



DUPLIC α Real Time Legionella ssp Detection KIT

Ref. EBR011032 – 32 tests



Instruction Manual

CE-IVD

Euroclone S.p.A. has a Quality System certified by Dasa-Raegister in compliance with EN ISO 9001 (2000)

Paq. 1 di 10 Rev_1

1. KIT DUPLIC α ^{RealTime} TECHNOLOGY
2. ADVANTAGES
3. CONTENT OF THE KIT
4. STORAGE AND STABILITY
5. REAGENTS REQUIRED BUT NOT SUPPLIED
6. PRECAUTIONS and WARNINGS
7. OPERATING PROCEDURE
8. TROUBLESHOOTING

1. DUPLIC α ^{RealTime} TECHNOLOGY

DUPLIC α ^{RealTime} Legionella ssp was designed to identify the sequence of the gene 16s belonging *Legionella ssp*.

DUPLIC α ^{RealTime} Legionella ssp is a DNA-based test for the detection of the *Legionella ssp*. The reagents for the amplification are ready to use and provided with 3 reactions mix:

- AMPLIFICATION MIX: with Taq DNA polymerase, nucleotides, MgCl₂ e buffer.
- OLIGO MIX with a primers and fluorogenic probes.
- INTERNAL CONTROL OF AMPLIFICATION: it allows to value the success of the reaction of amplification, it is identified by a hydrolysis probe.

The polymerase chain reaction (PCR) is a technique that makes it possible to amplify a target piece of DNA generating several copies of a template.

Most commonly, PCR is carried out in three steps: **denaturation**, **annealing** (primers binding) and **extension**. These steps, which are carried out in a thermal cycler that allows heating and cooling, represent a basic cycle of amplification; the continual repetition of a basic cycle produces billions of fragments of the examined DNA. The denaturation of DNA which is carried out at high temperatures is followed by the annealing of specific primers to the target DNA: the Taq enzyme polymerase recognizes the 3'-ends of these primers and polymerizes the DNA using the dNTPs, thus creating several copies of the template. In all PCR-real time systems the amplified products are detected by using a fluorescent dye that in fluorogenic probes is called "*reporter*".

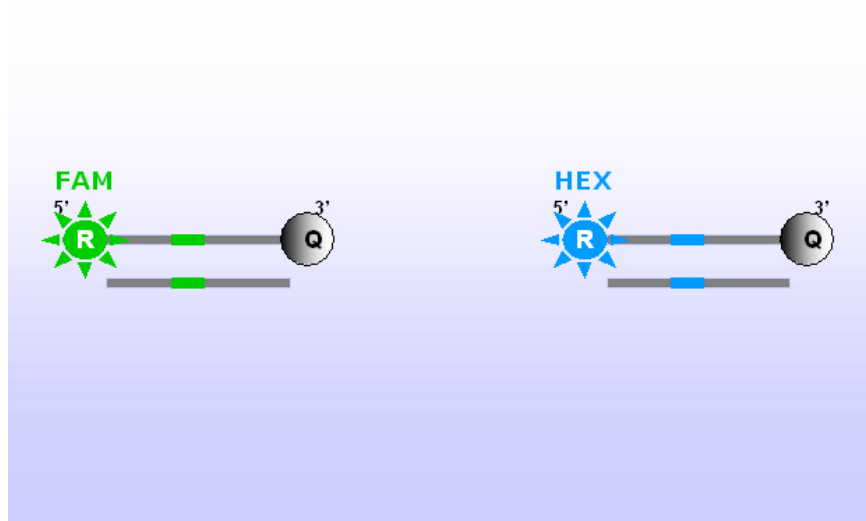


Fig. 1

The kit **DUPLIC α ^{RealTime} Legionella ssp** allows to find out bacterial DNA by using specific fluorogenic sequence probes. The fluorogenic probes are made of an oligonucleic sequence which presents at its 5'-end a fluorescent dye called «*reporter*», while at the 3'-end there is a second dye called «*quencher*». The test can detect the specific product of amplification by monitoring the increase of the fluorescence signal which is proportional to the quantity of the product amplified.

The kit takes advantage of an Oligo Mix containing specific probes *for Legionella ssp* linked at its 5'-end to a FAM molecule (6-carboxyfluorescein). In addition to this, the Oligo Mix contains internal control (IC) specific probes, linked at its 5'-end to a HEX molecule (hexa-chloro-fluoresceine). The two probes have at their 3'-ends a quencher called BHQ-1 (fig.1). If excited, the integral probe does not emit fluorescence, because the closeness of the quencher to the reporter prevents this last from emitting fluorescence (quenching effect).

