IVD

For *In Vitro* Diagnostic Use Only

Respiratory Pathogens Detection Kit

Instructions for Use

REF KS625-HXDA24/KS625-HXDB24/KS625-HXDC24

FileNo.: T-KRC10A-04

Version No.: D/0

CAUTION:

This kit is only for professional use. Please read this manual carefully before use.

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Table of Contents

1. Product Name ..... - 1 -
2. Product Specifications ..... - 1 -
3. Intended Use ..... - 1 -
4. Test Principle ..... - 1 -
5. Kit Components ..... - 1 -
6. Storage conditions and validity period ..... - 2 -
7. Applicable instruments ..... - 2 -
8. Warning and precautions ..... - 3 -
9. Sample Requirements ..... - 3 -
10. Operating procedure ..... - 3 -
11. Reference distribution ..... - 7 -
12. Quality Control ..... - 8 -
13. Result Analysis ..... - 8 -
14. Assay limitations ..... - 9 -
15. Product performance index ..... - 10 -
16. Symbol description ..... - 11 -
17. References ..... - 12 -

1. Product Name

Respiratory Pathogens Detection Kit

2. Product Specifications

Specification A: 1 test/tube, 12 tests/box

Specification B: 8 tests/strip, 24 tests/box

Specification C: 8 tests/strip, 24 tests/box

3. Intended Use

The Respiratory Pathogens Detection Kit is *in vitro* assay. The assay is for the detection of nucleic acid of 13 types of respiratory pathogens, including Influenza A virus, Influenza B virus, Respiratory Syncytial Virus, Human Rhinovirus, Adenovirus, Human Metapneumovirus, Boca virus, Coronavirus, *Mycoplasma Pneumoniae*, 2019-nCoV, *Bordetella pertussis*, *Bordetella parapertussis* and Parainfluenza Virus in human throat swab specimens. The kit is for qualitative detection only.

Human respiratory tract infections are caused by a variety of pathogenic organisms. Numerous viruses, including influenza viruses A, B, parainfluenza viruses (PIV-1, -2, -3 and -4), human respiratory syncytial virus (RSV), human metapneumovirus (hMPV), human coronaviruses (CoV), human rhinoviruses (RhV), adenoviruses(species B, C, and E) and bocavirus are associated with respiratory tract infections. Since December 2019, aglobal pandemic illness caused by severe acute respiratory syndrome coronavirus 2 (2019-nCoV) spread quickly around the world. Accurate and rapid diagnostic tests are critical for achieving control of the disease Covid-19.*Mycoplasmapneumoniae* (MP) is an important etiologic baterium for community-acquired pneumonia in children and adults. Pertussis, a disease that has been well described since the Middle Ages, has a worldwide distribution and may infect individuals with all ages. Pertussis is a highly contagious acute respiratory illness caused by *Bordetella pertussis* or *Bordetella parapertussis*.

4. Test Principle

The Respiratory Pathogens Detection Kit is based on the PCR-Melting curve analysis technology. A combination of PCR primers and dual labelled fluorescent probes is used for each pathogen. After reverse transcription, PCR amplification and melting curve analysis, pathogens in the corresponding fluorescence detection channels will be identified by their respective Tm values.

5. Kit Components

The kit contains 24 tests:

Table 1：Kit Components

Product name	Content	Key component	Specification A		Specification B		Specification C	
			Specification	Quantity	Specification	Quantity	Specification	Quantity
Respiratory Pathogens Detection Kit	Res-PCR Mix	Primers, probes, reverse transcriptase, DNA polymerase, UDG enzyme, RNA inhibitors, dNTPs, etc.	1 test/tube	12tubes	8 tests/strip	3 strips	8 tests/strip	3 strips
	Res-Dissolve Buffer	K^+, Mg^{2+}, Cl^- and other salt ion solutions	Pre-filled in the EXT iCassette	500μL	500 μL/tube	1 tube	500 μL/tube	1 tube
	Res-Positive Control	Pseudovirus containing Influenza A virus, Adenovirus, *Mycoplasma Pneumoniae* and Internal control gene fragments	1 test/tube	1 tube	1 test/tube	1 tube	1 test/tube	1 tube
	DNase/RNase-Free Distilled Water	DNase/RNase-Free Distilled Water	450 μL/tube	1 tube	450 μL/tube	1 tube	450 μL/tube	1 tube

Note: Different specifications and batch numbers of the same component cannot be mixed.

