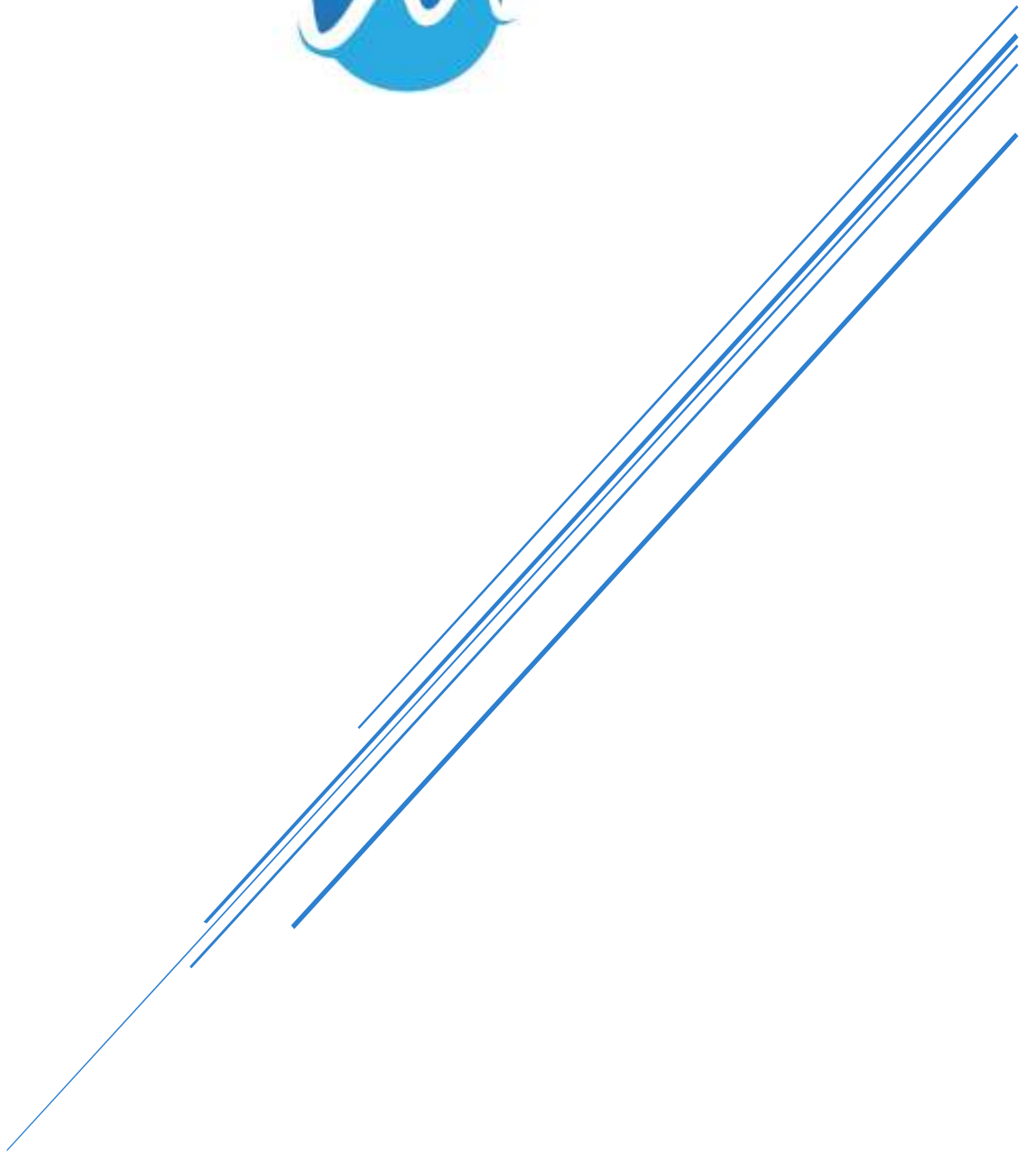


MARINABIOLAB



REF

RUO

MRNP004

MARINABIOLAB HPV DETECTION KIT

Instructions for Use

For research use only

Not for use in vitro diagnostic procedures

Marinabiolab Biyoteknoloji Sanayi Ticaret Limited Sirketi

Address

Yukarı Dudullu Mah. Alemdağ Cad. No:742/A, 34775 Ümraniye/ İstanbul/Türkiye
Phone: +1 (888) 212-1742

Email/Web

accounting@marinabiolab.com / www.marinabiolab.com

ISTANBUL-2024

Notice to User:

Before using our product, please read and understand the "Warnings and Precautions" section completely.

