Life Technologies™
Your Molecular & Cell Technology Partner

Helicobacter pylori and toxin (VacA/CagA) Real Time PCR Kit
User Manual

LT028130DS50

For use with ABI Prism®7000/7300/7500/Step One Plus; iCycler iQ™4/iQ™5; Smart Cycler II;Bio-Rad CFX 96;Rotor Gene™6000; Mx3000P/3005P;MJ-Option2/Chromo4; LightCycler®480 Instrument

Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, New Delhi-110034 (INDIA) Ph: +91-11-42208000, 42208111, 42208222 Mobile: +91-9810521400 Fax: +91-11-42208444
Email: customerservice@lifetechindia.com Website: www.lifetechindia.com

1. Intended Use

Helicobacter pylori and toxin (VacA/CagA) Real Time PCR Kit is used for the detection of *H pylori* and toxin gene VacA/CagA by real time PCR systems in samples like gastric mucosa, nasopharyngeal swab, saliva, bacteria solutions and etc.

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During 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 to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

Helicobacter pylori is a Gram-negative, microaerophilic bacterium found in the stomach. It is also linked to the development of duodenal ulcers and stomach cancer. More than 50% of the world's population harbor *H. pylori* in their upper gastrointestinal tract. H. pylori's is thought to have evolved to penetrate the mucoid lining of the stomach. Research has been conducted to identify the bacterial factors and the deregulated host cell pathways that are responsible for the progression to more severe disease states. The cytotoxin-associated gene A (CagA), and the vacuolating cytotoxin A (VacA) gene, are linked to increased pathogenicity of *H. pylori* strains.

Helicobacter pylori and toxin (VacA/CagA) Real Time PCR Kit contains a specific ready-to-use system for the detection of Helicobacter pylori and toxin gene VacA/CagA by polymerase chain reaction (PCR) in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of 3 kinds of target genes. Fluorescence is emitted and measured by the real time systems´optical unit during PCR. The detection of amplified *Helicobacter pylori* DNA fragment , toxin gene VacA DNA fragment and toxin gene CagA DNA fragment are performed in fluorimeter **channel FAM, HEX /VIC/JOE and Cal Red610/ROX/TEXAS RED.** DNA extraction buffer is available in the kit. In addition, the kit contains a system to identify possible PCR inhibition by measuring the channel **CY5** fluorescence of the internal control (IC). An external positive control contained.

4. Kit Contents

Ref.	Type of Reagent	Presentation 50 rxns
1	DNA Extraction Buffer	2 vials, 1.5ml x2
2	Hp-Toxin Reaction Mix	1 vial, 950µl x2
3	PCR Enzyme Mix	1 vial, 12µl x2
4	Molecular Grade Water	1 vial, 400µl x2
5	Internal Control (IC)	1 vial, 30µl x2
6	Hp-Toxin Positive Control	1 vial, 30µl x2

Analysis sensitivity: 5× 10^3 copies/ml;

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors .If you use the DNA extraction buffer in the kit, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.

5. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (>3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Reaction Mix should be stored in the dark.

6. Additionally Required Materials and Devices

- Biological cabinet
- Real time PCR system
- Trypsin digestive Solution
- Vortex mixer
- Real time PCR reaction tubes/plates
- Cryo-container
- Pipets (0.5 µl – 1000 µl)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and Freezer
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)
- Tube racks

7. ⚠ Warnings and Precaution

- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Avoid aerosols

8. Sample Collection, Storage and transport

- Collect samples in sterile tubes;
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

9. Procedure

9.1 DNA-Extraction

DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use.

9.1.1 Gastric mucosa sample

1) Take the patient's gastric mucosa lesions, and add 1.0 ml normal saline into the sample. Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant.
2) Add 100µl DNA extraction buffer and 4µl (20mg/ml) proteinase K, close the tube then vortex for 10 seconds, incubate the tube at 56 ℃for 3 hours.
3) Incubate the tube for 10 minutes at 100 °C.
5) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

9.1.2 Nasopharyngeal swabs sample

1) Wash the swabs in 1.0ml normal saline and vortex vigorously. Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
2) Add 1.0ml normal saline and suspend the pellet with vortex vigorously. Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
3) Add 100µl DNA extraction buffer, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge. **4)** Incubate the tube for 10 minutes at 100°C. **5)** Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