6. Storage conditions and validity period

1) The kit can be stored at 2℃～30℃ for up to 12 months. Keep away from sunlight. Avoid freezing.

2) The kit should be used within 30 minutes once opened because the reagents will become invalid due to moisture.

3) See the packaging label for the “Date of manufacture” and “Use-by date”.

7. Applicable instruments

Table 2 Applicable instruments

Specification	Applicable instrument	Manufacturer
Specification A	Automated Fully Enclosed qPCR Instrument： Galaxy Lite, Galaxy Pro (NL-CA002-2020-50082) (NL-CA002-2020-54910)	IGENESIS(Shanghai) Co., Ltd.
Specification B	Real-Time PCR System： SLAN-96S, SLAN-96P (ICAS-21100004)	Shanghai Hongshi Medical Technology Co., Ltd.
Specification C	cobas z 480 analyzer (DOC-2016-40)	Roche Molecular Systems, Inc.

Material Required But Not Provided

- EXT iCassette (IGENESIS for Specification A, others for Specification B and Specification C)
- DNase, RNase and human DNA-free pipette tips
- Adjustable pipette
- Pipe rack
- Vortex mixer
- Microcentrifuge

8. Warning and precautions

1) This kit is only used for detection *in vitro* only. Please read the instructions carefully before use.

2) Technologist should have been professionally trained and have corresponding skills and qualifications. Always wear appropriate personal protective equipment, including but not limited to disposable powder-free gloves, a lab coat, and protective eyewear. Protect skin, eyes and mucus membranes. Change gloves often when handling samples.

3) Handle all samples, used cartridges and transfer pipettes as if they are capable of transmitting infectious agents. Dispose of them in accordance with all national, state and local health and safety regulations and laws.

4) If the kit comes with damage, please do not use it and contact with KingCreate Biotechnology.

9. Sample Requirements

9.1 Sample type

Throat swab

9.2 Sample collection

When collecting throat swab samples, the medical staff should wipe the posterior pharyngeal wall and tonsils on both sides with moderate force and avoid touching the tongue. Then quickly put the swab into an external spiral covered sampling tube containing 3mL of preservation solution or medium. It is recommended to split the sample into two tubes at the time of collection, one of which shall be kept for review.

The samples used for virus separation and nucleic acid detection should be tested as soon as possible. Otherwise, the samples in the storage medium at 4℃ can be tested within 24 hours. The samples can be stored at -70℃ or below for a long time. Repeated freezing and thawing should be avoided during specimen transportation and storage.

10. Operating procedure

10.1 Specification A Test procedure (Applicable to Galaxy Pro or Galaxy Lite)

10.1.1 Reagent preparation

1) Positive and Negative controls preparation:

a: Take two EXT iCassette and replace the qPCR tubes on the iCassette with the “Res-PCR Mix” tube, and make sure that the “Res-PCR Mix” tube is tightened.

b:Open the package of the Res-Positive Control, pipetting 200 μL of DNase/RNase-Free Distilled Water to dissolve it, mix thoroughly as positive control.

c: Take another 200 μL of DNase/RNase-Free Distilled Water as the Res-Negative Control.

d: Unscrew the Lid of the EXT iCassette counterclockwise, pipetting 200 μL of prepared Res-Positive Control and Res-Negative Control and slowly add it to the Sample Compartment correspondingly, as shown in the figure 1 below (Note: Slow addition can avoid the formation of aerosols, which can result in a lower yield of nucleic acid extraction), tighten the Lid of the EXT iCassette clockwise to ensure that the cap is tightly shut.

