 229E	No homology
Human coronavirus OC43	No homology
Human coronavirus HKU1	No homology
Human coronavirus NL63	No homology
SARS-coronavirus	No homology
MERS-coronavirus	No homology
Adenovirus (e.g. C1 Ad. 71)	No homology
Human Metapneumovirus (hMPV)	No homology
Parainfluenza virus 1-4	No homology
Influenza A & B	No homology
Enterovirus (e.g. EV68)	No homology
Respiratory syncytial virus	No homology
Rhinovirus	No homology
Chlamydia pneumoniae	No homology
Haemophilus influenzae	No homology
Legionella pneumophila	No homology
Mycobacterium tuberculosis	No homology
Streptococcus pneumoniae	No homology
Streptococcus pyogenes	No homology
Bordetella pertussis	No homology
Mycoplasma pneumoniae	No homology
Pneumocystis jirovecii (PJP)	No homology
Candida albicans	No homology
Staphylococcus epidermis	No homology
Streptococcus salivarius	No homology

Wet Tested Cross Reactivity Analysis

Organism	Source	Result
Hepatitis C virus RNA for nucleic acid amplification techniques (6th WHO International Standard)	NIBSC (Cat. No: 18/184)	Not Detected
Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification Techniques (1st International Standard)	NIBSC (Cat. No: 09/162)	Not Detected
4th WHO International Standard for HBV DNA for NAT	NIBSC (Cat. No: 10/266)	Not Detected
First WHO International Standard for SARS-CoV-2 RNA	NIBSC (Cat. No: 20/146)	Not Detected
Human coronavirus (229E)	NIBSC (Cat. No: 09/132)	Not Detected
Rhinovirus	NIBSC (Cat. No: 08/324)	Not Detected
Human Adenovirus	NIBSC (Cat. No: 16/324)	Not Detected
Influenza Virus (A/Christchurch/1/2003, H1N1)	NIBSC (Cat. No: 07/296)	Not Detected
Influenza Virus (A/Wyoming/3/2003, H3N2)	NIBSC (Cat. No: 07/298)	Not Detected
Influenza Virus (B/Jiangsu/10/2003)	NIBSC (Cat. No: 07/300)	Not Detected
Human Respiratory syncytial virus A2	NIBSC (Cat. No: 08/120)	Not Detected
Parainfluenza virus type 1	NIBSC (Cat. No: 08/176)	Not Detected
Parainfluenza virus type 2	NIBSC (Cat. No: 08/178)	Not Detected
Parainfluenza virus type 3	NIBSC (Cat. No: 08/118)	Not Detected
Parainfluenza virus type 4	NIBSC (Cat. No: 08/180)	Not Detected

Cross-Contamination The potential cross-contamination between samples was evaluated. Five different runs were performed by testing alternating high positive and negative samples 5 high positive Measles morbillivirus and Rubella virus sample and 5 Measles morbillivirus and Rubella virus negative samples were used in every run. No cross-contamination was observed, and none of the samples exhibited evidence of containing PCR inhibitors as indicated by the amplification of internal control.
Clinical Comparative Study Total 102 clinical samples were tested. According to the results, the data gathered by RevoDx Measles morbillivirus / Rubella virus Multiplex qPCR Kit is compatible with the results of other CE-marked devices.

Additional Materials Required

- RevoDx Pathogen DNA/RNA Purification Kit (Cat. No: IP202302; idil biotech, Turkey) or RevoDx Magnetic Pathogen DNA/RNA Purification Kit (Cat. No: IP202303; idil biotech; Turkey)
- Real-Time PCR Detection System,
- Suitable protection (protective lab coat, disposable gloves, protective goggles, etc.)
- Micropipettes (0.5 µl – 1000 µl),
- DNase/RNase-free micropipette tips with filters,
- DNase/RNase-free 1.5 ml microcentrifuge tubes,
- Vortex mixer,
- Desktop microcentrifuge for PCR plates/strip tubes,
- Desktop microcentrifuge for 2.0 ml tubes,
- PCR Workstation,

Real-Time PCR reaction tubes or plates:

Protocol

Viral RNA Purification RevoDx Pathogen DNA/RNA Purification Kit or RevoDx Magnetic Pathogen DNA/RNA Purification Kit should be used for viral RNA extraction from clinical specimens. Using other purification kits may adversely affect the performance characteristics of the kit. Please follow the manufacturer's instructions as stated in the kit manual. The operations should ideally be done in three separate areas. (i.e. for DNA/RNA purification, PCR setup, amplification) to prevent contamination.

