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Instructions for Use

COVID-19 RT-qPCR Detection Kit Plus

PRODUCT NAME
COVID-19 RT-qPCR Detection Kit Plus
Catalog Number: QP019T-0100

Manufactured by:
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Table of Contents

1 Intended Use 3
2 Principle of Detection 3
3 Kit Contents 4
4 Storage and Handling Requirements 4
5 Product Description 5
6 Quality Control 5
7 Limitations 5
8. Warning and Precautions 5
9. Protocol 7
10 Data Analysis and Interpretation 8
11 Performance Characteristics 9
12 Troubleshooting 15
13 Symbols used in Packaging 16
14 Method of Sterilization 16

1 Intended Use

The COVID-19 RT-qPCR Detection Kit Plus is a multiplex real-time RT-PCR (RT-qPCR) test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 for human respiratory tract specimens (such as nasal swabs, mid-turbinate nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) from individuals suspected of COVID-19 by their healthcare provider.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper and lower respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infective status. The agent detected may not be the definite cause of disease. Positive results do not rule out bacterial co-infection with other viruses. Laboratories are required to report all positive results to the appropriate public health authorities. Negative results do not preclude SARS-CoV-2 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. The COVID-19 RT-qPCR Detection Kit Plus is intended for use by qualified trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

2 Principle of Detection

This product is a fluorescent probe-based RT-qPCR assay system. Firstly, the RNA of SARS-CoV-2 will be reverse transcribed into cDNA by reverse transcriptase, and then PCR amplification will be performed with cDNA as template. During amplification of the template, the probe will be degraded due to the 5´-3´ polymerase activity and exonuclease activity of DNA polymerase, then the separation of fluorescent reporter and quencher enables the fluorescent signal to be detected by instrument. The N1 gene of SARS-CoV-2 will be detected qualitatively by FAM channel and the internal control RP (human gene) will be detected by HEX channel. Internal control is used in the kit for quality control starting from sample collection.

Instrument: Thermo Fisher QuantStudio™ 5 Real-Time PCR System with software QuantStudio™ Design and Analysis cloud software or QuantStudio™ Design and Analysis desktop software.

3 Kit Contents

Table 1. COVID-19 RT-qPCR Detection Kit Plus Contents

Component	Description	Amount Supplied (per 100 rnxs)
2X RT-qPCR MasterMix	Multiplex assay primers/probes for N1 and RP (human) genes with HotStart DNA polymerase, dNTPs, and buffer	1000μL x 1
RScript Enzyme Mix	RScript reverse transcriptase with RIBOAssure RNase inhibitor	20μL x 1
Positive Control	Pseudo-virus DNA containing N1 gene	50μL x 1
Negative Extraction Control	Cancer cell line with RP gene	1mL x 1
Nuclease-Free Water	DEPC-treated water	1mL x 1

4 Storage and Handling Requirements

- COVID-19 RT-qPCR Detection Kit Plus is shipped with dry ice and gel packs.
- All components of the kit arrive in solution.
- All components of the kit must be stored at -20°C upon arrival.
- Do not use kit components after expiration date printed on the box label.
- If there is damage to the packaging inside, outside or kit contents have been tempered with, or storage condition failed to meet above -20°C, do not use.
- Dispose of unused reagents and waste in accordance with country, federal, state, and local regulations.
- Repeated freezing and thawing may lead to inaccurate results.
- The kit is stable for up to 1 year from date of release.

Note: Inaccurate results may be obtained if the kit is not handled according to the instructions provided.

5 Product Description

- 2X RT-qPCR Master Mix: It is a multiplex assay containing primers/probes for N1 and RP (human) genes with Hot Start DNA polymerase, dNTPs & buffer.

- RScript Enzyme Mix: Unique blend of RScript reverse transcriptase with RIBOAssure RNase inhibitor.

- Positive Control: Ensures the assay is performed according to its use by evaluation with Pseudo-virus DNA containing N1 genes.

- Negative Extraction Control: Refers to the cancer cell line with RP gene.

- Nuclease-Free Water: DNase, RNase, and nuclease-free, as in reference to DEPC-treated water.

