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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

User guide

Biotoxis qPCR Detection Kit

Bacillus anthracis, Yersinia pestis, Francisella tularensis

Document reference: 009972-008-DU001-A

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Contents

1 INTENDED USE

The Biotoxis qPCR detection kit is a TaqMan® probe-based real-time PCR assay for the detection of *Bacillus anthracis*, *Yersina pestis,* and *Francisella tularensis* genomes in DNA samples from various origins: air, water or biological sample.

This assay is intended for research use only and not for use in diagnostic procedure.

2 INTRODUCTION TO THE SPECIFIC TARGETS

In the context of bioterrorism threat, the biological agents responsible for anthrax, plague and tularemia are considered as the most dangerous major risk for public health.

Bacillus anthracis is a Gram-positive, rod-shaped, non-motile, spore-bearing bacterium. *B. anthracis* is the only obligate pathogen within the *Bacillus* genus and causes Anthrax, an acute lethal infection of both ruminant animals and humans.

B. anthracis spores are soil borne and can survive for decades even under extreme and unfavorable conditions. Spores enter a host by inhalation, ingestion or via skin lesions. Subsequently, they penetrate the bloodstream, germinate and produce fatal toxins.

Cutaneous anthrax is the most common form in humans accounting for 90% of all reported cases and rarely fatal if treated. Individuals with ingested, gastrointestinal form undergo a similar disease progression but mortality rates are much higher. The mortality rate of inhaled, pulmonary anthrax is even higher. Macrophages in the alveolar provide entry to the bloodstream, which can result in septic shock and respiratory difficulties progressing to organ failure.

Anthrax is generally treated with antibiotics. Penicillin is privileged, especially with current vaccination, which offers only temporary protection.

Yersinia pestis (previously known as *Pasteurella pestis*) is a Gram-negative, rod-shaped coccobacillus, which can infect humans and various animals. It causes the fatal illness called plague. Human *Y. pestis* infection has three main forms: pneumonic, septicemic and bubonic plagues. All of them were responsible for many high-mortality epidemics throughout human history.

Rodents are the main reservoir of *Y. pestis*. A variety of species can be hosts; however, some are known to have a variable resistance that means they can be asymptomatic carriers. *Y. pestis* is usually transmitted to human via infected fleabites. The incubation period of *Y. pestis* is between 2 to 6 days, and death can occur in under two weeks.

Francisella tularensis is an aerobic, Gram-negative, non-motile and sporing coccobacillus. It is the causative agent of the disease tularemia, an infectious disease which is highly virulent in humans and rabbits. There are several subspecies of these bacteria that vary in their degree of virulence.

These bacteria use arthropods as vectors to infect humans and animals but can also be transmitted via the air. Infection can occur via the mucosal membranes of the lung and gastrointestinal tract and also through the skin.

Infection with these bacteria results in a debilitating disease even at low levels of contamination. There is no vaccine available against these bacteria and most cases of infection are treated with antibiotics

3 PRODUCT DESCRIPTION

Biotoxis qPCR detection kit provides a simple, reliable and rapid method for the detection of the three pathogens above in air, water, and biological samples. The assay uses the polymerase chain reaction (PCR) to amplify unique microorganism-specific DNA target sequences and TaqMan® probes to detect the amplified sequences.

The Biotoxis qPCR detection kit includes a positive control with a known quantity that can be used for standard curve construction. Extra care must be taken to avoid cross-contamination.

This kit has been developed for the in vitro detection of *B. anthracis, Y. pestis* and *F. tularensis*. The primers and probes sequences in this kit have been designed on a comprehensive bioinformatics analysis.

4 KIT CONTENTS AND STORAGE

Sufficient reagents are supplied for 96 reactions (25 µL reaction volume).

Components	Cap color	Volume	Storage
qPCR Mix	No color	1.25 mL	+ 4°C
Primers and probes Mix	**Blue**	375 µl	+ 4°C
Xplex 1 Plasmid (positive control)	**Red**	60 µL	+ 4°C
Water BPC Grade	**Yellow**	2 x 1.5 mL	+ 4°C

Note: The primers and probes are light sensitive and should be protected from light as much as possible.

The kit should be stored at +4°C on arrival. It is stable at + 4°C up to the expiration date.

5 ADDITIONAL MATERIALS REQUIRED

Real-Time PCR instrument

This kit has been validate on one of the following real-time PCR systems but other PCR systems can be used:

- CFX96 Touch™,Biorad
- Lightcycler® 480 instrument II, Roche

Pipettors and Tips

Vortex and centrifuge

PCR plates or PCR tubes (for real time PCR)

6 SUITABLE SAMPLE MATERIAL

The kit has been validated with the DNA extracted from Qiagen Dneasy Blood and Tissue kits. This kit has been designed to work well with all processes that provide high-quality DNA with minimal PCR inhibitors. We recommend to run at least one negative control with samples. To prepare a negative-control, replace the template DNA sample with Water BPC grade.