Full understanding of the warnings prevents operating errors and possible risks.

If you have any questions, you can contact us at the e-mail address "accounting@marinabiolab.com".

It is for research purposes only. (RUO) It cannot be used for diagnostic purposes.

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1. INTENDED USE

The kit detects and distinguishes genomic material (DNA or RNA) from human biological samples. The test kit is a real-time PCR (qPCR) test intended for the qualitative detection of nucleic acids of viral and bacterial agents in research Cervical/Vaginal/Penile samples.

NOTE: For Research Use Only (RUO). Not for use in diagnostic procedures.

2. TEST PRINCIPLE

The principle of real-time detection is based on the fluorogenic 5'nuclease test. DThroat swab, vaginal swab, urethral swab or urogenital g the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes the target DNA. This splitting results in the fluorescent signal produced by the split reporter dye, which is monitored in real time by the PCR system. The PCR cycle (Ct) in which an increase in the fluorescent signal is detected initially is proportional to the amount of the specific PCR product. Real-time monitoring of fluorescent intensities allows detection of the accumulated product without the need to reopen the reaction tube after amplification.

The MARINABIOLAB HPV qPCR Kit detects ; HPV Types (16,18,45,31,33,35,39,51,52,54,56,58,59,68,69) in a single run with four individual multiplex qPCR reactions. The kit is applied to the nucleic acid isolates obtained from research Cervical/Vaginal/Penile samples.

3. INSTRUMENTS AND SOFTWARE

The MARINABIOLAB HPV Detection Kit is validated using the Roche LightCycler 480 Instrument II, Corbett Rotorgene, Applied Biosystems 7500 Fast, Biorad CFX 96 Realtime PCR, CFX384 Touch Real-Time PCR Detection System, BioMolecular Systems Bio 4 - Channel+HRM, Drawell Gentier 96E, Applied Biosystems QuantStudio 5, Applied Biosystems QuantStudio 7, Applied Biosystems QuantStudio 12K and their software.

4. INSTRUMENTS SPECIFIC CONSUMABLES

The strips and/or plates recommended by the manufacturers of the devices whose brand and model information is given in Section 3 are compatible with "The MARINABIOLAB HPV Detection Kit".

5. KIT COMPONENTS

The kit components are provided in Table 1.

Oligo Mix	Parameters	Channels	50 RXN	100 RXN
HPV OLIGO	45,31,33,35,39,51,52,54,56,58,59,68 or 69	FAM	250 µl	500 µl
	18	HEX		
	16	ROX		
	RNase P (IPC)	CY5		

CONTENT		50 RXN	100 RXN
MASTER MIX	2X MASTER MIX FOR QPCR ASSAY	125 µL	250 µL
PC	WHOLE GENOME	150 µL	300 µL
NTC	NO TEMPLATE CONTROL	100 µL	200 µL

Table 1: Kit Components

6. STORAGE

- All reagents should be stored at -10~-30°C. It is not recommended to store at +4°C. Reagents for daily use can be stored at +4°C to avoid multiple freezing and thawing.
- All reagents can be used until the expiration date stated on the kit label. At least 12 months from the date of manufacture of all kits and reagents.
- Repeated thawing and freezing processes (> 5x) should be avoided because they will reduce the sensitivity of the test.
- Keep all reagents cold Penile swab, vaginal swab or Cervicall swab samples the run steps.
- The reaction mixture should be stored in the dark.

7. MATERIALS AND DEVICES REQUIRED

- Biosafety cabinet
- Vortex mixer
- Microcentrifuge
- Micropipette (2 or 10 µL, 10 and 100 µL)
- Multi micropipette, rack for 1.5 mL microcentrifuge tubes (5-50 µl), -20°C cold block for 2 x 96-wells.
- PCR Device
- 10% bleach solution (1:10 dilution, commercial 5.25-6.0% hypochlorite bleach) • Disposable powderless gloves and surgical gowns
- Pipette tip with aerosol barrier
- 1.5 mL microcentrifuge tube (DNase/RNase free)
- 96 well plates or 0.1/0.2 µl strips
- Plate Seal

8. WARNINGS AND PRECAUTIONS

Carefully read this instruction before starting the procedure.