2) Sample preparation:

a: Replace the qPCR tube on the EXT iCassette with the “Res-PCR Mix” tube, and make sure that the “Res-PCR Mix” tube is tightened.

b: Unscrew the Lid of the EXT iCassette counterclockwise, pipetting 200 μL of the sample to be tested to the Sample Compartment, as shown in the figure 1 below, tighten the Lid of the EXT iCassette clockwise to ensure that the cap is tightly shut.

Figure 1 EXT iCassette

10.1. 2 Experiment Operation

10.1.2.1 Power on: Press the switch button on front side of the instrument for about 5 seconds, the blue light is on, which means "the instrument is turned on", then power on the tablet PC and access the software on the

desktop of tablet PC.

10.1.2.2 Log in to the software: log in with an administrator account (Admin/123456), click the "OK" button, and the instrument will perform a self-check.

10.1.2.3 Instrument self-check: After the login is successful, the instrument starts the self-check function. When the self-check is completed, click the "Open" button to open the compartment door of the instrument to prepare for loading the EXT iCassette.

10.1.2.4 Put the EXT iCassette with loaded samples insert the sample tray and put the tray into the instrument and click the "Close " button to close the compartment door.

10.1.2.5 After the compartment door is closed, input the sample information at the corresponding channel position of the tray on the software .

10.1.2.6 Click the "Start Running" button, the instrument scans the corresponding QR code on the EXT iCassette and the software automatically load the script , click the "OK" button to confirm the script and start the experiment.

10.1.2.7 After the experiment starts running, the status of the experiment will be displayed on the main interface.

10.1.2.8 After the experiment is completed, the results will be automatically interpreted by software. For the detailed steps of the test operation, please refer to the user manual of the instrument.

10.2 Specification B Test procedure (Applicable to SLAN-96S or SLAN-96P).

10.2.1 Open the package of Res-PCR Mix, add 20 μL of Res-Dissolve Buffer, and mix thoroughly.

10.2.2 Take the Res-Positive Control tube and add 200 μL of DNase/RNase-Free Distilled Water, mix thoroughly. The remaining DNase/RNase-Free Distilled Water will be used as the sample of Res-Negative Control.

10.2.3 Take 200 μL of the sample or Res-Positive Control and Res-Negative Control, to extract nucleic acid according to the instructions of the EXT iCassette.

10.2.4 Add 5 μL extracted nucleic acid of the sample to the Res-PCR Mix, cover the tube tightly, mix on a vortex for 5~10 seconds, centrifuge briefly. The total volume of each reaction is 25 μL.

10.2.5 Place Res-PCR Mix in SLAN-96S or SLAN-96P, set up according to the following reaction program.

Table 3：SLAN-96S or SLAN-96P Reaction procedure

Stage	Condition	Cycle

Reverse transcription	50℃ 15 minutes	1
Hot Start	95℃ 5 minutes	1
Amplification	95℃ 15 seconds	45
	58℃ 30 seconds (Use FAM, HEX, ROX and CY5 channel to collect fluorescent signals)	
	72℃ 30 seconds	
Melting Curve analysis	60℃ 2 minutes 60℃~90℃, Collect fluorescence signals at a heating rate of0.06℃/s	1

10.2.6 The Tm value of the test results will be automatically interpreted by SLAN-96S or SLAN-96P software.

10.3 Specification C Test procedure (Applicable to cobas z 480 analyzer)

10.3.1 Open the package of Res-PCR Mix, add 20 µL of Res-Dissolve Buffer, mix thoroughly.

10.3.2 Take the Res-Positive Control tube and add 200 µL of DNase/RNase-Free Distilled Water, mix thoroughly. The remaining DNase/RNase-Free Distilled Water will be used as the sample of Res-Negative Control.

