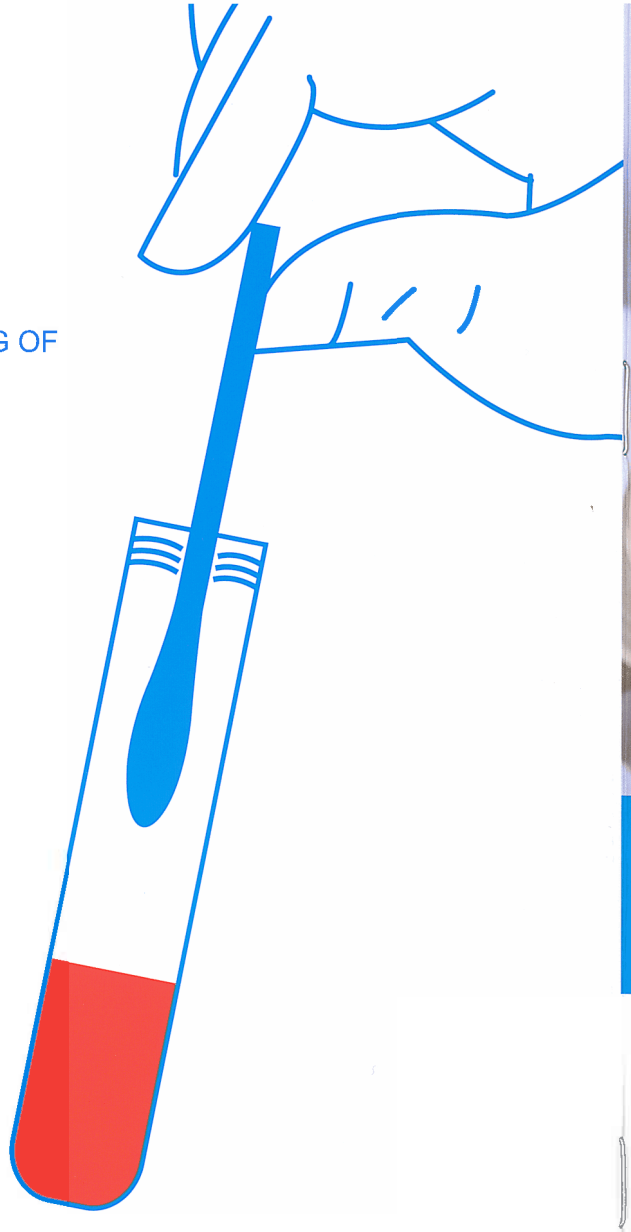
INNOVATE PROCESSING OF
SAMPLE PREPARATION



BIOCOMMA LIMITED

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C016-2EN

biocomma®
Transport and Preservation Medium

biocomma® transport and preservation medium, is intended for the collection, storage and transport of 2019-nCoV, viruses, chlamydiae, mycoplasma or ureaplasma specimens from the collection site to the testing laboratory. Biocomma provides solution for COVID-19 sampling and extraction.

Better Filter & Better Sample Prep www.biocomma.com

Company profile

Biocomma Limited, founded in 2006, is a leading manufacturer of sample preparation, sample filtration and sample collection products, based on its three technology platforms of porous plastic filters, separation materials and precision injection molding. Biocomma is ISO9001:2015 & ISO13485: 2016 certified and a National High and New Tech Enterprise.

Biocomma owns two subsidiaries, one R & D center and three manufacturing facilities, supplying more than 1,500 products. For the past fourteen years, we have served over 4,000 customers and provided OEM and custom services for dozens of well-known brands around the world.

CONTENTS

biocomma

Transport and Preservation Medium

No nucleic acid extraction, direct PCR analysis, efficient virus inactivation

Oder information:

Cat.#	Description	Qty.
YMJ-TE11	1*1 mL Direct to PCR VTM (5 mL tube), 1* nasopharyngeal swab	1 mL/pcs,50 pcs/Box

Preface

Respiratory tract infection is one of the most common clinical diseases, with a wide variety of pathogens, complex transmission routes, four seasons and any age. Common pathogens of respiratory infectious diseases mainly include viruses, bacteria, mycoplasma and chlamydia. The main methods of respiratory pathogen detection include virus isolation culture, bacterial culture and drug sensitivity test, antigen detection, antibody detection, nucleic acid detection and so on. Nucleic acid detection has the characteristics of early diagnosis, high sensitivity and specificity. It can quickly detect pathogens, confirm the cause, and timely treatment to avoid delaying the disease.