- Only for research use only
- It should be used by laboratory personnel trained in research use only procedures.
- Follow standard precautions. All patient samples and Positive Controls should be considered potentially infectious samples and handled accordingly.
- This test should be performed in accordance with Good Laboratory Practice.
- Do not use the kit after the expiration date.
- Do not eat, drink, smoke, apply cosmetics or touch contact lenses in areas where reagents and human samples are handled.
- Handle all samples as infectious, using safe laboratory procedures.
- Perform all studies of live virus samples in a Class II (or higher) biological safety cabinet (BSC).
- Wear personal protective equipment such as (but not limited to) gloves, eye protection, and lab coat when handling materials including samples, pipettes, other equipment, and reagents while performing this test.
- Misleading results may occur if the clinical sample or Real-Time reagents used in the amplification step are contaminated by accidental addition of the amplification product (amplicon). Work-flow in the laboratory should proceed in one direction.
- Provide separate areas when dispensing the reaction mix and dispensing samples to avoid cross-contamination.
- Replace aerosol barrier pipette tips between all manual liquid transfers.

9. QUALITY CONTROL

Controls that will be provided with the test kit are provided.

Control	Descriptions	Results
PC	Viral/Bacterial genome	+
NTC	No template Control	-
IPC	Rnase P Gene	+
EXC	Bacillus Atrophaeus	-

Table 2: Control Design

NTC consists of using nuclease-free water instead of RNA/DNA in RT-PCR reactions. NTC reactions for all mixtures of oligos should not exhibit fluorescent growth curves that cross the threshold line. If any of the NTC reactions exhibit a growth curve that exceeds the cycle threshold, reaction mixture contamination may have occurred. If this happens, repeat the operation strictly adhering to the manual. Positive Control (PC) consists of in vitro replicated DNA. Contains specific gene regions for target regions. RNase P (Internal Control), All clinical samples should exhibit fluorescent growth curves that cross the threshold in the RNase P reaction within 40.00 cycles <35.00 Ct), this indicating the presence of the human RNase P gene. Failure to detect RNase P in any clinical specimen may indicate:

- Incorrect extraction of nucleic acid from clinical materials resulting in RNA loss and/or RNA degradation
- Lack of adequate human cellular material due to insufficient collection or loss of sample integrity.
- Improper assay setup and execution.
- Reagent or equipment failure.
- If the RP test does not produce a positive result for human clinical specimens, interpret as follows:
The result should be considered valid even if the pathogens are positive and RP is negative. It is possible that some samples do not exhibit RNase P growth curves due to the low cell numbers in the original clinical sample. A negative RP signal does not preclude the presence of pathogens in a clinical sample. If all pathogen markers and RNase P are negative for the sample, the result should be considered invalid for the sample. Used for Bacillus Atrophaeus Extraction control.

10. APPLICATION PROTOCOL

10.1. Collecting the Specimens

Follow the sample collection device manufacturer's instructions for proper collection methods. Research samples are collected in accordance with the specimen collection guidelines.

10.2. Transporting and Storing Specimens

Research samples are transported and stored in accordance with the specimen labeling, storage & handling guidelines. Research specimens (Cervical swab, vaginal swab or penile swab) should be delivered to the lab within 2 hours of collection or refrigerated and transported to the lab as soon as possible. For transfers longer than 2 hours, ship the specimens to the laboratory on an ice pack if a specimen is frozen at -20 °C or lower, ship overnight to the laboratory on dry ice. It is important that specimens are not exposed to continuous freeze-thaw exposure.

10.3. Preparation of Nucleic Acid Samples

We recommend using DNA/RNA Extraction Kit for isolation steps. Automatic or manual extraction system can be used.

The amount and purity of DNA extracted in Cervical/Vaginal/Penile samples should be checked.

10.4. qPCR

Clean the room and cabinet where the reagents will be stored, place Oligo Mix, Master Mix and controls on ice or cold block. Keep area cool dThroat swab, vaginal swab, urethral swab or urogenital samples preparation and use.

Thaw mixtures before starting.

Before using Oligo Mix and Master Mix, the vial can be centrifuged for 5 seconds to prevent some of the contents from remaining at the tip of the vial, and then place the tube on a cold rack.

Label the 1.5 mL eppendorf tube for each run.

Determine the number of reactions (N) to set up per test. Overreaction mixing is necessary for Negative Controls, Positive Controls reactions, and pipetting error. Use the following guide to determine N.