6 Quality Control

In order to evaluate the quality control, the test includes Positive and Negative controls. They might also be used for laboratory verification.

Table 2. Positive and Negative Control

Products of quality control	Requirements of Quality Control	
	FAM Channel	HEX Channel
Positive Control of SARS-CoV-2	Ct<40	ND
Negative Extraction Control	ND	Ct<40

ND = Not Detected

7 Limitations

- This kit should be transported under 4°C. We are unable to demonstrate the kit quality if temperature which is transported in is over 37°C.
- Improper sample collection, shipping and storage may cause false-positive.
- Detection may be affected by sample collection methods and the stage of infection.
- Always use new pipette tips with aerosol barriers.

8. Warning and Precautions

- The contamination of laboratory environment and reagents, or cross contamination during specimen treatment may lead to false positive result.

- Quickly prepare the reaction mix on ice or in the cooling block.
- The decrease of detection effect even the false negative result may occur if there are any mistakes in the transportation, storage, and operation of reagents. SARS-CoV-2 early infection or other respiratory virus infection cannot be excluded in patients with negative results.
- For in vitro diagnostic use.
- For prescription use only.
- Laboratories are required to report all positive results to the appropriate public health authorities.
- Handle all specimens as if infectious and use safe laboratory procedures.
- Inappropriate sample collection, transfer, storage, and operation may lead to inaccurate test results.
- RNA extraction shall be carried out as soon as possible after sample collection to avoid degradation.
- The disposal of this kit will not cause any special risk or harm.

9. Protocol

1. Prepare the PCR Reaction: Thaw and assemble the following components in a 0.2 ml PCR tube on ice just prior to use: 2X RT-qPCR MasterMix and RScript Enzyme Mix.
Caution: Do not add more than one RNA sample into a single qPCR tube. Mix gently. If necessary, centrifuge briefly.

Table 3: PCR reaction preparation components

Component	Sample(s)	Positive Control	No Template Control	Negative Extraction Control
RNA Sample	5 µl	-	-	-
2X RT-qPCR MasterMix	10 µl	10 µl	10 µl	10 µl
RScript Enzyme Mix	0.2 µl	0.2 µl	0.2 µl	0.2 µl
Positive Control Template	-	5 µl	-	-
Negative Extraction Control	-	-	-	5 µl
Nuclease-free H_2O	4.8 µl	4.8 µl	9.8 µl	4.8 µl
Total Volume	20 µl			

2. Prepare the Controls: Use the Nuclease-free H_2O for the No Template Control while using Positive Control for the Positive Control setup. Cap tubes tightly and place them in the thermal cycler.

3. Running Method: Set the thermal cycler for 45 cycles as follows:

Table 4. Steps and cycles

Steps	Temperature	Time	Cycle(s)
cDNA Synthesis	42°C	15 minutes	1
Pre-Denaturation	95°C	5 minutes	1
Denaturation	95°C	5 seconds	45
Annealing	60°C	30 seconds	
Instrument Cooling	40°C	10 seconds	1

Note: Optimal conditions for amplification will vary depending on the thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.

10 Data Analysis and Interpretation

Base line and threshold settings

The COVID-19 RT-qPCR Detection Kit Plus has been validated using the Thermo Fischer QuantStudio™ 5 Real-Time PCR System with software QuantStudio™ Design and Analysis cloud software or QuantStudio™ Design and Analysis desktop software.

Test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

Considering the performance difference in real-time PCR instruments, thresholds for two fluorescence signals (FAM and HEX) are set automatically by the machine based on the fluorescent value of the positive control for each PCR run.

Results Interpretation

As two channels (FAM, HEX) are used in this one tube qPCR assay, we recommend to perform the channel calibration as requested by its manufacturer. Please refer to the instrument's user manual to perform this calibration. Choose the FAM and HEX channels for each sample to be tested with the COVID-19 RT-qPCR Detection Kit Plus. Select "None" for ROX passive reference on any qPCR machine requiring ROX as the reference dye.