The nucleic acid detection generally involves five steps: sampling, sample preservation, transportation, nucleic acid extraction and detection. Sampling is the first step for nucleic acid detection and directly affects the detection performance of viruses. The virus sampling tube is a universal sampling product, which can be used for the collection and transportation of various virus specimen, mycoplasma, chlamydia, and ureaplasma samples.

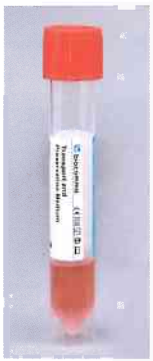
Most of the virus transport and preservation medium are internationally common formulas. The viruses stored in this type of sampling tubes are active and still have the potential risks of secondary infection. In addition, traditional sampling swabs are generally mattress-type core structures. It is difficult to release the specimen and affect its activation.

biocomma® Virus Transport and Preservation Medium include classic, inactivated, direct to PCR and pool testing methods. Classic VTM is suitable for virus cultivation, molecular biology test and immunology test; Inactivated VTM can inactivate virus efficiently and avoid the risk of aerosol infection, molecular biology test; When use direct to PCR VTM, the samples could be direct to PCR test without nucleic acid extraction; Pool testing VTM is suitable for group nucleic acid testing for 3~10 person per group.

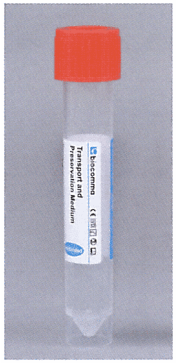
biocomma® virus transport and preservation medium have been supplied to more than 30 countries around the world, providing millions sets to support the prevention and control of the COVID-19 in 2020.

Introduction

biocomma® transport and preservation medium is intended for the collection, storage and transport of 2019-nCoV, viruses, chlamydiae, mycoplasma or ureaplasma specimens.



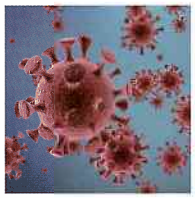
Classic VTM
Suitable for virus culture, molecular biology detection, immunological detection.

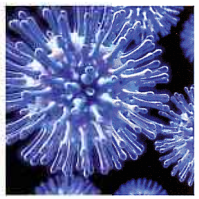


Inactivated VTM
Efficient inactivation of viruses, avoid the risk of aerosol infection, suitable for molecular biology detection.

Direct to PCR VTM
Efficient virus inactivation, no need to do nucleic acid extraction, direct PCR detection

Pool Testing VTM
Suitable for group nucleic acid testing for 3~10 person per group





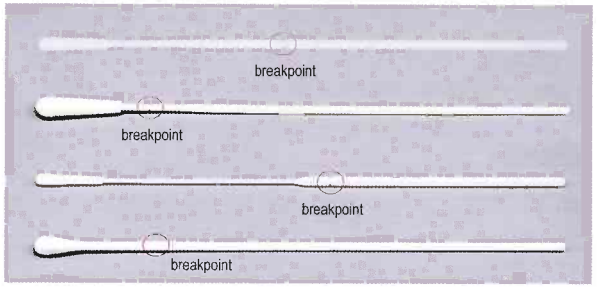
Composition:

1. Medium: biocomma® Classic VTM adds antibiotics, BSA, cryoprotectant, biological buffer and amino acid on the basis of Hank's solution, which has better maintenance capability of virus integrity than Hank's solution;

biocomma® Inactivated VTM adds cryoprotectants, biological buffers, amino acids, guanidine salts, and RNA protectants on the basis of Hank's solution, which can be used to lyse viruses and release nucleic acids directly, thereby eliminating RNase and making virus to lose its abilities of infection, causing disease, and reproduction without affecting the protein primary structure of the virus.

