RevoDx Bacterial Respiratory Pathogen Detection Kit

Instruction for Use

Qualitative Detection of Respiratory Pathogen DNA

For in vitro diagnostic use

For professional use only

Product numbers:
IP202603-50 – 50 tests
IP202603-100 – 100 tests

CE IVD

Product Components

	Component Name	50 Tests	100 Tests
1	ResBac MM 1	700 µl	1400 µl
2	ResBac MM 2	700 µl	1400 µl
3	ResBac MM 3	700 µl	1400 µl
4	ResBac Enzyme Mix	150 µl	300 µl
5	ResBac Positive Control	100 µl	100 µl
6	ResBac 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 Bacterial Respiratory Pathogen Detection 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. ResBac MM vials 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 Bacterial Respiratory Pathogen Detection Kit is a real-time PCR test intended for the qualitative detection and identification of nucleic acids of the specific bacterial pathogens from human nasopharyngeal swab, oropharyngeal swab, nasopharyngeal aspirate/lavage, bronchoalveolar lavage (BAL), bronchial aspirate (BAS), sputum and cerebrospinal fluid (CSF) specimens from individuals with signs and/or symptoms of respiratory infection.

Positive results do not rule out co-infection with other pathogens. The agent detected may not be the definite cause of disease. Negative results do not preclude the 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 Bacterial Respiratory Pathogen Detection 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.

The following pathogens are detected by RevoDx Bacterial Respiratory Pathogen Detection Kit:

Bacteria
• Moraxella catarrhalis
• Haemophilus influenzae
• Bordetella pertussis
• Chlamydophila pneumoniae
• Legionella pneumophila
• Mycoplasma pneumoniae
• Streptococcus pyogenes
• Streptococcus pneumoniae

Product Use Restrictions

- For prescription use only
- For in vitro diagnostic use only
- Potential mutations in the target regions of the pathogen genomes covered by the oligos in the kit may lead to false negative test results.
- This kit has been validated for use with human nasopharyngeal swab, oropharyngeal swab, nasopharyngeal aspirate/lavage, bronchoalveolar lavage (BAL), bronchial aspirate (BAS), sputum and cerebrospinal fluid (CSF) specimens. Test with other sample types may result in inaccurate results.
- 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 Respiratory-6 version 3.0 Pathogen Detection assay 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. A plot of the log of initial target copy number for a set of standards versus Ct is a straight line.

The method is performed directly on DNA/RNA extracted from the patient specimens. The detection of Respiratory pathogen DNA/RNA are done in 2 different reactions in which human RNase P is simultaneously detected. Respiratory real-time PCR assay utilizes human RNase P as an internal control, which controls for target isolation and amplification. The following table summarizes the target pathogens in 3 different reaction tubes:

Tube#	Target organism	Dye Channel
ResBac MM 1	Moraxella catarrhalis	FAM
	Haemophilus influenzae	HEX
	Bordetella pertussis	ROX
	Internal control	Cy 5
ResBac MM 2	Chlamydophila pneumoniae	FAM
	Legionella pneumophila	HEX
	Mycoplasma pneumoniae	ROX
	Internal control	Cy 5
ResBac MM 3	Streptococcus pyogenes	FAM
	Streptococcus pneumoniae	ROX
	Internal control	Cy 5

General Description

Molecular diagnosis of respiratory tract infections is a groundbreaking field in the medical world that aims to revolutionize how we detect and treat these common ailments. By utilizing advanced technology and techniques, scientists are now able to identify the specific pathogens responsible for causing respiratory infections with unprecedented accuracy. This means that healthcare providers can make more informed decisions when it comes to prescribing the appropriate treatment options for patients.

Gone are the days when doctors would rely solely on symptoms and physical examinations to diagnose respiratory tract infections. With molecular diagnosis, they can now analyze the genetic material of viruses or bacteria present in patient samples such as sputum, nasal swabs, or throat swabs. By detecting the unique DNA or RNA sequences of these pathogens, healthcare professionals can determine the exact cause of the infection, allowing for targeted treatments and better outcomes.

This innovative approach has numerous advantages over traditional methods. It not only provides quicker and more accurate results but also enables the identification of previously undetectable pathogens. Moreover, it allows for the detection of multiple pathogens simultaneously, which is especially beneficial in cases where co-infections are suspected. This information is vital in determining the most effective course of action, whether it be antiviral medications or antibiotics.

Molecular diagnosis of respiratory tract infections also plays a crucial role in reducing unnecessary antibiotic prescriptions. Overuse and misuse of antibiotics have led to the emergence of drug-resistant bacteria, posing a significant global health threat. By accurately identifying the pathogens causing an infection, healthcare providers can avoid prescribing antibiotics when they are not needed, thus helping prevent the development of antibiotic resistance.

