Instructions for Use

COVID-19 RT-qPCR Detection Kit (IVD)

PRODUCT NAME
COVID-19 RT-qPCR Detection Kit (IVD)
Catalog Number: QP019R-0100

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1. Intended Use

The COVID-19 RT-qPCR Detection Kit (IVD) 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 (IVD) 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 N gene of SARS-CoV-2 will be detected qualitatively by FAM channel, the RdRp gene of SARS- CoV-2 will be detected qualitatively by ROX 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: Bio-Rad CFX96™ Touch Real-Time PCR Detection System with software Maestro version 1.1

3. Kit Contents

Table 1. COVID-19 RT-qPCR Detection Kit (IVD) Contents

Component	Description	Amount Supplied (per 100 rnxs)
2X qPCR MasterMix	Multiplex assay primers/probes for N, RdRp and RP (human) genes with Hot Start DNA polymerase, dNTPs & buffer	1000µL x 1
RScript Enzyme Mix	RScript reverse transcriptase with RNase inhibitor	20 µL x 1
Positive Control	Pseudo-virus DNA containing N and RdRp genes	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 (IVD) 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 qPCR Master Mix:* It is a multiplex assay containing primers/probes for N, RdRp and RP (human) genes with Hot Start DNA polymerase, dNTPs & buffer.
- *RScript Enzyme Mix:* Unique blend of RScript reverse transcriptase with RNase inhibitor.
- *Positive Control:* Ensures the assay is performed according to its use by evaluation with Pseudo-virus DNA containing N and RdRp 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	ROX Channel
Positive Control of SARS-COV-2	Ct<40	Undetected	Ct<40
Negative Extraction Control	Undetected	Ct<40	Undetected

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

For Instructions on how to set the Thermal Cycler, please refer to the below section.

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	10 seconds	45
Annealing	60°C	60 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 (IVD) has been validated using the Bio-Rad CFX96™ Touch Real-Time PCR Detection System with software Maestro version 1.1.
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 three fluorescence signals (FAM, HEX, and ROX) are set manually by the operator based on the fluorescent value of the positive control for each PCR run.

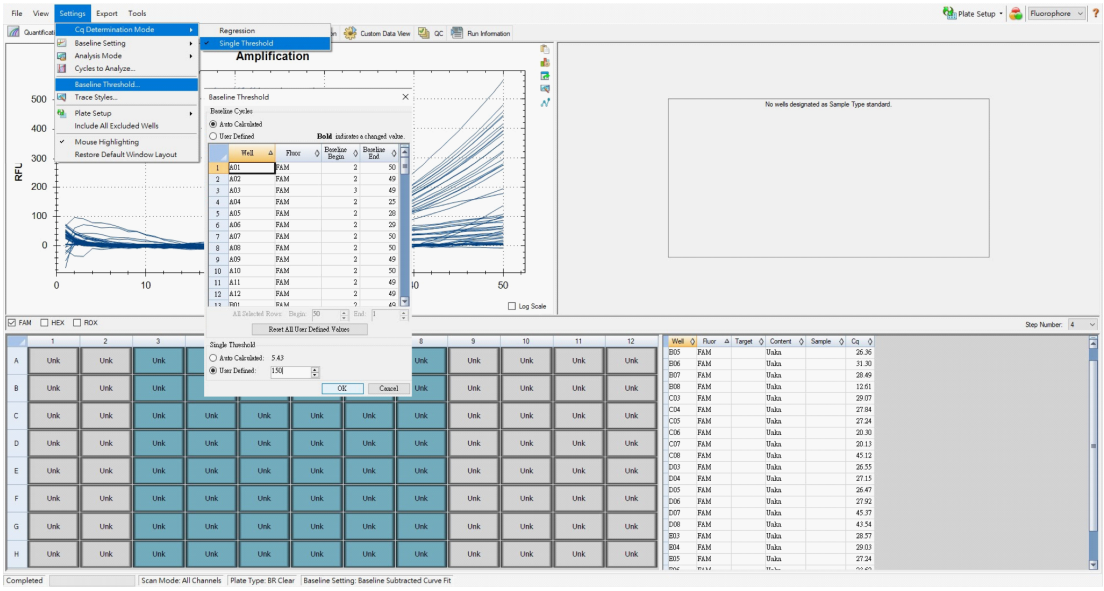
The baseline setting can be adjusted manually in case of production of background noise signal in PCR initiation phase. For adjusting manually, thresholds should be adjusted to fall within exponential phase of the fluorescence curves and above any background noise signal.

To adjust the threshold baseline manually, please follow the instructions below:

1 - Check the box in front of the reporter (FAM, HEX, ROX) that needs to be adjusted.