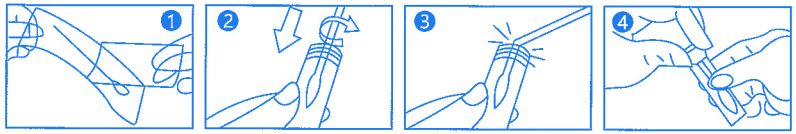
biocomma® Direct to PCR VTM is used to quickly release the nucleic acid of samples such as nasopharyngeal swabs, oral swabs and saliva. After the nucleic acid is released, it can be directly used as a nucleic acid amplification template for PCR, qPCR, RT-qPCR and isothermal amplification without nucleic acid purification.

2. Swabs: Optional oropharyngeal or nasopharyngeal swabs, the swab tip has a higher collection and release rate to ensure the accuracy of PCR test result. The plastic shaft has an unique breakpoint design, and the breaking process is microdebris-free.



3. Sampling tube: The tube is made of thickened premium polypropylene, which could be frozen at temperatures as low as -196°C under liquid nitrogen. The height of sampling tube is greater than 8 cm, which is to prevent the contamination from drop splashing out.

Instructions for Use:



Unwrap the package, take out the swab.

Use the swab to collect samples and put it back into the tube.

Break the swab rod and leave the sampling parts inside the tube.

Screw the lid tightly, and mark on the tube.

◆ Before sampling, fill out the sample information on the label;

◆ According to different experimental purposes, use swab to collect samples at the corresponding part;

◆ After sampling, quickly place swabs into the corresponding Transport and Preservation Medium tube, break the plastic shaft at the breakpoint and tighten the tube cap;

◆ The detailed sampling method is as follows:

a) Nasopharyngeal Swabs: gently insert the swab tip into the nasal palate of nasal passages, and slowly turn to exit after a while. Use another swab to collect samples from the other nostril, then quickly immerse the swab tip in the sampling liquid, break the sampling swab at the breakpoint and discard the handle end.

b) Oropharyngeal Swabs: wipe the bilateral pharyngeal tonsils and posterior pharyngeal wall with a swab and exit, then quickly immerse the swab tip in the sampling liquid, break the sampling swab at the breakpoint and discard the handle end.

◆ Transportation and storage conditions:

a) When collect specimens with biocomma® Classic Transport and Preservation Medium, sampling tube should be transported to the laboratory within 48 hours at 2-8°C. Specimens used for virus isolation and nucleic acid testing should be tested as soon as possible. Specimens 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 (If there is no storage condition at -70°C, please store it temporarily in the refrigerator at -20°C).

b) When collect specimens with biocomma® Inactivated Transport and Preservation Medium, sampling tube should be delivered to the laboratory within 7 days at room temperature (5-25°C), it will be better if there are ice bags during transportation. For a better performance, specimens used for nucleic acid detection should be tested within 24 hours.

c) When collect specimens with biocomma® direct to PCR VTM, sample can be stored at 5-25°C, but not more than 5 days. For long-term storage, put it at -70°C and below for freezing. It's better to test the samples within 24 hours after collection.

Precautions:

◆ This product is only used for in vitro diagnosis(IVD).

◆ The liquid in the sampling tube is the transport medium, and it cannot be dipped with a sampling swab before sampling.

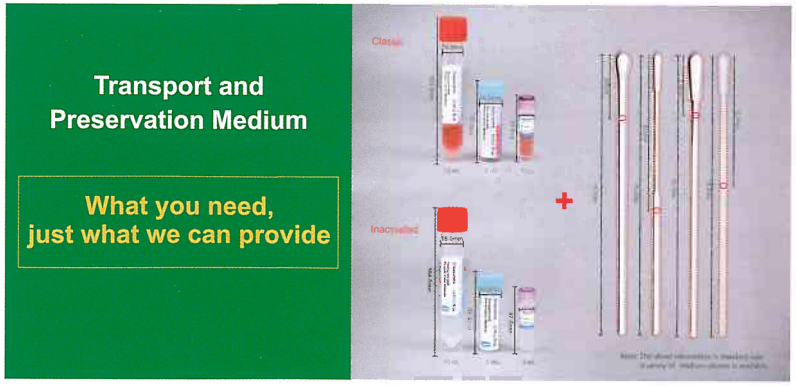
◆ The waste sample collection solution should be sterilized.

◆ If it is found that the transport medium is out of date, the liquid is discolored, turbid, or leaks, it is forbidden to use.