If the number of samples, together with the controls (n) is between 1 and 14; $N = n+1$ If the number of samples is greater than (n) 15 with controls; $N = n+2$

For each run, calculate the amount of reagents to be added to the mixture (N = number of reactions)

Transfer the reagents to the labeled 1.5 mL microcentrifuge tubes sequentially. After adding the reagents, mix the reaction mixture by pipetting. **DO NOT USE VORTEX.**

Reaction mixes are obtained by mixing 5 µL of oligo mix and 2,5 µL of master mix per reaction. Dispense 7,5 µL of Reaction Mixes into appropriate wells.

Note: Each Sample must be added separately to each reaction mix.

10.5. Nucleic Acid Sample Addition

Vortex the sample tubes for approximately 10 seconds.

Samples should be added to the specific assay being tested in columns 2-11 (columns 1 and 12 are for control) as shown in Table 3.

Carefully pipette 2,5 µL of the first sample into the well labeled for that sample. Keep other sample wells closed dThroat swab, vaginal swab, urethral swab or urogenital isolate addition. Change pipette tips after each addition.

To prevent cross contamination and allow sample tracking, tightly close the well into which the sample was added.

Frequently change gloves to avoid contamination.

Repeat steps #3 and #4 for the remaining samples.

Cover the entire reaction plate and move the reaction plate to the positive control processing area.

Pipette 2,5 µL of positive control solution into column 12.

Securely close the wells after addition of control DNA.

NOTE: If using 8-tube strips, label the TAB of each strip to indicate sample position.

Briefly centrifuge the reaction tube strips for 10-15 seconds. Place on cold rack after centrifugation.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NC	PC
B												
C												
D												
E												
F												
G												
H												

Table 3: PCR Plate

10.6. Real- Time PCR Program Setup

	HPV Detection Kit		
	Temperature °C	Time (sec.)	Cycles
Initial Activation	95	600	1
Denaturation	95	30	40
Separation	55	60	
Extention	72	20	

Table 4: HPV Detection Kit Thermal Profile

11. RESULT INTERPRETATION

Negative: The sample tested is negative for the tested agent.

Positive: The sample tested is positive for the tested agent.

Contamination: Repeat the analysis paying attention to the “Warnings and precautions” section.

Invalid: Sampling isn’t successfully done or there is a problem Vaginal swab, Cervical swab or Penile swab samples the sample transportation. A new sample from the same source should be collected and tested again.

Reagent Problem: Test the PC(s) provided with the kit setting up the PC reactions. If the test result is positive, the run is valid.

Cq Value	Results
Cq<18	High Positive
18<Cq<27	Positive
27<Cq<35	Low Positive
35<Cq	Negative or repeat test

Table 5: Result Interpretation

12. PERFORMANCE CHARACTERISTICS

12.1. LOD Study and Results

Limit of detection (LoD) studies determine the lowest detected concentration of target parameters at which greater or equal to 96% of all replicates test positive.

A study was conducted to determine the concentrations to be tested in the LoD study. Cervical/Vaginal/Penile samples were used in this study. Using a pool of confirmed negative Cervical/Vaginal/Penile samples. Copies/mL were prepared with artificial contamination to determine the LoD concentration range. Samples at these concentrations were tested in triplicate and the minimum concentration. The highest concentration to be used in the LoD that gave positive results in all three tests was determined. The lowest concentration at which all triple tests are positive is 500 copies/mL for all targets in Cervical/Vaginal/Penile samples.

15 different LoD samples were prepared at 4 different concentrations to include each targeted organism. Reference from the culture collection of the Medical analysis laboratories approved by the Ministry of Health of the Republic of Turkey was used in all validation studies of the kit. Additionally, ATCC and ZEPTO strains were used in this study. Cervical/Vaginal/Penile samples that were negative for all agents targeted by the kit were obtained by pooling them separately. The LoD of the MARINABIOLAB HPV Detection Kit in Cervical/Vaginal/Penile samples is 100-500 copies/mL for all samples targets.