10.3.3 Take 200 µL of the sample or Res-Positive Control or Res-Negative Control, to extract nucleic acid according to the instructions of the EXT iCassette.

10.3.4 Add 5 µL extracted nucleic acid of the sample to the Res-PCR Mix, cover the tube tightly, mix on a vortex for 5~10 seconds, centrifuge briefly. The total volume of each reaction is 25 µL.

10.3.5 Place the Res-PCR Mix on cobas z 480 analyzer, click "Tools" button on the software, select "Detection Formats", click "New" button, name "Res-Four Color", and select "Filter Combination Selection" according to Table 4. Make your selections and names, and click "Close" when you're done.

Table 4 Filter Combination Selection

	Emission						
Excitation		510	580	610	645	670	700

	465	☑	☐	☐	☐	☐	☐
	498	☐	☐	☐	☐	☐	☐
	540	☐	☑	☑	☐	☐	☐
	610	☐	☐	☐	☐	☑	☐
	680	☐	☐	☐	☐	☐	☐
		Name “FAM”	Name “HEX”	Name “ROX”		Name “CY5”	

10.3.6 Click "New Experiment" on the software, set up the reaction program according to the following, and set the reaction system to 25 μL.

Table 5 Cobas z 480 analyzer Reaction procedure

Stage	**Condition**	**Cycle**
Reverse transcription	50℃ 15 minutes	1
Hot Start	95℃ 5 minutes	1
Amplification	95℃ 15 seconds	45
	58℃ 30 seconds (Use FAM, HEX, ROX and CY5 channel to collect fluorescent signals)	
	72℃ 30 seconds	
Melting analysis	60℃ 2 minutes 60℃~90℃, Collect fluorescence signals at a heating rate of 0.06℃/s	1

11. Reference distribution

The Tm reference distribution for 13 respiratory pathogens in each channel is as follows:

Table 6 Tm value of respiratory pathogens in each channel

Channel	**Pathogen**	**Tm reference value range**
FAM (Channel 1)	2019-nCoV (ORF)	64℃≤Tm≤68℃(1)

	Bordetella pertussis (B. pertussis)	69℃≤Tm≤73℃ (2)
	Influenza A virus (Flu A)	74℃≤Tm≤78℃ (3)
	Influenza B virus (Flu B)	79℃≤Tm≤82.5℃ (4)
	Respiratory Syncytial Virus (RSV)	84℃≤Tm≤87℃ (5)
HEX (Channel 2)	*Bordetella parapertussis* (B.parapertussis)	68℃≤Tm≤72.5℃ (2)
	Rhinovirus (RhV)	73℃≤Tm≤77℃ (3)
	Adenovirus (Adv)	78℃≤Tm≤82℃ (4)
	Human Metapneumovirus (hMPV)	83℃≤Tm≤87.5℃ (5)
ROX (Channel 3)	2019-nCoV (N)	64℃≤Tm≤67.5℃ (1)
	Boca virus (Boca)	68℃≤Tm≤72℃ (2)
	Coronavirus (CoV)	73℃≤Tm≤77℃ (3)
	Mycoplasma Pneumoniae (MP)	79℃≤Tm≤83℃ (4)
	Parainfluenza virus (PIV)	83℃<Tm≤87℃ (5)
CY5 (Channel 4)	Internal Control (HBB)	69℃≤Tm≤73℃

12. Quality Control

12.1 Res-Positive Control: Four melting curves will appeared in FAM, HEX, ROX and CY5 channels, which correspond to those for Flu A, adenovirus, *Mycoplasma pneumoniae* and internal control respectively.

12.2 Res-Negative Control: There are no melting curves in FAM, HEX, ROX and CY5 channels.

12.3 Before to analyse the tests results, one ought to see if both the Res-Positive Control and Res-Negative Control are met with the expectation in 12.1 and 12.2; otherwise, the current experiment is invalid and the experiment shall be carried out again.