Order information:

Cat. #	Description	Qty.
YVJ-TE	1* 3 mL of classic medium, 1* rayon swab	50 pcs/Box
YVJ-TE2	1* 3 mL of classic medium, 1* nasopharyngeal swab	50 pcs/Box
YVJ-TE4	1* 3 mL of classic medium, 1* nasopharyngeal swab+1 *oropharyngeal swab	50 pcs/Box
YVJ-E	1* 3 mL of classic medium	50 pcs/Box
YMJ-TE	1* 3 mL of inactivated medium, 1* rayon swab	50 pcs/Box
YMJ-TE2	1* 3 mL of inactivated medium, 1* nasopharyngeal swab	50 pcs/Box
YMJ-TE4	1* 3 mL of inactivated medium, 1* nasopharyngeal swab+1 *oropharyngeal swab	50 pcs/Box
YMJ-E	1* 3 mL of inactivated medium	50 pcs/Box
YMJ-TE11	1*1 mL direct to PCR VTM, 1* nasopharyngeal swab	50 pcs/Box
YMJ-TE5	1* virus sampling tube (15 mL), containing 5 mL inactivated preservation medium, no swab	50 pcs/Box
SW21E	Disposable rayon swab	50 pcs/PK

Note: The sampling tube can be 2 mL/5 mL/10 mL/15 mL, etc. A variety of medium volume (1 mL/2 mL/3 mL/5 mL/10 mL) is available You can use the swab included in the medium, or from other brands.

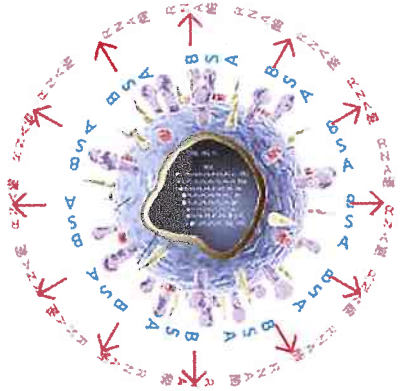


Quality Assurance

biocomma® transport and preservation medium owns comprehensive evaluation and test data, automated production and strict quality control system. At present, our products are steady supplied to more than 30 countries around the world.

Test Data:

1. biocomma® classic transport and preservation medium adds protein stabilizing component BSA. BSA can form a protective film on the surface of the virus shell, thereby making virus difficult to decompose to ensure the integrity virus and increase the positive rate.



2. biocomma® transport and preservation medium adopts stable antibiotics at room temperature, and the combination of multiple antibiotics was applied to prevent bacterial and fungal contamination effectively, thereby solving the room-temperature storage of sampling tubes.

Table 1: The effect of using normal temperature antibiotics in the transport and preservation medium

	Experimental group: Sampling tube with antibiotic + bacterial suspension	Positive control group: Sampling tube without antibiotic + bacterial suspension	Negative control group: Sampling tube with antibiotic + blank culture solution
Parallel 1	- -	+++	- -
Parallel 2	- -	+++	- -
Parallel 3	- -	+++	- -

Note: The bacteriostatic effect after adding 0.5 MCF of *Candida albicans* suspension in the sampling tube for 7 days.
- -: Fungal growth is completely inhibited, +++: Fungal growth is good.

3. biocomma® inactivated transport and preservation medium adds appropriate concentration of guanidine salt and RNA protectant. The guanidine salt can destroy the hydrogen bond and the hydrophobic bond between the non-polar side chains of amino acid residues, denature the viral protein, and quickly destroy the viral protein shell. The RNA protector can effectively improve the integrity of the viral RNA and achieve effect for both inactivate virus and protect its RNA.

Table 2: Effect comparison for three kinds of bacteria by the classic/inactivated sampling tube

	Test Group: inactivated sampling tube + bacterial suspension	Test Group*: Classic sampling tube + bacterial suspension	Positive Control Group: Classic sampling tube + bacterial suspension	Negative Control Group: inactivated sampling tube + blank culture medium
Parallel A-1	- -	- -	++	- -
Parallel A-2	- -	- -	++	- -
Parallel A-3	- -	- -	++	- -
Parallel B-1	- -	- -	++	- -
Parallel B-2	- -	- -	++	- -
Parallel B-3	- -	- -	++	- -
Parallel C-1	- -	- -	++	- -
Parallel C-2	- -	- -	++	- -
Parallel C-3	- -	- -	++	- -