Furthermore, this cutting-edge technique holds promise for future advancements in personalized medicine. As our understanding of the human genome continues to expand, researchers are discovering genetic variations that affect susceptibility to certain diseases, including respiratory tract infections. Molecular diagnosis can help identify individuals who may be at higher risk due to their genetic makeup, allowing for earlier interventions and tailored preventive measures.

In conclusion, molecular diagnosis of respiratory tract infections is a game-changer in the field of medicine. By harnessing the power of genetics and advanced technology, we are paving the way for more accurate diagnoses, targeted treatments, and improved patient outcomes. This approach not only enhances our understanding of infectious diseases but also contributes to the global efforts in combating antimicrobial resistance. As this field continues to evolve, it holds tremendous potential for transforming how we diagnose and manage respiratory tract infections, ultimately improving the health and well-being of individuals worldwide.

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

Limit of Detection (LoD) - Analytical Sensitivity Study:

To determine the limit of detections (LoD), a dilution series of each pathogen was prepared to give the final concentrations of 2430, 810, 270, 90 and 30 copies/mL by. spiking respiratory specimens collected from negative individuals to mimic clinical specimens. Bacterial DNA was purified using Pathogen DNA/RNA Purification Kit. Each dilution was tested in 24 replicates. Limit of Detection (LoD) values were calculated by probit analysis. The Limit of Detection (LoD) value was calculated by probit analysis. The Limit of Detection (LoD) value was 150 copies/mL. This LoD value was confirmed by testing an additional 20 replicates spiked at 150 copies/mL. All 20 replicates produced the positive results for each target, and the LoD was therefore confirmed to be 150 copies/mL.

Inclusivity:

An *in silico* inclusivity analysis of the RevoDx Bacterial Respiratory Pathogen Detection Kit primers and probes was performed for the sequences of each pathogen available from NCBI databases. The alignments demonstrated that the regions recognized by the designed primers and probes have 100% homology with all available pathogen sequences from the National Center for Biotechnology Information (NCBI) databases/databanks.

Cross Reactivity:

Cross-reactivity of the RevoDx Bacterial Respiratory Pathogen Detection Kit was evaluated using both in silico analysis and by wet testing. The in silico analysis of the RevoDx Bacterial Respiratory Pathogen Detection Kit primers and probes against the sequences of 24 pathogens showed the kit would be specific to the specific targets and not cross-react with these pathogens. The 31 pathogens listed below were wet tested with the RevoDx Bacterial Respiratory Pathogen Detection 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	Result
Bacillus subtilis	No homology
Mycobacterium tuberculosis	No homology
Streptococcus salivarius	No homology
Pneumocystis jirovecii (PJP)	No homology
Entamoeba dispar	No homology
Proteus spp.	No homology
Saccharomyces cerevisiae	No homology
Schizosaccharomyces pombe	No homology
Aspergillus niger	No homology
Salmonella spp.	No homology
Serratia marcescens	No homology
JC virüs	No homology
BK virüs	No homology
Parvovirus B19	No homology
Human Norovirus	No homology
VZV	No homology
HIV-1	No homology
HIV-2	No homology
HCV	No homology
HBV	No homology
Ebola virüs	No homology
Human Cytomegalovirus	No homology
Epstein-Barr Virus	No homology
Human Parechovirus	No homology