9.1.3 Saliva sample

1) Take 1.0 ml sample to a tube, add 50µl digestion buffer (1mol/L Tris, 0.5mol/L EDTA, 10% N-Lauroyl Sarcosine Sodium),incubate the tube at 37℃ for 20 min, add 12µl Proteinase K and incubate at 56℃ for 3 hours. **2)** Centrifuge the tube at 13000rpm for 5 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet. **3)** Add 100µl DNA extraction buffer, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge. **4)** Incubate the tube for 10 minutes at 100°C. **5)** Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

9.1.4 Bacteria solution sample

1) Take 100~500µl bacteria solutions into a tube, centrifuge the tube at 13000rpm for 5 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet.
2) Add 1.0ml normal saline into the pellet, close the tube then vortex for 10 seconds. Centrifuge the tube at 13000rpm for 5 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet. **3)** Add 100µl DNA extraction buffer, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge. **4)** Incubate the tube for 10 minutes at 100°C. **5)** Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

Attention:

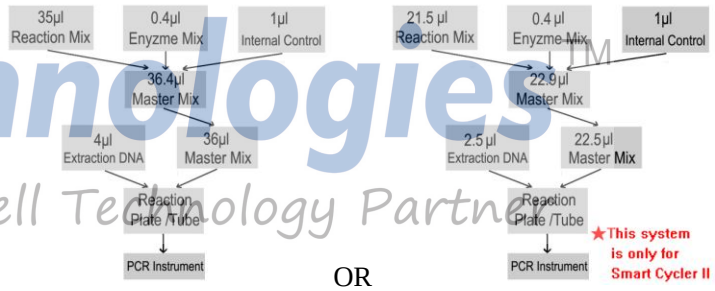
A. During the incubation, make sure the tube is not open, as the vapor will volatilize into the air and may cause contamination in case the sample is positive.
B. The extraction sample should used in 3 hours or store at -20°C for one month.
C. DNA extraction kits are available from various manufacturers. You can also use your own extraction systems or the commercial kit depending on the yield. For DNA extraction, please comply with the manufacturer's instructions.

9.2 Internal Control

It is necessary to add internal control (IC) in the reaction mix. Internal Control (IC) allows the user to determine and control the possibility of PCR inhibition.
Add the internal control (IC) 1µl/rxn and the result will be shown in the channel Cy5.

9.3 PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



OR

※PCR system without CY5 channel may be treated with 1µl Molecular Grade Water instead of 1µl IC.

1) The volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.
2) Pipet **36µl (22.5µl for Smart Cycler II)** Master Mix with micropipets of sterile filter tips to each *Real time* PCR reaction plate/tubes. Separately add **4µl (2.5µl for Smart Cycler II)** DNA sample, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument:

37°C for 2min	1cycle
94°C for 2min	1cycle
93°C for 15sec, 60°C for 1min (Fluorescence measured at 60°C)	40cycles

Selection of fluorescence channels	
FAM	Hp
HEX/VIC/JOE	CagA
Cal Red 610/ROX/TEXAS RED	VacA
CY5	IC

5) ⚠ If you use ABI Prism® system, please choose **"none"** as **passive reference** and **quencher.**

10. Threshold setting: just above the maximum level of molecular grade water.

11. Quality control: Negative control, positive control and internal control must be performed correctly, otherwise the sample results is invalid.

Channel / Control	FAM	HEX/VIC/JOE	Cal Red 610	CY5
	Ct value			
Molecular Grade Water	UNDET	UNDET	UNDET	25~35
TP Positive control	≤35	≤35	≤35	——

12. Data Analysis and Interpretation : The following sample results are possible:

	Ct value				
	FAM	HEX	Cal Red 610	CY5	Result Analysis
1#	UNDET	UNDET	UNDET	25~35	Below the detection limit or negative
2#	≤38	UNDET	UNDET	——	*H. pylori* positive;
3#	≤38	≤38	UNDET	——	*H. pylori* and Toxin CagA positive;
4#	≤38	UNDET	≤38	——	*H. pylori* and Toxin VacA positive;
5#	≤38	≤38	≤38	——	*H. pylori* and Toxin CagA/VacA positive;
6#	38~40			25~35	Re-test; If it is still 38~40, report as 1#
7#	UNDET				PCR Inhibition; No diagnosis can be concluded.