Note: The bacterial suspension of Parallel A is *E. coli*, the bacterial suspension of Parallel B is *Bacillus subtilis*, and the bacterial suspension of Parallel C is *Staphylococcus aureus*; Add 0.5 MCF *E. coli/Bacillus subtilis/Staphylococcus* aureus bacterial suspension to the classic/inactivated sampling tubes respectively, and then transfer to the corresponding solid medium for 24 hours of growth; The Test Group* is the growth condition after adding the bacterial suspension and treating at 56 ℃ for 30 minutes, and then transferring to the corresponding solid medium for 24 hours.
- - : Aseptic growth; ++: Colonies grow well

Figure 1: Comparison of effect of different medium on *E. coli*

Note:
2-1: Inactivated medium + *E. coli*, after 24 hours of culture, aseptic growth
2-2: Classic medium + *E. coli*, after 24 hours of culture, the colony grows well

The above data can explain that the inactivation effect of the inactivated medium on *E. coli* can achieve the effect of 56°C, 30 min treatment.

4. Preserve pseudovirus (10^6copies/mL) with biocomma® direct to PCR VTM, and taking the preservation medium for qPCR amplification, no inhibition occured.

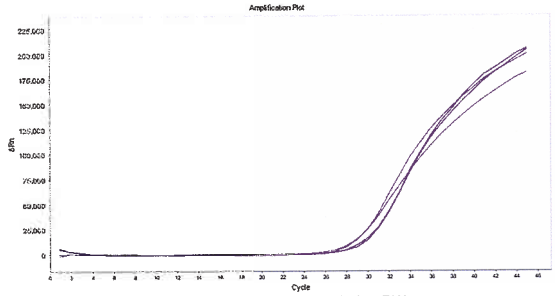
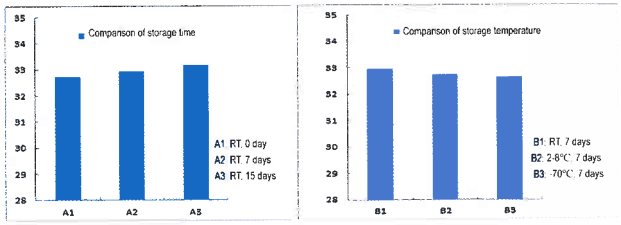


Figure 2: qPCR analysis of pseudovirus RNA

5. Dilute the simulated virus sample to 10^5 copies/mL, preserve it with biocomma® inactivated transport and preservation medium (YMJ-TE), and then do nucleic acid extraction and qPCR detection to analyze the effect of storage time and storage temperature on the preservation effect of the sampling tube. The results are as follows:



4-1 4-2

Figure 3: Effect on preservation in different storage time and temperature

4-1: From the qPCR results, with the increase of storage time, the CT value increases by 0.23 on average, with the amplitude 0.73%, which does not affect the judgment of the test results.
4-2: From the qPCR results, in different storage temperature, the CT value differs by 0.15, which does not affect the judgment of the test results.

6. Oral swabs were collected and stored in biocomma® transport and preservation medium (classic/inactivated), and samples were stored at 4°C for 0 days and 3 days, virus DNA/RNA extraction kits (Cat. No.: MNP027-1E) were used to extract RNA, electrophoresis detection analysis is as follows:

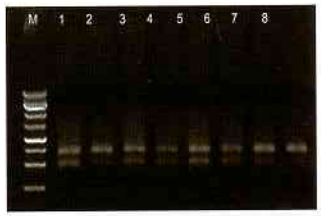


Figure 4: Electrophoresis detection analysis of RNA
Lane 1-2: RNA from classic medium after 0 days of storage
Lane 3-4: RNA from inactivated medium after 0 days of storage
Lane 5-6: RNA from classic medium after 3 days of storage
Lane 7-8: RNA from inactivated medium after 3 days of storage

7. Storing pseudovirus with biocomma® transport and preservation medium (classic/inactivated), put the classic type tube into -20°C and inactivated type into room temperature, extract RNA for qPCR, the results are as below:

