For Medical Professional Use Only

Multiplex Real time PCR Kit for Carbapenem Resistance Genes

INSTRUCTION FOR USE

Product Name

Multiplex Real time PCR Kit for Carbapenem Resistance Genes

Product Description

The kit is used for in vitro qualitative detecting the NDM/OXA-48/KPC/VIM/IMP resistance genes for Carbapenem in rectal swabs and pure colonies specimens.

The kit contains two tubes. Primer sets and FAM labeled probe are designed for specific detection of NDM gene, VIC labeled probe for VIM gene, ROX labeled probe for OXA-48 gene. The other Primer sets and FAM labeled probe are designed for specific detection of VIM gene, VIC labeled probe for IMP gene. Human RNase P gene extracted concurrently with the test sample provides an internal control to validate nucleic extraction procedure and reagent integrity. Probe targeting human RNase P gene is labeled with CY5.

Size

48 Tests/kit, 96 Tests/kit

Kit Contents

Large packageComponents	Main components	48 Tests/kit	96 Tests/kit
PCR reaction buffer A	KPC、NDM、OXA-48、 Internal reference gene、Primers、 probes、Buffer	960 µL × 1 tube	960 µL × 2 tube
PCR reaction buffer B	VIM、IMP、Internal reference gene、Primers、probes、Buffer	960 µL × 1 tube	960 µL × 2 tube
Positive control	Mixture of target bacterial liquid nucleic acid	500 µL × 1 tube	1000 µL × 1 tube
Negative control	TE Buffer	500 µL × 1 tube	1000 µL × 1 tube

Single tube packae	Main components	48 Tests/kit	96 Tests/kit
PCR reaction buffer A	KPC、NDM、OXA-48、 Internal reference gene、Primers、 probes、Buffer	1 test × 48 tubes	1 test × 96 tubes
PCR reaction buffer B	VIM、IMP、Internal reference gene、Primers、 probes、Buffer	1 test × 48 tubes	1 test × 96 tubes
Positive control	Mixture of target bacterial liquid nucleic acid	500 µL × 1 tube	1000 µL × 1 tube
Negative control	TE Buffer	500 µL × 1 tube	1000 µL × 1 tube

Precautions:

1. Components with different lot numbers cannot be used together.
2. User prepared DNA extraction Kit.

Storage and Expiration

This kit expires in 12 months when stored at -20±5℃ and in prevention of light.

Avoid repeated freeze-thaw cycles (5 times maximum).

Manufacture date and expiration date are printed on the label.

Applicable Instruments

Real-Time PCR System: Molarray MA-6000, ABI 7500, ViiA™ 7, QuantStudio 5, QuantStudio 6/7 pro, QuantStudio 6/7 flex, Agilent Mx3000P/3005P, Rotor-Gene™ 6000/Q, Bio-Rad CFX96 Touch™/iQ™ 5，Hongshi SLAN-96S/96P，AGS8830, AGS4800

Specimen Handling and Storage

1. Acceptable Specimens: Rectal swabs and pure colonies
2. Collect samples in sterile tubes.
3. Contamination should be avoided during collection, storage and transportation of the samples.
4. Specimens can be stored at 2~8 ℃ for up to 24 hours after collection. If a delay in extraction is expected, store specimens at -20℃ or lower. Avoid repeated freeze-thaw cycle of the sample and make sure sample is completely thawed before DNA extraction.
5. Transport samples in sealed box with dry ice or ice bag.

Protocol

Precaution: All the reagents should be thawed completely before use, then vertex and spin down at 6,000rpm.

1. Sample treatment (Sample treatment zone)

Extract DNA with Bacteria DNAextraction kit. DNA extracted can be stored at -20℃ or lower for 6 months. Positive control and negative control can be used without extraction.

2. Preparation of amplification reagents (Mix preparation zone)

Take 2N (N=negative control number + DNA sample number +positive control number) PCR tubes, add 20µL PCR reaction buffer A and B to each tube (take 2n tube directly to the next step for single tube package).

Components	Volume per test
PCR reaction buffer A	20 µL
PCR reaction buffer B	20 µL

Centrifuge the PCR tubes at 6,000rpm for 30s and transport to sample treatment zone.

3. Sample loading (Sample treatment zone)

Add 20 µL DNA sample, negative control and positive control to the above PCR tubes respectively. Cap the tubes tightly and centrifuge at 6,000rpm for 10s and then transport to PCR amplification zone.

* Precaution: Adding samples in the following order is recommended: negative control-> DNA sample -> positive control.