13. Result Analysis

13.1 Analysis of negative results

If there is no melting curve in the FAM, HEX, ROX channels, but there is a melting curve in the CY5 channel, it is determined that the sample has no pathogen detected within the detection range, so the test result is negative.

If there are no melting curve in the FAM, HEX, ROX and CY5 channels, it suggests the sample may be improperly collected or stored. It is recommended to re-collected or re-extract nucleic acid before re-testing.

13.2 Analysis of positive results

When there is a melting curve appears in the FAM, HEX, ROX channels within the range of detection, the

corresponding pathogen is determined to be positive. When two or more melting curves appear at the same time, it is determined that the sample contains two or more pathogens. When judging the positive result of this sample, there is or no corresponding melting curve in the CY5 channel.

Table 7 Examples of interpretation of positive results

Situation	Channel	Melting Curve Range					Results Interpretation
		1	2	3	4	5	
Situation 1	FAM	-	-	+	-	-	Influenza A virus positive
	HEX	-	-	-	-	-	
	ROX	/	-	-	-	-	
	CY5	/	±	/	/	/	
Situation 2	FAM	-	-	-	-	-	Rhinovirus, *Mycoplasma pneumoniae* positive
	HEX	-	-	+	-	-	
	ROX	/	-	-	+	-	
	CY5	/	±	/	/	/	
Situation 3	FAM	-	-	-	-	-	Normal negative
	HEX	-	-	-	-	-	
	ROX	/	-	-	-	-	
	CY5	/	+	/	/	/	
Situation 4	FAM	-	-	-	-	-	Abnormal detection, re-sampling or re-extraction of nucleic acid before testing
	HEX	-	-	-	-	-	
	ROX	-	-	-	-	-	
	CY5	/	/	/	/	/	

Note: "1", "2", "3", "4" and "5", please refer to Table 6 for the corresponding Tm reference value range, "+" means there is a melting curve, "-" means no melting curve, "±"indicates the presence or absence of a melting curve, and "/" indicates that the Tm value has no detection target.

14. Assay limitations

1) This kit is used to assist in the diagnosis of 13 different respiratory pathogen infections. The test results must be interpreted by a trained healthcare professional within the context of all relevant clinical, laboratory and epidemiological findings. they are not intended to be used as the sole basis for diagnosis, treatment or other patient management decisions.

2) Improper sample collection, transportation or nucleic acid extraction may lead to false negative results.

3) Mutation or sequence variation may also lead to false negative results.

4) This kit can only detect pathogens within the detection range of corresponding pathogens. In addition, the assays are not designed to differentiate subtypes in the same species of the pathogen.

5) A negative result may indicate there is no such pathogen in the sample, or may indicate the concentration of the pathogen in the sample is below the detection limit of the assay.

6) False positive may be resulted from contamination during the test procedure.

7) Due to the difference in detection sensitivity and specificity of different technical platforms, the test results may be inconsistent with those by other kits from different manufacturers.

8) This kit is a qualitative test and does not provide a quantitative value for the pathogens in the sample.

15. Product performance index

15.1 Enterprise reference: 13 positive references (QNP01~QNP13) were detected and the coincidence rate of positive references was 100%. 5 negative references (QNN01~QNN05) were detected and the negative coincidence rate was 100%. 1 precision reference (QNR01) was tested repeatedly and the results were consistent. 5 detection limit references (QNL01-QNL05) meet the requirements of detection limit.