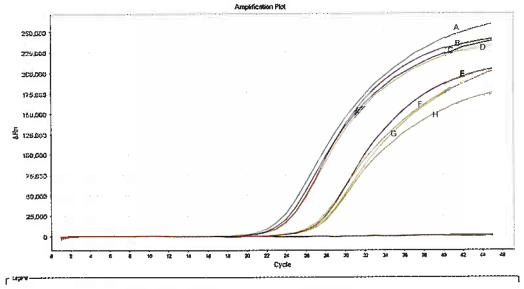


Figure 5: qPCR analysis of pseudovirus RNA

A-B: classic medium, 10^6 copies/mL C-D: inactivated medium, 10^6 copies/mL
E-F: classic medium, 10^5 copies/mL G-H: inactivated medium, 10^5 copies/mL

8. Preserve pseudovirus (10^6copies/mL) at room temperature with biocomma® direct to PCR VTM, take a sample for qPCR detection after 1/2/3/4/5 days, the results are as following diagram:

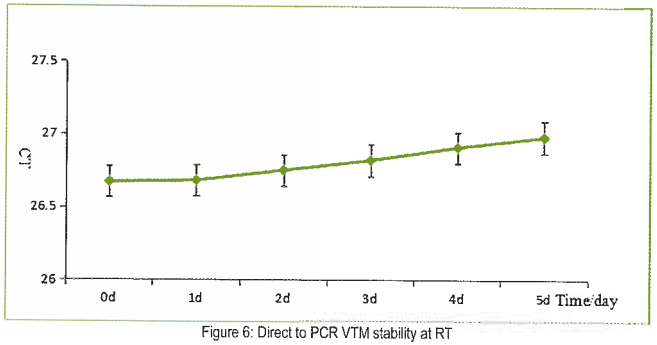


Figure 6: Direct to PCR VTM stability at RT

Raw Material Option:

We have more than 14 years experience in custom mold services for plastic column tubes and porous plates. The injection molding environment of virus sampling tubes has been certified by ISO9001. The raw materials for the production of column tubes are USP VI (US National Pharmacopoeia Level 6) resins, guaranteed the stability of virus sampling tube products from the source. The screw cap design is adopted to enhance the sealing of the column tube and avoid liquid leakage; the sampling tube can be tapered to facilitate the operation.

Automatic Production Line:

biocomma® transport and preservation medium (classic/inactivated) adopts automatic production line, with an average daily production capacity of 500,000 pieces, fully automatic blister packaging, beautiful appearance, easy to use, good sealing performance and convenient transportation.

Excellent Quality Control System:

We have established a complete quality control system for transport and preservation medium, and each batch of products produced is verified and approved by the quality inspection management. We use ERP system for product management, each batch of products are implemented batch number management, from raw materials to finished products which can be traced back to the whole process, each batch number products are retained sample management according to regulations.

Application

2019-nCoV Sample Collection by biocomma® Transport and Preservation Medium

1. Material and Equipment Preparation

1.1 Sample: Collect oral sample from a suspected 2019-nCoV patient in a top hospital in Chongqing, China, sample is an oral swab.

1.2 Preservation Medium: Use biocomma® Transport and Preservation Medium(YVJ-TE3) to collect samples according to the 2019-nCoV diagnosis and treatment plan (Fifth version)

1.3 Nucleic Acid Extraction and Detection Reagent: Both nucleic acid extraction and detection reagent come from Sansure Biotech.

1.4 Real-time fluorescent PCR detection system: Bio-RAD CFX-96

2. Experimental Methods and Procedures

2.1 Reagent Preparation

2.1.1 Take out all the components in the kit and put it at room temperature to equilibrate it to room temperature, then mix well and set aside.

2.1.2 Base on the quantity of samples to be tested, negative control, positive control, take the corresponding amount of reaction solution and enzyme according to the proportion (2019-nCoV-PCR reaction solution 26 μL/rnx + 2019-nCoV-PCR-enzyme mixed solution 4 μL/rnx), and fully mix them into PCR-mix, which is used after instant centrifugation.

2.2 Sample Preparation

2.2.1 Put the transport medium tube with swab head (do not open the lid) into the oven at 56 ℃ for 30 minutes to inactivate the virus.

2.2.2 Process nucleic acid extraction according to the reagent instruction.

2.2.3 Add 30 μL of PCR-mix and 20 μL of the processed sample to the PCR reaction tube in sequence, and then test it on the PCR system.