Targets	Sample Type	Concentration (copy/mL)	Results Target of	Results of RNase P (IC)
HPV TYPE 16	Cervical/Vaginal/Penile samples	500	24/24	24/24
		250	21/24	24/24
		100	13/24	24/24
		0	0/24	24/24
HPV TYPE 18	Cervical/Vaginal/Penile samples	500	24/24	24/24
		250	23/24	24/24
		100	14/24	24/24
		0	0/24	24/24
HPV TYPE 45	Cervical/Vaginal/Penile samples	500	24/24	24/24
		250	21/24	24/24
		100	18/24	24/24
		0	0/24	24/24
HPV TYPE 31	Cervical/Vaginal/Penile samples	500	24/24	24/24
		250	22/24	24/24
		100	18/24	24/24
		0	0/24	24/24
HPV TYPE 33	Cervical/Vaginal/Penile samples	500	24/24	24/24
		250	23/24	24/24
		100	15/24	24/24
		0	0/24	24/24
HPV TYPE 35	Cervical/Vaginal/Penile samples	500	24/24	24/24
		250	21/24	24/24
		100	13/24	24/24
		0	0/24	24/24
HPV TYPE 39	Cervical/Vaginal/Penile samples	500	24/24	24/24
		250	19/24	24/24
		100	16/24	24/24
		0	0/24	24/24
HPV TYPE 51	Cervical/Vaginal/Penile samples	500	24/24	24/24
		250	21/24	24/24
		100	18/24	24/24
		0	0/24	24/24
HPV TYPE 52	Cervical/Vaginal/Penile samples	500	24/24	24/24
		250	19/24	24/24
		100	13/24	24/24
		0	0/24	24/24
HPV TYPE 54	Cervical/Vaginal/Penile samples	500	24/24	24/24
		250	19/24	24/24
		100	15/24	24/24

		0	0/24	24/24
HPV TYPE 56	Cervical/Vaginal/Penile samples	500	24/24	24/24
		250	23/24	24/24
		100	19/24	24/24
		0	0/24	24/24
HPV TYPE 58	Cervical/Vaginal/Penile samples	500	24/24	24/24
		250	19/24	24/24
		100	15/24	24/24
		0	0/24	24/24
HPV TYPE 59	Cervical/Vaginal/Penile samples	500	24/24	24/24
		250	20/24	24/24
		100	13/24	24/24
		0	0/24	24/24
HPV TYPE 68	Cervical/Vaginal/Penile samples	500	24/24	24/24
		250	22/24	24/24
		100	13/24	24/24
		0	0/24	24/24
HPV TYPE 69	Cervical/Vaginal/Penile samples	500	24/24	24/24
		250	20/24	24/24
		100	17/24	24/24
		0	0/24	24/24

Table 6: LoD Study Results

12.2. Device Equivalence Study

Device equivalence study was carried out to observe the differences between the results to be obtained using the kit in different instruments. The results of device equivalence studies showed no significant difference between mentioned instruments

13. INCLUCIVITY (ANALYTICAL SENSITIIVITY)

13.1. In-Silico Tests

The analytical specificity of the Marina Biolab HPV Detection Kit was extensively checked using oligonucleotides. Sequences were checked one by one. Comparative analysis with publicly available sequences was performed to ensure that all target genotypes of interest were obtained. BLAST search of oligonucleotide sequences in available ;

HPV TYPE 16
HPV TYPE 18
HPV TYPE 45
HPV TYPE 31
HPV TYPE 33
HPV TYPE 35
HPV TYPE 39
HPV TYPE 51
HPV TYPE 52
HPV TYPE 54
HPV TYPE 56
HPV TYPE 58
HPV TYPE 59
HPV TYPE 68
HPV TYPE 69

genomes were examined in the NCBI database.

BLAST tool was used. As a result of the in silico tests applied. The primer and probe sequences used for each target were able to detect the entire organism/strain genomes.

13.2. Wet Tests

Not applicable

14. EXCLUSIVITY/CROSS REACTIVITY (ANALYTICAL SPECIFICITY)

14.1. In-Silico Tests

In silico tests were performed using the NCBI Primer Blast tool (<https://www.ncbi.nlm.nih.gov/tools/primerblast/>) by checking the cross-reacting organism/strain and oligonucleotide sequences tested.

BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) was also used to compare all target regions.

The genomes of the cross-reacting organisms/strains were compared with the genomes of the targeted organisms.

In Silico Testing showed that the kit did not cross-react with any organisms/species.

14.2. Wet Tests

High priority pathogens from the same genetic family were subjected to wet testing. (1000000 copies/mL).

Reference strains and nucleic acid isolates were obtained from culture-confirmed cases. (these samples are; HSGM, ATCC, ZEPTO)

The following organisms were used in wet studies;

HPV TYPE 16

HPV TYPE 18

HPV TYPE 45

HPV TYPE 31

HPV TYPE 33

HPV TYPE 35

HPV TYPE 39

HPV TYPE 51

HPV TYPE 52

HPV TYPE 54

HPV TYPE 56

HPV TYPE 58

HPV TYPE 59

HPV TYPE 68

HPV TYPE 69

All samples were tested in triplicate and produced no cross-reactivity.