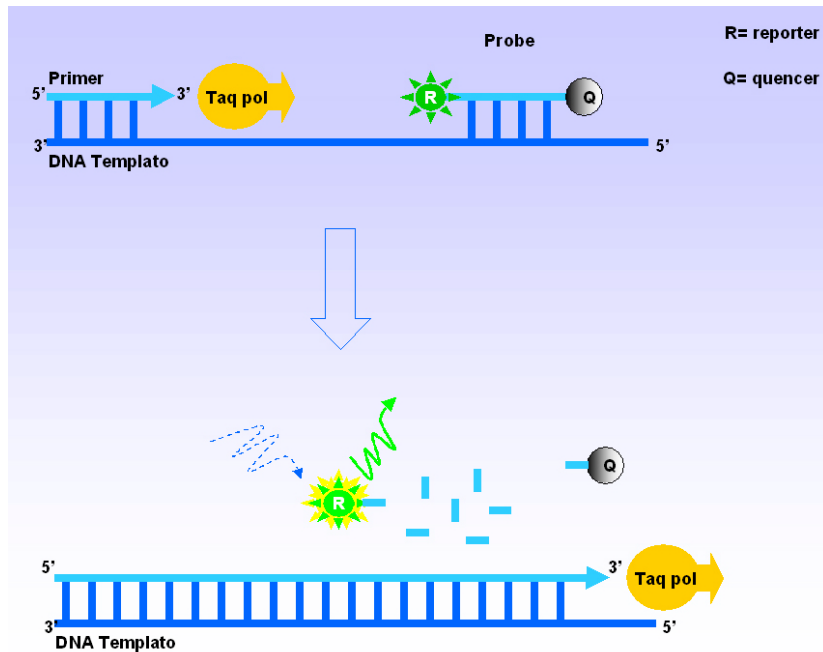


Fig.2

2. ADVANTAGES

- ◆ **Ease of handling:** The Oligo-Mix and Amplification mix of Kit contains all the reagents for PCR; you will need to add the DNA produced.
- ◆ **Real Time PCR:**
 1. increases the system sensibility;
 2. combines DNA amplification with detection of the products in a single tube;
 3. Reduces detection times (the detection takes place during the amplification phase);
 4. Minimized risk of cross contamination
 5. High throughput and the possibility to automatize the procedure
 6. removes the significant contamination risk caused by opening tubes for post-PCR-manipulation (Carry-over).

3. CONTENT OF THE KIT

Reagents	Colour code
Amplification mix	Blue Cap
Oligo Mix*	Green Cap
Control 1 (Positive C. of amplification)	Red Cap
Control 2 (Internal C. of amplification)	Yellow Cap
Reaction blank (B)	White Cap

*store the tube far from light

This kit was designed to perform 32 reactions, included an internal control of amplification that must be co-amplified with any sample and that allows to identify inhibitions in the reactions of amplification.

The kit allows to perform 4 runs, each of which include: 6 samples, 1 controls and 1 reaction blank.

4. STORAGE AND STABILITY

Twelve month at 2-8°C.

5. REAGENTS REQUIRED BUT NOT SUPPLIED

Equipments and materials required for DNA extraction:

- Tubes of 1.5 ml
- Micropipette 1000 µl
- Micropipette 10-100 µl
- Plugged tips
- Rack for 1.5 ml tubes
- Centrifuge (12 000 rpm)
- Incubator
- Vortex

Materials for amplification:

- Micropipette 1-10 µl
- Micropipette 10-100 µl
- Plugged tips
- Rack for 0.2 ml tubes
- Optical microplate for real Time PCR
- Tubes of 0.2 ml with optical caps
- Thermalcycler for Real Time PCR

6. PRECAUTIONS and WARNINGS

- The kit can be used only after a training for instruction of use.
- Laboratory equipments (pipettes, pipes, etc.) cannot be moved from a working area to another.
- Don't use the reagents after the date of expiration.
- Mix the reagents of the kit before use.
- Periodically verify the calibration of micropipette and the operative of instruments..
- Change gloves frequently.
- Periodically wash the working area with hypochlorite 5%
- Use powder-free gloves, don't leave fingerprints on optical caps. Don't write on caps as this may cause an overlap with fluorescences detection.