2.3 PCR Amplification

Select FAM (ORF-1ab gene) and ROX (N gene) (Reporter: FAM/ROX, Quencher: none) channels to detect 2019-nCoV nucleic acid; select HEX or VIC channel (Reporter: HEX/VIC, Quencher: none) detection Internal standard; set Sample Volume to 50.

PCR System Parameter Programming

	Experimental procedures	Temperature	Time	Cycles
1	Reverse transcription	50℃	30min	1
2	Pre-denaturation	95℃	1min	1
3	Denaturation	95℃	15sec	45
	Annealing, extension, and fluorescence collection	60℃	30sec*	
4	Instrument Cooling (optional)	25℃	10sec	1

* is the setting of fluorescence acquisition

3. Result Analysis

3.1 Baseline confirmation

The instrument automatically generates a baseline. If the instrument misjudges the baseline and the curve shape is abnormal, manually select the area where the fluorescence signal does not fluctuate greatly. The starting cycle number should avoid the signal fluctuations at the beginning of the fluorescence collection, and the ending cycle number should be earlier than the earliest appearance. The CT value of the exponentially amplified sample decreases by 1~2 cycles (generally takes 2~10 or 2~15 cycles of fluorescence signal).

3.2 Threshold setting

In principle, the threshold line just exceeds the highest point of the normal negative control amplification curve (irregular noise line), and the CT value = 0.0 shall prevail. It can also be adjusted between 15.0~40.0 according to the noise of the instrument.

3.3 Results analysis

3.3.1 Analyze whether the internal standard of the HEX channel has an amplification curve, Ct≤40 means that the test is valid and the analysis can be continued;

(A) If a typical S-type amplification curve is detected in the FAM or ROX channel, and Ct≤40, it means that the COVID-19 virus test is positive;

(B) If neither FAM nor ROX channels detect a typical S-type amplification curve, or Ct>40, it means that the COVID-19 virus test is negative.

3.3.2 Gray area result judgment: If the fluorescence signal of a sample in the FAM and ROX channels has a significant increase, but the Ct > 40, the sample is in the gray area and needs to be re-examined. If the retest result is still in the gray area, it is judged as positive.

3.3.3 If the internal standard does not detect a signal in the HEX channel or Ct>40, it means that the concentration of the test sample is too low or there is an interfering substance to inhibit the reaction, and the experiment must be prepared again.

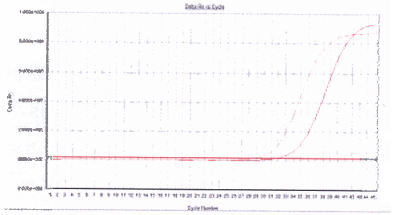
3.3.4 For a negative sample, the internal standard test should be positive. If the internal standard test is negative, the test result of the sample is invalid. The cause should be found and eliminated, and the sample should be re-prepared to repeat the experiment (if the repeated test result is still invalid, please contact the reagent company).

4. Sample processing after amplification

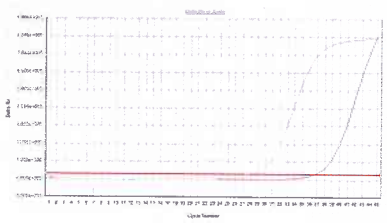
4.1 After the amplification is completed, wear new gloves, discard the complete PCR reaction tube into the dirt bucket and soak it for more than 30 minutes, and then do centralized treatment ;

4.2 Use 75% alcohol to sterilize the instrument and desktop, irradiate the instrument with ultraviolet light for 1h; leave the amplification room and discard the PE gloves and do hand disinfection. After returning to the treatment area, disinfect the corridor floor.