15.2 Limit of Detection (LOD)

Table 8 Limit of Detection for respiratory pathogens

Pathogen	LOD	Pathogen	LOD
Influenza A virus (Flu A)	500 copies/mL	Rhinovirus (RhV)	500 copies/mL
Influenza B virus (Flu B)	500 copies/mL	Parainfluenza virus (PIV)	500 copies/mL
Respiratory Syncytial Virus (RSV)	500 copies/mL	Human metapneumovirus (hMPV)	500 copies/mL
Adenovirus (Adv)	500 copies/mL	*Mycoplasma pneumoniae* (MP)	500 copies/mL
Boca virus (Boca)	500 copies/mL	Coronavirus (CoV)	500 copies/mL
2019-nCoV(ORF)	500 copies/mL	2019-nCoV (N)	500 copies/mL
Bordetella pertussis (B. pertussis)	500 copies/mL	*Bordetella parapertussis* （B.parapertussis）	500 copies/mL

15.3 No cross-reactivity was found with pathogens at the following concentrations:

Table 9 Cross-reactivitywith common pathogens in human respiratory track

Cryptococcus 1.65E+08	Cytomegalovirus 6.90E+08	Herpes simplex virus type 1 6.02E+08	*Aspergillus flavus* 3.20E+08
Varicella zoster virus 6.43E+08	Epstein-Barr virus 2.56E+08	*Streptococcus pyogenes* 5.45E+08	*Streptococcus salivarius* 6.70E+08
Streptococcus pneumoniae 2.28E+08	*Corynebacterium* 6.38E+06	*Haemophilus influenzae* 5.72E+08	*Klebsiella pneumoniae* 3.51E+08
Lactobacillus 6.88E+07	*Legionella pneumophila* 7.13E+06	*Moraxella catarrhalis* 1.63E+08	*Pneumocystis* 2.04E+08
Neisseria 6.23E+06	*Staphylococcus aureus* 7.37E+06	*Staphylococcus epidermidis* 2.59E+08	*Candida albicans* 4.31E+08

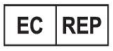
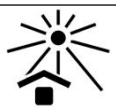
15.4 This kit does not interfere with the following concentrations of interfering substances:

Endogenous substances including whole blood (5%) and mucin (20 μg/mL) showed no detectable interference with the kit.

Following exogenous substances such as phenylephrine (100 μg/mL), oxymetazoline (100 μg/mL), beclomethasone (50 μg/mL), dexamethasone (100 μg/mL), flunisolide (10 μg) /mL), triamcinolone acetonide (100 μg/mL), budesonide (50 μg/mL), mometasone (50 μg/mL), fluticasone (50 μg/mL), histamine hydrochloride (50 μg/mL), intranasal live influenza Viral vaccine (30 μg/mL), benzocaine (50 μg/mL), menthol (32 μg/mL), zanamivir (100 μg/mL), ribavirin (100 μg/mL), oseltamivir (100 μg/mL), peramivir (100 μg/mL), mupirocin (100 μg/mL), and tobramycin (50 μg/mL), showed nodetectable interference with this kit.

16. Symbol description

IVD	*In vitro* diagnostic medical device
	Manufacturer

	Authorized representative in the European Community
	Use-by date
	Batch code
	Date of manufacture
	Catalogue number
	Caution
	CE Mark
	Consult instructions for use
	Keep away from sunlight
	Keep dry
	Positive control
	Contains sufficient for <24> tests
	Upwards
	Do not use if package is damaged
	Limit of temperature
	Handle with care

17. References

1.Huang Q, Liu Z, Liao Y, et al. Multiplex Fluorescence Melting Curve Analysis for Mutation Detection with

Dual-Labeled, Self-Quenched Probes. PloS ONE(2011), 6(4):e19206.

2.Gunson Rn, Collins TC, CarmanWF, et al. Real-time RT-PCR detection of 12 respiratory viral infections in four triplex reactions. JClinVirol, 2005,33(4):341-344.

3.Kim, S.R., C.S. Ki and N.Y. Lee. Rapid detection and identifcation of 12 respiratory viruses using a dual priming oligonucleotide system-based mμltiplex PCR assay. Journal of virological methods, 2009. 156(1): p. 111-116.

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File No.: T-KRC10A-04

Version No: D/0

January 26, 2022

Website: www.kingcreate.com.cn