7. OPERATING PROCEDURE

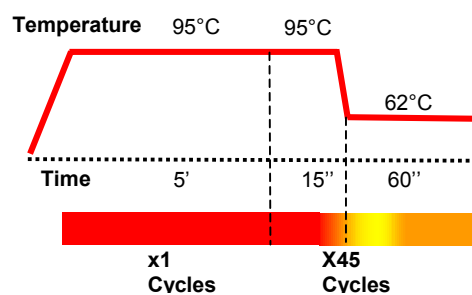
a) Thermal cycler set up

Refer to the specific handbook of the equipment used but be sure to set the following thermal profile.

Thermal Profile

Time	Temperature	N° Cycles
5 min	95°C	1
15 sec	95°C	45
60 sec	62°C	

Fig.4



We recommend to switch on the instrument, setting the thermal profile and having the plate ready before preparing the reaction mix.

b) Preparation of PCR mix

For each experiment prepare a PCR mix for the 2 controls (1 and 2), 1 reaction blank, n+1 samples. The reagents of the mix have to be mixed under this ratio:

REAGENT	VOLUME (μl)
Amplification mix	10
Oligo mix	10
Control 2 (Internal C.)	1
Extracted DNA	4

Once the mix is ready, aliquot 21ul of Master Mix in the tubes or in the microplates for PCR and add in each tube 4ul of extracted DNA and of controls; set the tubes inside the instrument and start the program of amplification you had set before. At the end of the program remove the tubes from the thermal cycler.