5. Experimental results and analysis

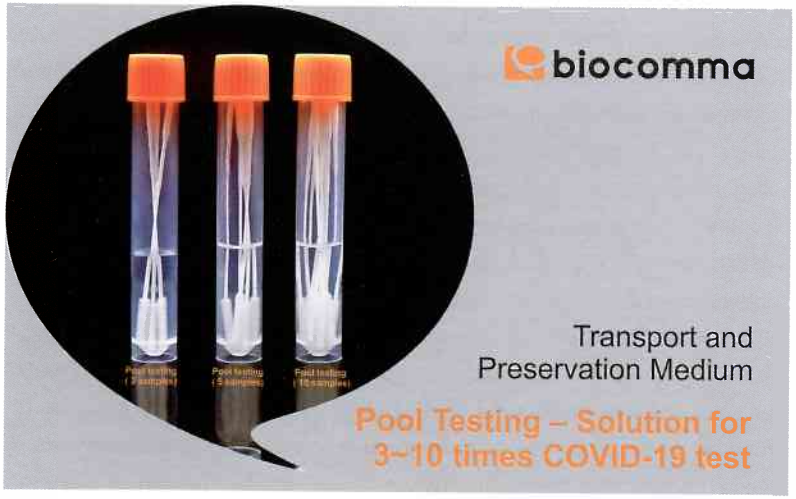


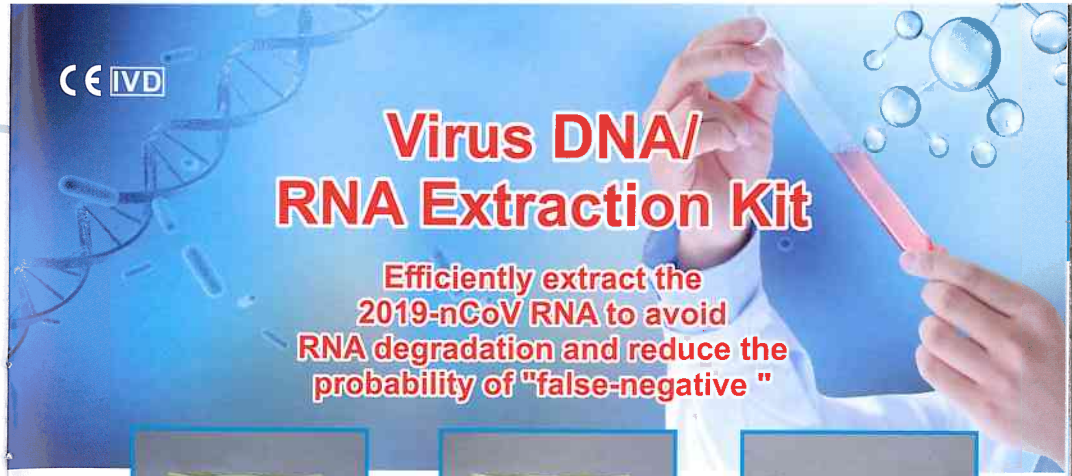
ORF1ab Gene



N Gene

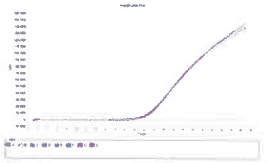
Analysis of the internal standard Ct≤40 of the HEX channel indicates that the test is valid and the analysis can be continued. The ORF1ab gene and N gene are both detected as typical S-type amplification curves, and Ct=29, indicating that the COVID-19 virus nucleic acid test result is positive.





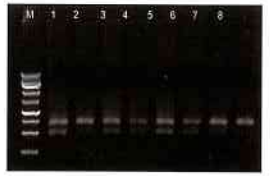
Typical application 1:

Virus DNA was extracted from African swine fever virus (ASFV) positive serum using a pre-packed viral DNA/RNA extraction kits (Cat. No.: BNP027-2E). The results of qRT-PCR are shown below:



Typical application 2:

Oral swabs were collected and stored in classic/inactivated virus transport media, and samples were stored at 4° C for 0 and 3 days, Viral DNA/RNA extraction kits (Cat. No.: MNP027-1E) were used to extract RNA, electrophpresis detection analysis is as follows:



Lane1-2: RNA from classic transport medium after 0 days of storage
Lane3-4: RNA from inactived transport medium after 0 days of storage
Lane5-6: RNA from classic shipping medium after 3 days of storage
Lane7-8: RNA from inactived transport medium after 3 days of storage

Order Information:

Cat. #	Description	Qty.
MNP027-1E	Virus DNA/RNA extraction kit (Spin Column)	50preps/box
BNP027-1E	Virus DNA/RNA extraction kit (Magnetic Beads)	50preps/box
BNP027-2E	Virus DNA/RNA extraction kit (Prepacked Magnetic Beads, MB32)	32preps/box
BNP027-3E	Virus DNA/RNA extraction kit (Prepacked Magnetic Beads, MB96)	96preps/box