2 - Go to Settings > Cq Determination Mode > Single Threshold

3 - Return to Settings > Baseline Threshold > Activate the User defined > Adjust the Threshold value to 150 (as shown in the figure below).



Results Interpretation

As three channels (FAM, HEX, ROX) 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, HEX, and ROX channels for each sample to be tested with the COVID-19 RT-qPCR Detection Kit (IVD). 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)	RdRP (ROX)
No Template Control	Assay or extraction reagent contamination	Negative Ct ND	Negative Ct ND	Negative Ct ND
Positive Control	Improper assay setup and reagent failure, including primer and probe degradation	Positive Ct <40	Negative Ct ND	Positive Ct < 40
Negative Extraction Control	Cross-contamination during extraction	Negative Ct ND	Positive Ct < 40	Negative Ct ND

Table 6. Expected Performance of Patient Samples

SARS-CoV-2			Interpretation	Action
N	RdRP	RP		
+	+	+/-	Positive	Report result to health authority.
If only one of targets is positive		+/-	Presumptive positive *	Repeat RT-qPCR of samples or repeat from extraction step. If the test still yields an invalid result, recommended collection of new specimen(s) from the patient is needed.
-	-	+	Negative	SARS-CoV-2 not detected. Report result 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.

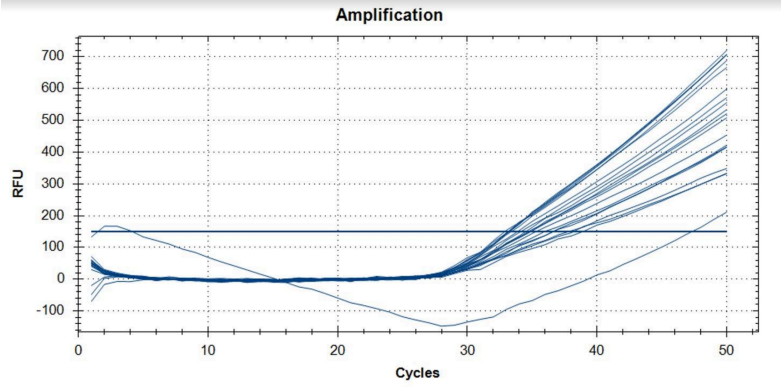
*A test is considered positive where N and RdRP gene's result is positive (+), otherwise the results are considered presumptive/ inconclusive and re-testing needs to be carried out.

11. Performance Characteristics

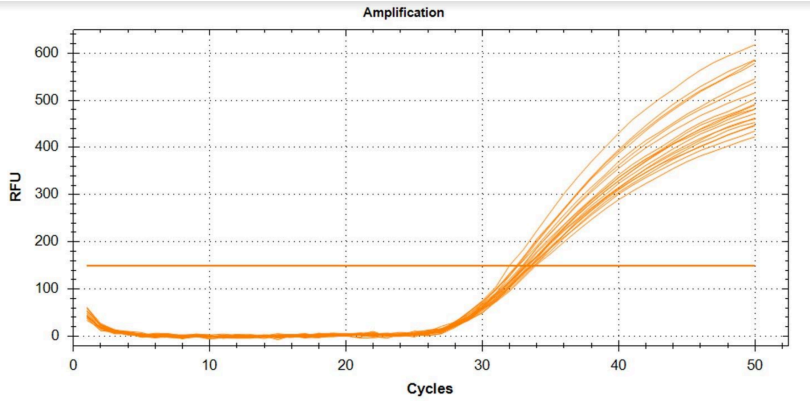
Analytical Sensitivity and Limit of Detection (LoD)

A study was performed to assess the performance of COVID-19 RT-qPCR Detection Kit (IVD). A testing of 20 replicates at the tentative limit of detection (LoD) concentration was carried out with the N gene, 100 copies/reaction and it was further confirmed by testing 20 additional replicates of samples at 100 copies/reaction with the RdRp gene by spiking it in.

Table 7. Limit of Detection COVID-19 RT-qPCR Detection Kit (IVD) for N and RdRP gene



Target	Concentration	Detection Rate	Mean Ct
SARS-CoV-2-N gene	100 copies	95 % (19/20)	32.22