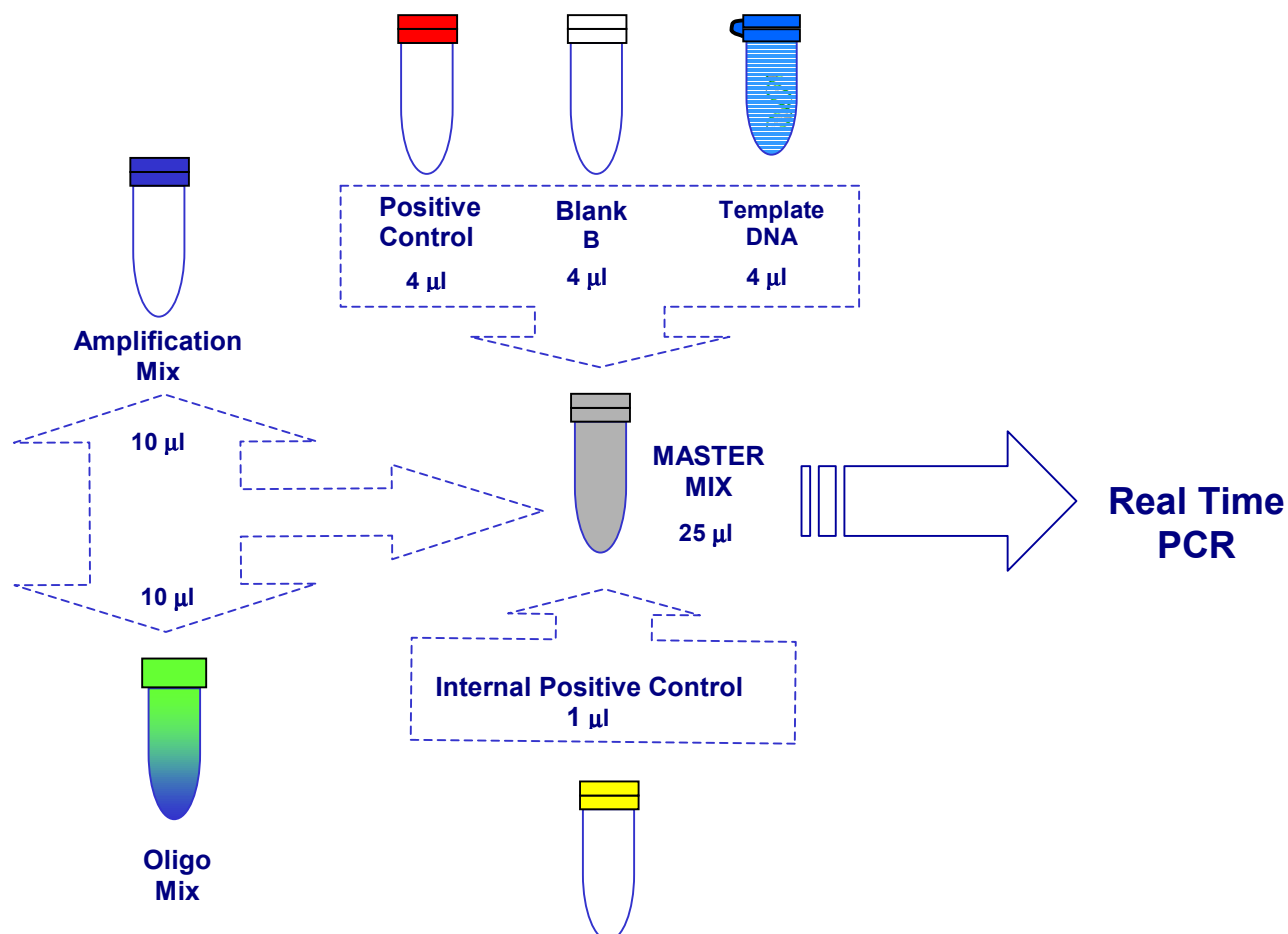


Fig.5

c) ANALYSIS and INTERPRETATION of the RESULTS

The fluorescence signal registered by the instrument detects the presence of the DNA, in particular the fluorescence detected in the FAM channel reveals the target DNA, while the fluorescence detected in the HEX channel reveals the amplification of the internal control (Control 2). When a signal at fluorophore FAM ($Ct > 0$) is detected, the sample is surely positive and the signal detected by HEX fluorophore ($Ct \geq 0$) is not relevant. When no signal at fluorophore FAM ($Ct = 0$) is detected, to confirm the negativeness of the result, the completed amplification of internal control, and

therefore the appearance of a fluorescent signal at HEX (ct > 0) fluorophore level, must be verified. Only in this case we can state that the sample is definitely negative. The absence of signal the amplification at the level of the two FAM-HEX (Ct=0) fluorophores indicates the presence of inhibition of the PCR. The sample must be repeated, and a dilution 1:10 of the target DNA is suggested.

FAM Fluorophore (L.ssp)	HEX Fluorophore (I.C.)	RESULT
Ct > 0	Not relevant (Ct ≥ 0)	POSITIVE
Ct = 0	Ct > 0	NEGATIVE
Ct = 0	Ct = 0	INIBITION

8. TROUBLESHOOTING

Problem 1: No signal

1. Wrong channel/filter was chosen.
2. Pipetting error due to omitted reagents or samples.
3. Inhibitory effect of the sample: genomic DNA with a insufficient purification and/or insufficient extraction
4. Check for correct conservation of the kit.
5. Control the performances of the Thermal Cycler

Problem 2: Fluorescence intensity too low

1. Deterioration of dyes and or primers in the device due to unsuitable storage condition
2. Very low starting amount of genomic DNA and/or low purity.

Problem 3: Fluorescence intensity varies

1. The prepared PCR master mix is not well mixed
2. Air bubbles trapped in the PCR tubes

RELATED PRODUCTS

EBR001032	Duplica RealTime Mycobacterium tuberculosis	32 tests
EBR010032	Duplica RealTime Mycobacterium Avium complex	32 tests
EBR008032	Duplica RealTime Adenovirus	32 tests
EBR007032	Duplica RealTime Legionella pneumophila	32 tests
EBR011032	Duplica RealTime Legionella ssp	32 tests
EBR006032	Duplica RealTime Chlamydia pneumoniae	32 tests
EBR012032	Duplica RealTime Mycoplasma pneumoniae	32 tests
EBR005032	Duplica RealTime Chlamydia trachomatis	32 tests
EBR009032	Duplica RealTime Thricomonas vaginalis	32 tests
EBR013032	Duplica RealTime Neisseria gonorrhoeae	32 tests
EBR014032	Duplica RealTime Mycoplasma genitalium	32 tests
EBR015032	Duplica RealTime Mycoplasma hominis	32 tests
EBR016032	Duplica RealTime Ureaplasma urealyticum	32 tests
EMR058050	Mycobacterium DNA Gene Releaser	50 test
EMR059050	EurocloneSpin Mycobacterium DNA purification Kit	50 test

**Kit components are Temperature sensible, store Kit
at 2 - 8°C**