Table 5. Expected Performance of Controls

Control Type	Used to Monitor	Expected Results and Ct Values	
		N (FAM)	**RP (HEX)**
No Template Control	Assay or extraction reagent contamination	Negative ND	Negative ND
Positive Control	Improper assay setup and reagent failure, including primer and probe degradation	Positive Ct <40	Negative ND
Negative Extraction Control	Cross-contamination during extraction	Negative ND	Positive Ct < 40

ND = Not Detected

Table 6. Expected Performance of Patient Samples

SARS-CoV-2		Interpretation	Action
N1	**RP**		
+	+/-	Positive	Report results to health authority.
-	+	Negative	SARS-CoV-2 not detected. Report results to health authority
-	-	Invalid Result *	Repeat from extraction step. If the repeated result remains invalid, recommend collection of a new specimen(s) from the patient.

* If the samples obtained are repeatedly invalid or presumptive, a new test must be obtained.
*A test is considered positive where N gene's result is positive (+), otherwise the results are considered presumptive/inconclusive and re-testing needs to be carried out.

11 Performance Characteristics

11.1 Inclusivity – Analytical Reactivity

Table 7. *In silico* analysis for Inclusivity Blast resalt.

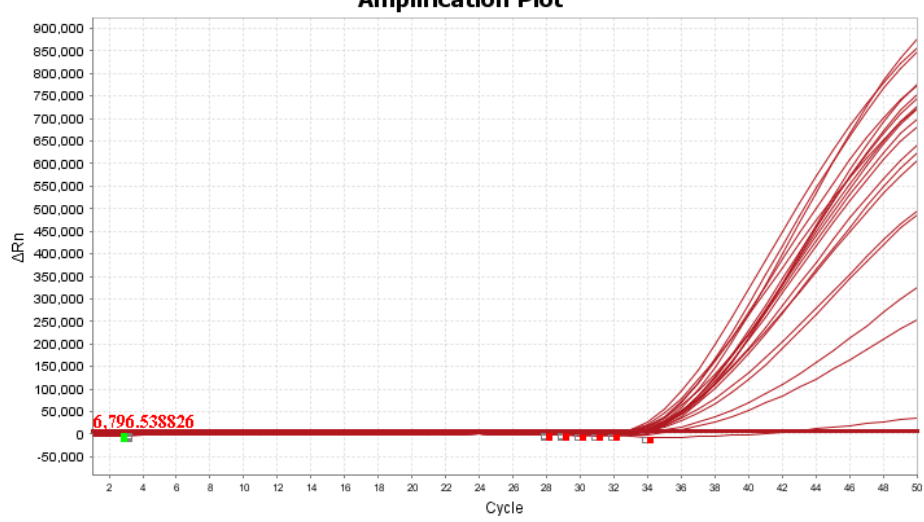
Strain	PANGOLIN	Genbank	In silico analysis of % identify to 2019-nCoV_N1 Forward Primer	In silico analysis of % identify to 2019-nCoV_N1 Reverse Primer	In silico analysis of % identify to 2019-nCoV_N1 Probe	The likelihood of being detected
SARS CoV 2 isolate CGMH-CGU-60	B.1.1.7	MZ277393.1	100%	100%	100%	Can be detected
SARS CoV 2 isolate SS1436	B.1.351	OK085750.1	100%	100%	96%	Can be detected
SARS CoV 2 isolate Dtt17	P.1	MZ611960.1	100%	100%	100%	Can be detected
SARS CoV 2 isolate INSACOG-GBRC873	B.1.617.2	MZ895467.1	100%	100%	100%	Can be detected
SARS CoV 2 isolate Hospital_1_54	B.1.617.1	MZ724426.1	100%	100%	96%	Can be detected
SARS CoV 2 isolate TG808823	B.1.427	MZ915615.1	100%	100%	100%	Can be detected
SARS CoV 2 isolate NAMRU3_A844	B.1.525	MZ970734.1	100%	100%	100%	Can be detected

SARS CoV 2 isolate CO-CDPHE-2101395288	B.1.526	OK021801.1	100%	100%	100%	Can be detected
SARS CoV 2 isolate WV118809	C.37	MZ497816.1	100%	100%	96%	Can be detected
SARS CoV 2 isolate WV119718	P.2	MZ498354.1	100%	100%	100%	Can be detected