Wet Tested Cross Reactivity Analysis

Organism	Source	Concentration	Result
Pneumocystis jirovecii (PJP)	Clinical specimen	no unitage assigned	Not Detected
Entamoeba dispar	Clinical specimen	no unitage assigned	Not Detected
Mycobacterium tuberculosis	Clinical specimen	no unitage assigned	Not Detected
Aspergillus niger	Clinical specimen	no unitage assigned	Not Detected
Measles Virus	Clinical specimen	no unitage assigned	Not Detected
Candida albicans	Clinical specimen	no unitage assigned	Not Detected
Cryptococcus neoformans	Clinical specimen	no unitage assigned	Not Detected
Escherichia coli	Clinical specimen	no unitage assigned	Not Detected
Neisseria meningitidis	Clinical specimen	no unitage assigned	Not Detected
Legionella feeleii	Clinical specimen	no unitage assigned	Not Detected
Klebsiella pneumoniae	Clinical specimen	no unitage assigned	Not Detected
Chlamydia trachomatis	Clinical specimen	no unitage assigned	Not Detected
Mycoplasma hominis	Clinical specimen	no unitage assigned	Not Detected
Neisseria gonorrhoeae	Clinical specimen	no unitage assigned	Not Detected
Human Immunodeficiency Virus 1 (HIV-1)	NIBSC (Cat. No: 16/194)	1.25×10^5 IU/ml	Not Detected
Human Immunodeficiency Virus 2 (HIV-2)	NIBSC (Cat. No: 16/296)	2.8×10^5 IU/ml	Not Detected
4th WHO International Standard for HBV DNA for NAT	NIBSC (Cat. No: 10/266)	9.55×10^5 IU/ml	Not Detected
Hepatitis C virus RNA (6th WHO International Standard)	NIBSC (Cat. No: 18/184)	2.57×10^5 IU/ml	Not Detected
Human Cytomegalovirus (HCMV) (1st International Standard)	NIBSC (Cat. No: 09/162)	5×10^6 IU/ml	Not Detected
Epstein-Barr Virus (1st International Standard)	NIBSC (Cat. No: 09/260)	5×10^6 IU/ml	Not Detected
VZV (1st WHO International Standard)	NIBSC (Cat. No: 19/164)	1×10^7 IU/ml	Not Detected
EBOV RNA NP-VP35-GP (WHO Reference Reagent)	NIBSC (Cat. No: 15/222)	no unitage assigned	Not Detected
Parvovirus B19 (1st International Standard)	NIBSC (Cat. No: 09/110)	9.55×10^5 IU/ml	Not Detected
HSV-1	NIBSC (Cat. No: 16/368)	no unitage assigned	Not Detected
HSV-2	NIBSC (Cat. No: 17/122)	no unitage assigned	Not Detected
JC Virus (JCV) DNA (1st International Standard)	NIBSC (Cat. No: 14/114)	1.55×10^7 IU/ml	Not Detected
BK Virus (BKV)(1st International Standard)	NIBSC (Cat. No: 14/122)	2.04×10^7 IU/ml	Not Detected
HHV-6 Virus 1st WHO International Standard	NIBSC (Cat. No: 15/266)	5.63×10^7 IU/ml	Not Detected
Human Parechovirus	NIBSC (Cat. No: 08/322)	no unitage assigned	Not Detected
Human Norovirus	NIBSC (Cat. No: 08/318)	no unitage assigned	Not Detected
First WHO International Standard for Mycobacterium tuberculosis	NIBSC (Cat. No: 20/152)	2×10^6 IU/ml	Not Detected

Clinical Evaluation:

The performance of the RevoDx Bacterial Respiratory Pathogen Detection Kit was evaluated using archived respiratory specimens. For each pathogen, a total of 20 positive and 20 negative specimens were tested in a randomized and blinded fashion. All the 20 positive specimens and the 20 negative specimens were collected from a state hospital lab and had previously been tested with a validated comparator assay. Samples were extracted by RevoDx Pathogen DNA/RNA Purification Kit according to the product manual. Then, PCR reactions were setup by RevoDx Bacterial Respiratory Pathogen Detection Kit according to the product manual. BIO-RAD CFX96 Real-Time PCR Detection System was used for amplification, detection and analysis.

According to the test results, 100% agreement was observed with expected results.

Additional Materials Required

- RevoDx Pathogen DNA/RNA Purification Kit (Cat. No: IP202302; idil biotech,Turkey) or DirEXT OneStep Pathogen DNA/RNA Extraction Reagent (Cat. No: IP202319; 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,
- PCR Workstation,
- Real-Time PCR reaction tubes or plates,

Sample Preparation

This kit has been validated for use with human nasopharyngeal swab, oropharyngeal swab, nasopharyngeal aspirate/lavage, bronchoalveolar lavage (BAL), bronchial aspirate (BAS), sputum and cerebrospinal fluid (CSF) specimens. Clinical specimens should be treated as potentially infectious; and the precautions are recommended during sample collection and handling.
Clinicians (including healthcare assistants, nurses, doctors and professionals allied to medicine) have the responsibility of using the correct procedure during the collection and safe transportation of samples to the laboratory. The validity of test results largely depends on good practice in the 'pre-test' stage and it is essential that documentation is accurate and comprehensive.
Specimens can be stored at 2-8°C for up to 72 hours after collection. If a delay in extraction is expected, store specimens at -15°C or lower. Extracted nucleic acid should be stored at -15°C or lower. Transportation of the specimens must conform to country or local regulations.