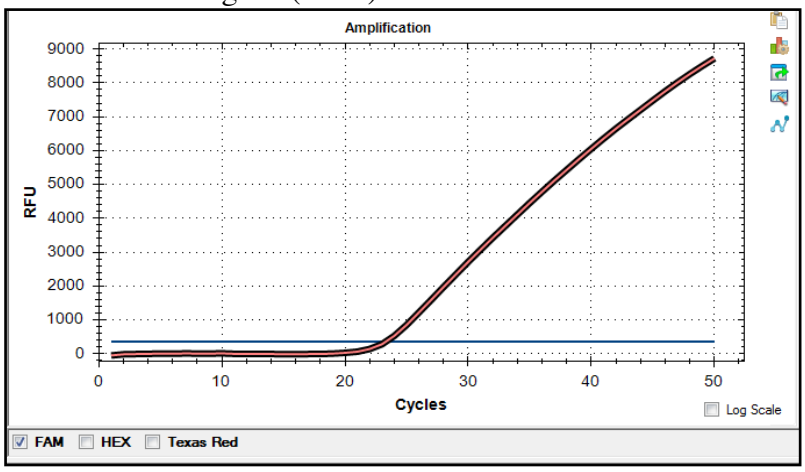
Target	Concentration	Detection Rate	Mean Ct
SARS-CoV-2-RdRp gene	100 copies	100 % (20/20)	33.48

Typical S-Shape Amplification Curve

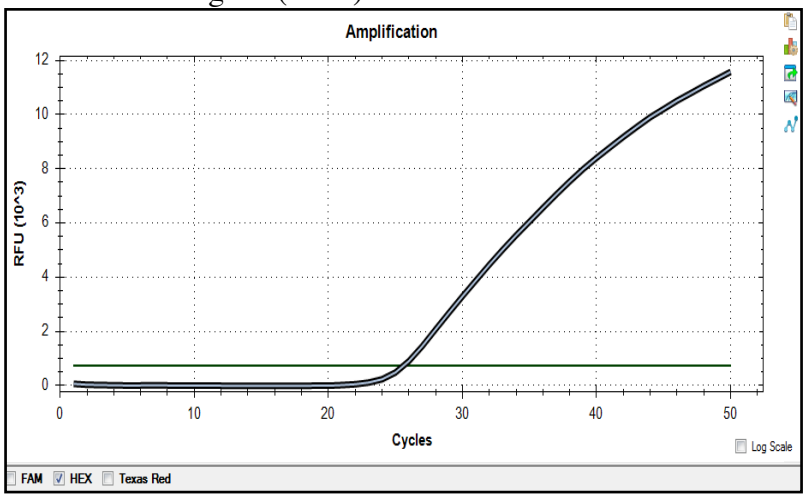
Considering the performance difference in real-time PCR instruments, thresholds for three fluorescence signals (FAM, HEX, and ROX) are set manually by the operator.

Table 8. The Typical S-shape Amplification Curve Example

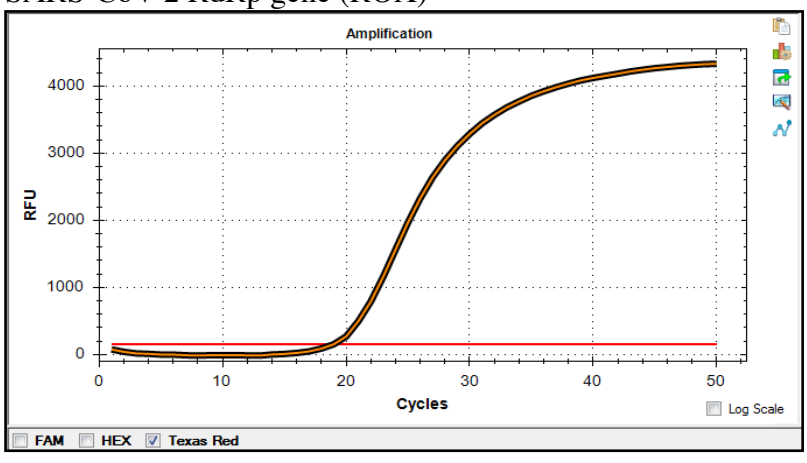
SARS-CoV-2-N gene (FAM)



For Human-RP gene (HEX)



SARS-CoV-2 RdRp gene (ROX)



11.1 Cross -Reactivity

To demonstrate that the COVID-19 RT-qPCR Detection Kit (IVD) 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 from the same family

Pathogen	Genome Sequence	Reactivity %	Notes
Human coronavirus 229E			No cross reactivity was found with in silico sequence comparison analyses.
Human coronavirus OC43			No cross reactivity was found with in silico sequence comparison analyses.
Human coronavirus HKU1	NC_006577.2 (Human coronavirus HKU1)	100 %	>80% homology No detected
Human coronavirus NL63			No cross reactivity was found with in silico sequence comparison analyses.
SARS-coronavirus	NC_045512.2 (SARS-CoV-2/01)	100 %	>80% homology
MERS-coronavirus			No cross reactivity was found with in silico sequence comparison analyses.