4. PCR amplification (PCR amplification zone).

4.1. Put the caped PCR tubes into real-time PCR machine for amplification.

4.2. ABI 7500,etc thermal cycling setting

Steps	Temperature	Duration	Cycle
1	50℃	3min	1
2	95℃	1min	1
3	95℃	5s	45
	60℃	35s	

4.3. A MA-6000,etc thermal cycling setting

Steps	Temperature	Duration	Cycle
1	50℃	3min	1
2	95℃	1min	1
3	95℃	3s	45
	60℃	13s	

Collect fluorescent signals at step 3:60℃; 35s for ABI 7500，while 13s for other fast Real-Time PCR Systems. The total volume: 40 µL.

NOTE: for ABI7500, ViiA™ 7, QuantStudio series instruments, choose ‘none’ as both passive reference and quencher.

4.4. Disposal after detection

Dispose the PCR tubes in a sealed bag after reaction and treat the used tubes as medical wastes.

5. Settings for result analysis

Set the baseline at a region before the exponential amplification where the fluorescent signals of all the samples are relatively stable (no significant fluctuations in all the samples); set the starting point (cycle number) away from the signal fluctuations at the starting phase of fluorescence collection; set the end point (cycle number) 1~3 cycles before the Ct of the first sample to enter exponential amplification. 4~15 cycles are recommended.

Set the threshold right above the highest point of the negative control amplification curve (irregular noise curve).

6. Quality control criteria

Prior to evaluating the specimen results, the Positive Control and Negative Control should be interpreted using the interpretation table below, and the Positive Control and Negative Control curve must be performed correctly, otherwise the sample result is invalid.

Channels / Controls	Cycle threshold（Ct）value			
	FAM	**VIC**	**ROX**	**CY5**
Negative control	Ct > 40 or UNDET	Ct > 40 or UNDET	Ct > 40 or UNDET	Ct > 40 or UNDET
Positive control	Ct ≤ 35	Ct ≤ 35	Ct ≤ 35	Ct ≤ 35

Channels / Controls	Cycle threshold（Ct）value		
	FAM	**VIC**	**CY5**
Negative control	Ct > 40 or UNDET	Ct > 40 or UNDET	Ct > 40 or UNDET
Positive control	Ct ≤ 35	Ct ≤ 35	Ct ≤ 35

Result Determination

FAM channel for NDM, detection result should be interpreted as below.

1. Positive: Ct ≤ 38 and amplification curve is S-shaped.
2. Suspected: 38 < Ct ≤ 40 and amplification curve is S-shaped, a second test is needed. Consider positive if Ct ≤ 40 and amplification curve is S-shaped for the second test. Considered negative if Ct > 40 or null Ct and Ct ≤ 35 in CY5 channel for the second test.
3. Negative: Ct > 40 or null Ct and Ct ≤ 35 in CY5 channel.
4. Re-test: Ct > 40 or null Ct and Ct > 35 in CY5 channel.

VIC channel for OXA-48, detection result should be interpreted as below.

1. Positive: Ct ≤ 38 and amplification curve is S-shaped.
2. Suspected: 38 < Ct ≤ 40 and amplification curve is S-shaped, a second test is needed. Consider positive if Ct ≤ 40 and amplification curve is S-shaped for the second test. Considered negative if Ct >40 or null Ct and Ct ≤ 35 in CY5 channel for the second test.
3. Negative: Ct > 40 or null Ct and Ct ≤ 35 in CY5 channel.
4. Re-test: Ct >40 or null Ct and Ct > 35 in CY5 channel.

ROX channel for KPC, detection result should be interpreted as below.

1. Positive: Ct ≤ 38 and amplification curve is S-shaped.
2. Suspected: 38 < Ct ≤ 40 and amplification curve is S-shaped, a second test is needed. Consider positive if Ct ≤ 40 and amplification curve is S-shaped for the second test. Considered negative if Ct >40 or null Ct and Ct ≤ 35 in CY5 channel for the second test.
3. Negative: Ct > 40 or null Ct and Ct ≤ 35 in CY5 channel..
4. Re-test: Ct >40 or null Ct and Ct > 35 in CY5 channel.

FAM channel for VIM, detection result should be interpreted as below.

1. Positive: Ct ≤ 38 and amplification curve is S-shaped.
2. Suspected: 38 < Ct ≤ 40 and amplification curve is S-shaped, a second test is needed. Consider positive if Ct ≤ 40 and amplification curve is S-shaped for the second test. Considered negative if Ct >40 or null Ct and Ct ≤ 35 in CY5 channel for the second test.
3. Negative: Ct > 40 or null Ct and Ct ≤ 35 in CY5 channel..
4. Re-test: Ct >40 or null Ct and Ct > 35 in CY5 channel.

ROX channel for IMP, detection result should be interpreted as below.