11.2 Analytical Sensitivity and Limit of Detection (LoD)

A study was performed to assess the performance of the COVID-19 RT-qPCR Detection Kit Plus. A testing with 20 replicates at the tentative Limit of Detection (LoD) concentration was carried out with 50 copies/reaction with the 95% detection rate. Samples were spiked with the SARS-CoV-2 RNA in the pooled negative clinical nasopharyngeal swab matrix, then extracted with QIAamp Viral RNA Mini Kit, and tested on the QuantStudio™ 5 Real-Time PCR System. The synthetic SARS-CoV-2 RNA was obtained from Twist Biosciences (Assay Ready Control 14,EPI_ISL_710528).

Table 8. Limit of Detection COVID-19 RT-qPCR Detection Kit Plus for N1 gene.



Target	Concentration	Detection Rate	Mean Ct
SARS-CoV-2-N1 gene	50 copies	95 % (19/20)	34.088

11.3 Cross -Reactivity

To demonstrate that the COVID-19 RT-qPCR Detection Kit Plus does not react with related pathogens, high prevalence disease agents and normal or pathogenic flora that are reasonably likely to be encountered in a clinical specimen, an *in silico* analysis was performed using the primer and probe sequences from the NCBI database.

Table 9. *In silico* analysis for high priority pathogens and organisms.

Pathogen	GenBank	Percent of Homology		
		SARS-CoV-2 N1 forward primer	**SARS-CoV-2 N1 reverse primer**	**SARS-CoV-2 N1 probe**
HCoV HKU1	NC_006577.2 MK167038 MH940245 KF686341 NC_006577	≦80% homology	≦80% homology	≦80% homology
HCoV OC43	NC_006213.1 ATCC VR-759 MK303620 MN310478 MG197719 MG197713 NC_006213	≦80% homology	≦80% homology	≦80% homology
HCoV NL63	NC_005831.2 MK334047 MN306040 NC_005831 JQ765567 JQ765573 JQ765564	≦80% homology	≦80% homology	≦80% homology
HCoV 229E	NC_002645.1 MF542265 MN369046 MN306046 KY996417	≦80% homology	≦80% homology	≦80% homology
SARS CoV	NC_004718.3 NC_004718.3 AY291451.1	≦80% homology	≦80% homology	≦80% homology
MERS CoV	NC_019843.3 MN120514.1 MH013216.1 KP719932.1	≦80% homology	≦80% homology	≦80% homology
Influenza A/B	NC_026422.1 NC_026423.1 NC_026424.1 NC_026425.1 NC_026426.1 NC_026429.1 NC_026427.1 NC_026428.1 NC_002023 NC_002021	≦80% homology	≦80% homology	≦80% homology

Pathogen	GenBank	Percent of Homology		
		SARS-CoV-2 N1 forward primer	SARS-CoV-2 N1 reverse primer	SARS-CoV-2 N1 probe
	NC_002022 NC_002017 NC_002019 NC_002018 NC_002016 NC_002020 NC_002204.1 NC_002205.1 NC_002206.1 NC_002207.1 NC_002208.1 NC_002209.1 NC_002210.1 NC_002211.1			
Adenovirus type 1、7	NC_011202.1 AC_000008.1 NC_011203.1 NC_001405	≦80% homology	≦80% homology	≦80% homology
Cytomegalovirus	NC_006273.2	≦80% homology	≦80% homology	≦80% homology
Enterovirus	NC_001430.1 JX070222 KU509997	≦80% homology	≦80% homology	≦80% homology
Epstein Barr Virus	NC_007605.1	≦80% homology	≦80% homology	≦80% homology
Human parainfluenza type 1、**2**、3、4	NC_003461.1 NC_003443.1 NC_001796.2 NC_021928.1 NC_003461 KM190939 NC_001796 NC_021928	≦80% homology	≦80% homology	≦80% homology
Measles Human metapneumovirus	NC_039199.1	≦80% homology	≦80% homology	≦80% homology
Mumps Virus	NC_002200.1	≦80% homology	≦80% homology	≦80% homology
Respiratory syncytial virus type B	NC_001803.1 KM360090	≦80% homology	≦80% homology	≦80% homology
Rhinovirus	KY369886.1 X02316 NC_001490	≦80% homology	≦80% homology	≦80% homology
Bordetella pertussis	NC_002929.2 NC_017223	≦80% homology	≦80% homology	≦80% homology
Chlamydia pneumoniae	NC_000922.1	≦80% homology	≦80% homology	≦80% homology
Corynebacterium sp.	NZ_CP069568.1 NZ_CP069569.1	≦80% homology	≦80% homology	≦80% homology
Escherichia coli	NC_000913.3	≦80% homology	≦80% homology	≦80% homology
Hemophilus influenzae	NC_000907.1 NC_017451	≦80% homology	≦80% homology	≦80% homology