Table 10. *In silico* analysis for high priority organisms likely present in a respiratory specimen

Organisms	Genome Sequence	Reactivity %	Notes
Adenovirus	AC_000008.1 (Human adenovirus 5)	80 %	≦80% homology No detected
Human Metapneumovirus (hMPV)			No cross reactivity was found with in silico sequence comparison analyses.
Parainfluenza virus 1-4			No cross reactivity was found with in silico sequence comparison analyses.
Influenza A & B			No cross reactivity was found with in silico sequence comparison analyses.
Enterovirus (e.g. EV68)			No cross reactivity was found with in silico sequence comparison analyses.
Respiratory syncytial virus			No cross reactivity was found with in silico sequence comparison analyses.
Rhinovirus			No cross reactivity was found with in silico sequence comparison analyses.
Chlamydia pneumoniae	NC_000922.1 (Chlamydia pneumoniae CWL029)	63 %	≦80% homology No detected
Haemophilus influenzae	NC_000907.1 (Haemophilus influenzae Rd KW20)	60%	≦80% homology No detected
Legionella pneumophila	NC_002942.5 (Legionella pneumophila subsp)	67.5 %	≦80% homology No detected
Mycobacterium tuberculosis	NC_000962.3 (Mycobacterium tuberculosis H37Rv)	70 %	≦80% homology No detected
Streptococcus pneumoniae	NC_003098.1 (Streptococcus pneumoniae R6)	69%	≦80% homology No detected
Streptococcus pyogenes	NC_002737.2 (Streptococcus pyogenes M1 GAS)	71.25 %	≦80% homology No detected
Bordetella pertussis	NC_002929.2 (Bordetella pertussis Tohama I)	68.3 %	≦80% homology No detected
Mycoplasma pneumoniae	NZ_CP010546.1 (Mycoplasma pneumoniae FH chromosome)	66.6 %	≦80% homology No detected

Organisms	Genome Sequence	Reactivity %	Notes
***Pneumocystis jirovecii* (PJP)**			No cross reactivity was found with in silico sequence comparison analyses.
Pooled human nasal wash - *to represent diverse microbial flora in the human respiratory tract*			No cross reactivity was found with in silico sequence comparison analyses.
Candida albicans			No cross reactivity was found with in silico sequence comparison analyses.
Pseudomonas aeruginosa			No cross reactivity was found with in silico sequence comparison analyses.
Staphylococcus epidermis			No cross reactivity was found with *in silico* sequence comparison analyses.
Streptococcus salivarius	NC_002737.2 (Streptococcus pyogenes M1 GAS)	72.5 %	≦80% homology No detected

Results of *in silico* analysis demonstrates that there is homology between the **Human coronavirus HKU1 and Adenovirus 5** in the primer/probes for RdRP, N and RdRp and Rp , but these primers are unable to amplify during PCR consequently.

For SARS-coronavirus, homology is seen for both, the reverse and forward primer. For HKU1, only the reverse primer has high homology not the forward primer. For Adenovirus, we see an exact 80 % homology found in RP, primer.

The primers and probes sequences of the N and RdRp genes were analyzed using sequences deposited in NCBI database. No *in silico* cross-reactivity was found from the organisms in the recommended list (see Table below) except SARS-CoV. Primers sequences of SARS-CoV-2 with N and RdRP genes have a 100% homology with that of SARS-CoV. *In silico* analysis shows the probe will not hybridize with the SARS-CoV sequence, thus the N primers/probe set should not cross-react with SARS-CoV.

Remarks: The following pathogens and microorganisms were not found on the database, with an indication that these have no homology with our COVID-19 Detection Kit (IVD) primers and probes.

Human coronavirus 229E	**Human coronavirus OC43**
Human Metapneumovirus (hMPV)	**Parainfluenza virus 1-4**
Respiratory syncytial virus	**Human coronavirus NL63**
Pseudomonas aeruginosa	**Influenza A & B**
Staphylococcus epidermis	***Candida albicans***
Rhinovirus	**Enterovirus (e.g. EV68)**
***Pneumocystis jirovecii* (PJP)**	**MERS-coronavirus**

Despite these remarks in SARS-CoV-2, the COVID-19 Detection Kit (IVD) is expected to detect the strains of SARS-CoV2.

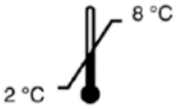
12. Troubleshooting

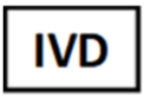
Refer to the table below to troubleshoot problems that you may encounter when quantifying nucleic acid targets with the kit.

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	
	Use-by date	 2005-09-15
	Batch code	
	Catalog number	
	Manufacturer	
	Date of Manufacture	 2001-06

Symbol	Used for
	Caution
	Consult instructions for use
	CE mark
	*In vitro* diagnostic medical device
	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.