Internal Control The presence of the internal control (IC) during the purification procedure is necessary. Internal Control includes in vitro transcribed RNA containing an insert. The internal control is utilized to monitor the efficiency of RNA extraction step as well as to check any PCR inhibition. For each sample, add 2.5 µl IC into Lysis Solution of Purification Kit. **Do not add IC directly into clinical sample.** Depending on final elution volume, the volume of IC to be added is calculated (0.05 µl IC/1 µl Elution Buffer). Bad signal or no signal might be observed in the internal control channel where high positive samples are amplified, because there is a competition between internal control template and target template while using PCR components. The Ct value of the internal control of a negative sample should be equal to 28 ± 4, otherwise, it indicates a problem during purification.

Positive Control Positive Control includes plasmid containing an insert. Positive Control is amplified in a separate Reaction Tube. To be able to evaluate the experiment, the Ct value of Positive Control should be equal to 26 ± 4, otherwise, it indicates a problem during amplification.

PCR Protocol

1. Thaw all components at room temperature except MR RM 2. Put MR RM 2 on ice. Mix each component thoroughly, then centrifuge briefly before use. Transfer all the reagents onto ice or cooling block.

2. The final volume of Master Mix is obtained by multiplying single reaction volumes of RM 1 and RM 2 by the total sample size. When calculating the total sample size, the number of negative/positive controls and the clinical samples should be taken into consideration. For possible pipetting errors, it is recommended to add an extra sample to the total sample size.

3. To prepare master mix, add 14 µl of MR RM 1 and 1 µl of MR RM 2 for each sample to the master mix tube. Vortex the tube and spin down briefly in a microcentrifuge. Add 15 µl of Master Mix into Real-Time PCR reaction tubes or capillaries for each sample. Add 5 µl RNA of each sample, negative control and positive control into the tubes. Spin down briefly.

4. Enter cycling conditions for Real-Time PCR Detection System: 50°C for 15 min; 95°C for 2 min, 1 cycle; 95°C for 20 sec, 60°C for 30 sec, 40 cycles (Table 1). Enter 20 µl as sample volume.

Table 1: Amplification program

Program Name	Cycles	Program
cDNA Synthesis	1	50ºC, 15 min
Hot Start	1	95ºC, 2 min
Amplification*	40	95ºC, 20 sec
		60ºC, 30 sec

*** Fluorogenic data should be collected at 60°C; FAM, CY5 and HEX channels should be chosen**

7. Fluorogenic data is collected at 60°C. FAM, CY5 and HEX channels should be selected.

8. Start run.

9. To program and analyze the results, refer to the User Manual of the instrument concerned.

Data Analysis

In order to evaluate the assay, the Ct value of Positive Control in the FAM and CY5 channels must be equal to 26±4, and Negative Control in all channels must be negative. Otherwise, the experiment should be repeated.

The results can be interpreted as:

Signal in any FAM channel (Measles morbillivirus RNA)	Signal in any CY5 channel (Rubella virus RNA)	Signal in HEX channel (Internal Control)	Interpretation
+	-	+/-	Measles morbillivirus RNA is positive
-	+	+/-	Rubella virus RNA is positive
-	-	+	Target RNA is not detected
-	-	-	Invalid result. This sample should be re-tested

Ordering Information

Product Name	Package	Cat. No.
RevoDx Measles morbillivirus / Rubella virus Multiplex qPCR Kit	50 tests	IP202606-50
RevoDx Measles morbillivirus / Rubella virus Multiplex qPCR Kit	100 tests	IP202606-100