Pathogen	GenBank	Percent of Homology		
		SARS-CoV-2 N1 forward primer	SARS-CoV-2 N1 reverse primer	SARS-CoV-2 N1 probe
Lactobacillus sp.	NZ_CP027194.1 NZ_CP047411.1	≦80% homology	≦80% homology	≦80% homology
Legionella spp	NC_002942.5 NC_021350	≦80% homology	≦80% homology	≦80% homology
Moraxella catarrhalis	NC_014147.1	≦80% homology	≦80% homology	≦80% homology
Mycobacterium tuberculosis (avirulent)	NC_000962.3 NC_017524 NC_022350 NC_022350	≦80% homology	≦80% homology	≦80% homology
Mycoplasma pneumoniae	NZ_CP010546.1 NC_000912 NC_016807	≦80% homology	≦80% homology	≦80% homology
Neisseria meningitidis	NZ_CP021520.1	≦80% homology	≦80% homology	≦80% homology
Neisseria sp.	NZ_CP023429.1	≦80% homology	≦80% homology	≦80% homology
Pseudomonas aeruginosa	NC_002516.2 NC_008463 NC_009656	≦80% homology	≦80% homology	≦80% homology
Staphylococcus aureus (Protein A producer)	NC_007795.1	≦80% homology	≦80% homology	≦80% homology
Staphylococcus epidermidis	NZ_CP035288.1 NZ_CP035290.1 NZ_CP035289.1 NC_004461	≦80% homology	≦80% homology	≦80% homology
Streptococcus pneumoniae	NC_003098.1 NC_011900 NC_018594 NC_014494	≦80% homology	≦80% homology	≦80% homology
Streptococcus pyogenes	NC_002737.2 NC_017596 NC_011375	≦80% homology	≦80% homology	≦80% homology
Streptococcus salivarius	NZ_CP015283.1 NZ_CP015284.1 NC_017595 NZ_CP015283	≦80% homology	≦80% homology	≦80% homology

11.4 Clinical Evaluation

A study was performed to assess the clinical performance of the COVID-19 RT-qPCR Detection Kit Plus. A testing with 20 replicates, considered as low positive with 100 copies/reaction, 10 replicates, considered as high positive with 2500 copies/reaction, and 30 replicates, as unspiked, was conducted. Samples were spiked with the SARS-CoV-2 RNA in the pooled negative clinical nasopharyngeal swab matrix, then extracted with QIAamp Viral RNA Mini Kit, and tested on the QuantStudio™ 5 Real-Time PCR System. The synthetic SARS-CoV-2 RNA was obtained from Twist Biosciences (Assay Ready Control 14, EPI_ISL_710528). The clinical samples were provided by Shuang Ho Hospital, Ministry of Health and Welfare, Republic of China (Taiwan).

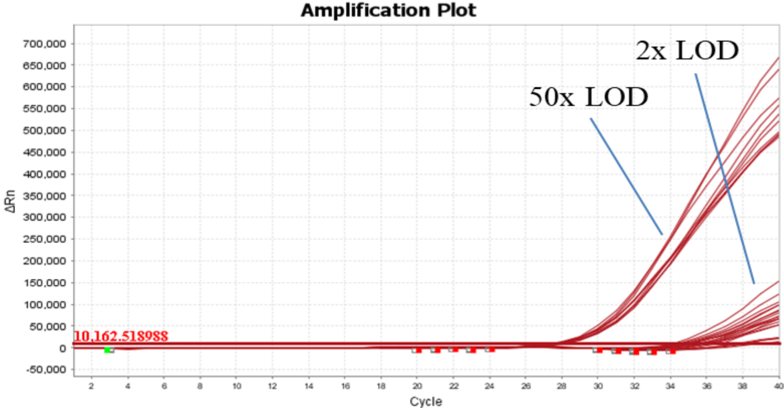


Figure 1. The result of specimens spiked with synthetic SARS-CoV-2 RNA.