Protocol

DNA/RNA Extraction: RevoDx Pathogen DNA/RNA Purification Kit or DirEXT OneStep Pathogen DNA/RNA Extraction Reagent should be used for Bacterial DNA extraction from human nasopharyngeal swab, oropharyngeal swab, nasopharyngeal aspirate/lavage, bronchoalveolar lavage (BAL), bronchial aspirate (BAS), sputum and cerebrospinal fluid (CSF) specimens. 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: An internal (Hs_RPP30) control targeting RNase P is needed to verify that nucleic acid is present in every sample and is used for every sample processed. This also serves as the extraction control to ensure that samples resulting as negative contain nucleic acid for testing.

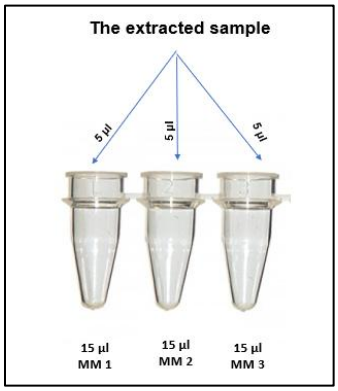
Positive Control: To be able to evaluate the experiment, the Ct values of Positive Control should be equal to 26 ± 4, otherwise, it indicates a problem.

PCR Protocol

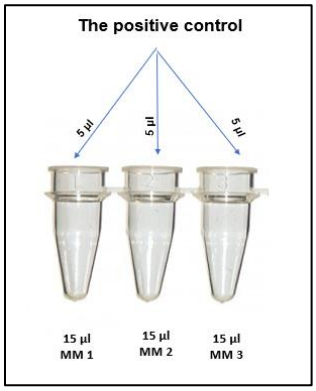
1. Thaw all components at room temperature except ResBac Enzyme Mix. Put ResBac Enzyme Mix on ice. Mix each component thoroughly, then centrifuge briefly before use. Transfer all the reagents onto ice or cooling block.

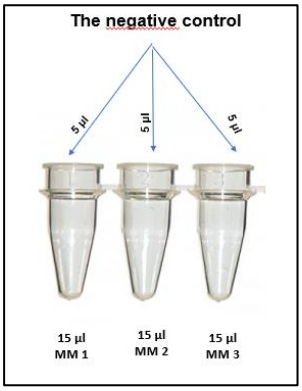
2. The final volumes of Master Mixes are obtained by multiplying single reaction volumes of any ResBac MM and ResBac Enzyme Mix by the total sample size. When calculating the total sample size, the number of negative controls, positive controls and clinical samples should be taken into consideration.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

3. To prepare each master mix, add 14 µl of ResBac MM and 1 µl of ResBac Enzyme Mix for each sample to the master mix tube. After preparing Master Mixes, vortex the tubes gently and spin down briefly. Add 15 µl of each Master Mix to PCR reaction tubes/plate. For each clinical specimen, 2 wells should be used. After the additions of Master Mixes into the wells, add 5 µl of the extracted sample into each well as shown in figure below. Close the cap of 8-Well Strips or seal the plate. Spin down briefly.



4. Repeat Step 3 for each extracted sample, negative control and positive control.





5. Enter cycling conditions for Real-Time PCR Detection System: 50°C for 15 min; 95°C for 2 min, 1 cycle; 95°C for 10 sec, 60°C for 20 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, 10 sec
		60ºC, 20 sec

*** Fluorogenic data should be collected at 60°C; FAM, HEX, ROX and Cy 5 channels should be chosen**

6. Fluorogenic data is collected at 60°C. FAM, HEX, ROX and Cy 5 channels should be selected.

7. Start run.

8. 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 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 for each Master Mix as:

Signal in any FAM / HEX / ROX channel	Signal in Cy 5 channel (RNase P gene)	Interpretation
+	+/-	Positive for specific pathogen
-	+	Pathogen is not detected
-	-	Invalid result. This sample should be re-tested for this Master Mix

For each Master Mix, the dye channels of the target organism/target gene are given in the following table:

Tube#	Target organism	Dye Channel
ResBac MM 1	Moraxella catarrhalis	FAM
	Haemophilus influenzae	HEX
	Bordetella pertussis	ROX
	Internal control	Cy 5
ResBac MM 2	Chlamydophila pneumoniae	FAM
	Legionella pneumophila	HEX
	Mycoplasma pneumoniae	ROX
	Internal control	Cy 5
ResBac MM 3	Streptococcus pyogenes	FAM
	Streptococcus pneumoniae	ROX
	Internal control	Cy 5

Ordering Information

Product Name	Package	Cat. No.
RevoDx Bacterial Respiratory Pathogen Detection Kit	50 tests	IP202603-50
RevoDx Bacterial Respiratory Pathogen Detection Kit	100 tests	IP202603-100