14.3. Endogenous Interference Substances Studies

The performance of the kit was examined for any potential interferences that may have been encountered throughout specimen collection and analysis or found in Cervical/Vaginal/Penilesamples. At concentrations around 3xLoD, each substance was introduced to synthetic samples containing relevant species. The quantity of substance introduced to the samples was at a concentration that was similar to or higher than the greatest amount anticipated in Cervical/Vaginal/Penilesamples. The majority of substances investigated had no impact on the results of the organism tests or control assays in the kit. According to the results of the interfering substance inhibition tests, anti-viral at 1% (v/v), antibiotics at 0.1% (w/v) may interfere with the MARINABIOLAB HPV DETECTION KIT qPCR Panel.

15. STABILITY STUDIES

The shelf life of the kit is estimated as 12 months. Real-Time stability testing is on-going, and the final shelf life will be established after the stability studies have been completed.

16. ASSAY LIMITATIONS

- A false negative result may occur if a specimen is improperly collected, transported, or handled.
- Performance of the MARINABIOLAB HPV DETECTION KIT has only been established in Cervical/Vaginal/Penile samples.
- Mutations within the target regions of the MARINABIOLAB HPV DETECTION KIT could affect primer and/or probe binding resulting in failure to detect the presence of agent.
- Inhibitors or other types of interference may produce a false negative result. Anti-viral at 1% (v/v), antibiotics at 0.1% (w/v) may interfere with the Marinabiolab HPV qPCR Panel.
- False negative results may also occur if inadequate numbers of organisms (lower than the LoD) are present in the specimen.

17. TROUBLESHOOTING

PROBLEM	CAUSE	SOLUTION
Internal Positive control and/or Positive control did not work.	Amplification Failure	Check that the thermal cycler settings and amplification program are correct. If there is no error in these, there may be a reagent problem; contact the manufacturer, renew the reagents, and repeat the reaction
In the Negative Control wells, target-specific and/or IC signals are detected.	Contamination of the PCR.	Contamination may be due to errors in sample handling, reagent contamination, or environmental contamination. - Decontaminate benchtop surfaces and other equipment where PCR is performed with 10% bleach solution. - Use fresh reagents and repeat the PCR. - Set up the Positive Control reactions last to
In unknown wells (sample wells), no IC signal is detected, but target-specific signal is detected.	A high copy number of target nucleic acid (NA) exists in samples, resulting in preferential amplification of the targetspecific NA.	No action is required. The result is considered positive
In unknown wells (sample wells), no IC and no targetspecific signal is detected	• Inhibition Problem • Extraction Problem • Sampling Problem	• Dilute the nucleic acid isolate 1/10 and repeat the PCR. If the diluted sample does not give a positive result in the IC channel, request for a new sample and repeat the NA extraction. If the problem persists, contact Technical Support. • Repeat the NA extraction and the PCR. If the problem persists, contact Technical Support. • Request for a new sample, repeat the NA extraction and the PCR. If the problem persists, contact Technical Support.

18. EXPLANATION OF SYMBOLS

Symbol	Title of Symbol	Symbol	Title of Symbol	Symbol	Title of Symbol
	Research use only		Batch code		Contains sufficient for <n> tests
	Manufacturer		Catalogue number		Keep away from sunlight
	Use-By Date		Non-Sterile		Protect from heat and radioactive sources
	Control		Consult <i>instructions for use</i> or consult electronic <i>instructions for use</i>		Do not use if package is damaged and consult <i>instructions for use</i>
	Negative Control		Caution		Keep dry
	Positive Control		Temperature limit		Keep it upright

Manufacturer Marinabiolab Biyoteknoloji Sanayi Ticaret Limited Sirketi Yukarı Dudullu Mah. Alemdağ Cad. No:742/A, 34775 Ümraniye/ İstanbul/Türkiye Phone: +1 (888) 212-1742 Email WEB accounting@marinabiolab.com www.marinabiolab.com	Technical Support Marinabiolab Biyoteknoloji Sanayi Ticaret Limited Sirketi Yukarı Dudullu Mah. Alemdağ Cad. No:742/A, 34775 Ümraniye/ İstanbul/Türkiye Phone: +1 (888) 212-1742 Email WEB accounting@marinabiolab.com www.marinabiolab.com
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