5. Positive: Ct ≤ 38 and amplification curve is S-shaped.
6. Suspected: 38 < Ct ≤ 40 and amplification curve is S-shaped, a second test is needed. Consider positive if Ct ≤ 40 and amplification curve is S-shaped for the second test. Considered negative if Ct >40 or null Ct and Ct ≤ 35 in CY5 channel for the second test.
7. Negative: Ct > 40 or null Ct and Ct ≤ 35 in CY5 channel.
8. Re-test: Ct >40 or null Ct and Ct > 35 in CY5 channel.

Limitations

1. This product is only used on the applicable instrument platform, and the test operation and result judgment shall be carried out according to the instructions.
2. The test results of this kit are only for clinical reference. The treatment of patients should be comprehensively considered in combination with their symptoms / signs, medical history, other laboratory tests and treatment reactions.

Performance Index

1. Sensitivity: 500 copies/mL.
2. Specificity

1) No cross reaction with mycoplasma pneumoniae （ ≥106 pfu/mL） 、 Chlamydia pneumoniae（≥10^6 pfu/mL） 、 Legionella （≥10^6 pfu/mL）、Pertussis bacilli（≥10^6 pfu/mL）、Haemophilus influenzae（≥10^6 pfu/mL）、Staphylococcus aureus（≥10^6 pfu/mL）、Streptococcus pneumoniae（≥10^6 pfu/mL）、Streptococcus pyogenes （≥10^6 pfu/mL） 、 klebsiella pneumoniae （≥10^6 pfu/mL） 、 Mycobacterium tuberculosis（≥10^6 pfu/mL）、Aspergillus fumigatus （≥10^6 pfu/mL）、Candida albicans（≥10^6 pfu/mL）、Candida glabrata （≥10^6 pfu/mL） 、 Cryptococcus neoformans（≥10^6 pfu/mL）

2) Interfering substances: the following interfering substances do not affect the test results of the kit.

Endogenous interfering substances: blood (2%), purified mucin (2.5%).

Exogenous interfering substances: phenylephrine (0.5 mg/ml), hydroxymethylzoline (0.005%) 、 sodium chloride (containing preservative) (145 mmol/L) 、 beclomethasone (0.98 ng/ml), dexamethasone (989 2ng/ml) 、 flunitrazone (1mg/ml) 、 triamcinolone acetonide (0.5 ng/ml) 、 budesonide (4 nmol/L) 、 mometasone (0.05%) 、 fluticasone (0.05%) 、 histamine hydrochloride (1mg/ml) 、 α-Interferon (100u/ml) 、 zanamivir (142ng/ml) 、 ribavirin (52.7g/ml) 、 oseltamivir (10ug/ml) 、 pramivir (53.8 ug/mll)、 lopinavir (13.5 ug/ml)、 ritonavir (26.23 ug/ml) 、 abidol (658.5 ng/ml) 、 levofloxacin (6.12 ug/ml) 、 azithromycin (20 ug/ml)、ceftriaxone(80 ug/ml)、meropenem (112 ug/ml)、 tobramycin (4ug/mll).

3. Precision: CV ≤ 5%

Precautions

1. Management of the lab should comply with national regulations on gene amplification testing labs.
2. This kit is used for *in vitro* diagnosis only. It is for medical professional use only.

3. To prevent contamination, the same item should not be used cross different zones. Clean the bench immediately after conducting experiments.
4. Reagents of the kit should be thoroughly thawed and centrifuge briefly before use.
5. Reaction tubes containing reaction aliquots should be capped or placed in sealed bags before being delivered to the sample zone.
6. When loading sample, pipette the sample directly into the reaction solution without contacting the tube wall. Cap the tube immediately after loading the sample.
7. After the amplification is completed, the reaction tubes should be taken out immediately, placed in designated sealed bags and disposed at designated locations.
8. Avoid generating bubbles when aliquoting reaction mixture. Ensure the reaction tubes are capped before loading them into the PCR machine to avoid contamination.
9. Pipette tips used in the experiments should be directly tossed into a waste tank containing 1% sodium hypochlorite and disposed together with other wastes after sterilization.
10. Disinfect the working benches and other items regularly with 1% sodium hypochlorite, 75% alcohol or ultraviolet lamp.

Company Information

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SYMBOL	DESCRIPTION	SYMBOL	DESCRIPTION
	Manufactured By	CE	CE Mark
EC REP	Authorized Representative	REF	Catalog Number
IVD	InVitro Diagnostic Medical Device		Potential Biological Hazards After Use
LOT	Batch Code		Do Not Reuse
	Expiration Date in Year-Month-Day Format		Date of Manufacture
	Temperature Limitation		Consult instructions for use
	caution		Keep away from sunlight