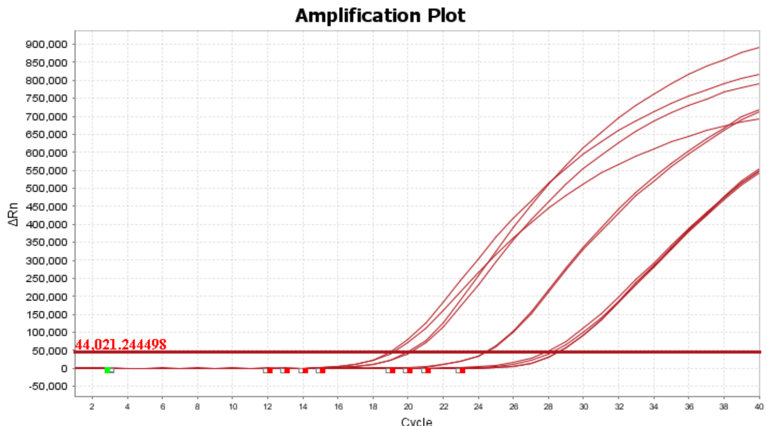


Figure 2. The result of clinical SARS-CoV-2 positive specimens.

Table 10. Clinical Evaluation for nasopharyngeal swabs.

RNA Concentration	Replicants	N1 gene		RP gene	
		Detection Rate	Mean Ct	Detection Rate	Mean Ct
Unspiked	30/30	0%	ND	100%	28.103
2X LoD (100 copies)	20/20	100%	35.935	100%	28.278
50X LoD (2500 copies)	10/10	100%	27.936		
Clinical Positive Sample	5/5	100%	23.929	100%	27.851
Clinical Negative Sample	5/5	0%	ND	100%	27.851

ND = Not Detected

12 Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when quantifying nucleic acid targets with the kit.

Table 11. Solutions for troubleshooting

Trouble	Cause	Solution
Poor Signal or No Signal	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Degraded Template Material	1. Do not store diluted template in water or at low concentrations. 2. Check the integrity of template material by automated or manual gel electrophoresis.
Signal in Negative Control	Contamination of Reaction Components with Target Sequence	1. To minimize the possibility of contamination of PCR components by PCR product or other template, designate a work area exclusively for PCR assay setup. 2. Use a solution of 15% bleach instead of ethanol to prepare the workstation area for PCR assay setup. Ethanol will only induce precipitation of DNA in your work area, while the 15% bleach solution will hydrolyze, as well as dissolve, any residual DNA.
Poor Reproducibility Across Replicate Samples	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
Low or High Reaction Efficiency	Insufficient Optimization	1. Use a thermal gradient to identify the optimal thermal cycling conditions.

13 Symbols used in Packaging

Symbol	Used for	Example of Usage
	Temperature limit	8 °C 2 °C
	Use-by date	2005-09-15
LOT	Batch code	LOT ABC123
REF	Catalog number	REF ABC123
	Manufacturer	Company Address
	Date of Manufacture	2001-06

Symbol	Used for
	Caution
	Consult instructions for use
RUO	Research use only
CE	CE mark
IVD	*In vitro* diagnostic medical device
EC REP	Authorized representative in the European Community

14 Method of Sterilization

Sample Collection, Storage and Transport

- Flocked swabs are preferred. Sterile dacron or rayon swabs with plastic or flexible metal handles may also be used. Do NOT use cotton or calcium alginate swabs or swabs with wooden sticks as they may contain substances that inactivate viruses and inhibit PCR.
- Always use sterile pipette tips with filters.
- Use 15% bleach to sterilize the environment.