7 PRINCIPLE OF THE TEST

7.1 Real Time PCR

The primers and probes mix provided exploits the TaqMan® principle. The 5'-reporter dye and 3'-quencher dual-labeled oligonucleotide (TaqMan® probe) hybridizes on a specific region within the amplified fragment. During amplification, the probe is cleaved, and the reporter dye (fluorophore) is released. The fluorescent signal intensity detected is proportional to the number of amplicons. The Ct value (the cycle at which the rise of the fluorescent signal from the baseline is first significant) is used for quantification purposes. Each target pathogen amplification is detected using the following channels: HEX for *B. anthracis* amplification, FAM for *Y. pestis* amplification and TexasRed® for *F. tularensis* amplification.

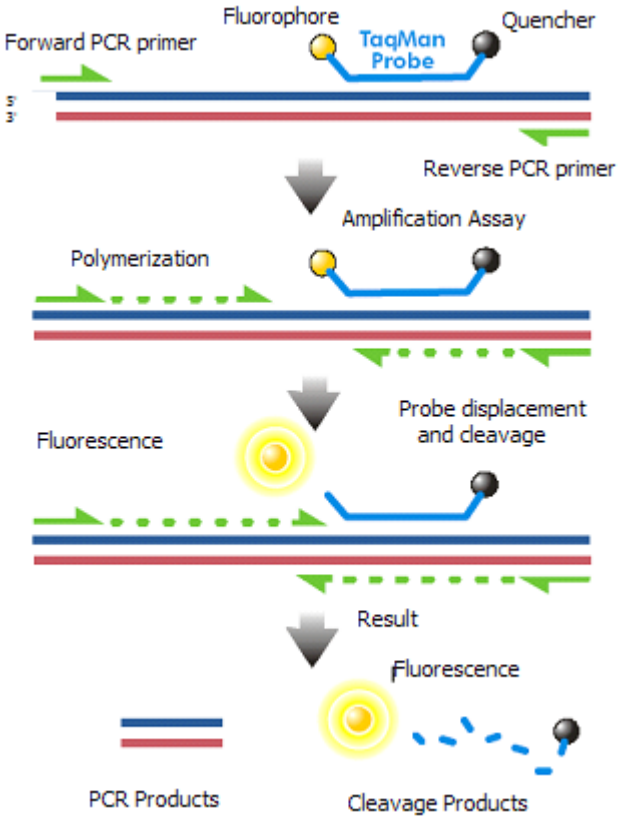


Figure 1: TaqMan® probe chemistry mechanism (source: Wikipedia)

7.2 Positive control

The kit contains a positive control template for quantity determination and positive control for the PCR set up. It can also be used to generate a standard curve of all three targets (*B. anthracis*, *Y. pestis* and *F. tularensis*). Alternatively, the positive control can be used directly when the quantitative analysis of the sample is not required.

Each time the kit is used, we recommend using at least one positive control reaction in the run. A positive result indicates that the primers and probes for

detecting the three targets genes worked properly in that particular experimental scenario.

If a negative result is obtained, the test results are invalid and the test must be repeated. Special care should be taken to ensure that the positive control does not contaminate any other kit component. Otherwise, it will lead to false-positive results. This could be avoided by managing the component in a Post PCR environment. Precaution should also be taken to prevent cross-contamination of other samples when adding the positive control to the run. This situation can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Note: experiments should be realized according to the laboratory specific regulation

7.3 Negative control

To validate any positive results, a negative control reaction should be included every time the kit is used. For this reaction, the water BPC grade should be used instead of a template. A negative result indicates that the reagents have not become contaminated while setting up the run.

8 QPCR PROTOCOL

8.1 Sample preparation

Before starting the sample preparation, have your experimental DNA sample ready

1. **For each DNA sample prepare a reaction mix according to the table below:**

Include sufficient reactions for positive and negative controls and dead volume. Mix by pipetting up and down gently.

Component	Volume (µl)
qPCR Mix	12.5
Primers and probes Mix	3.75
Water BPC Grade	3.75

2. **Pipette 20 µl of the mix into individual wells according to your qPCR experimental plate set up**
3. **Pipette 5 µl of DNA template into each well, according to your experimental plate set up**
4. **For negative control, wells use 5 µl of water BPC grade. For positive control wells use 5 µl of the plasmid (Red capped tube). The final volume in each well is 25 µl**
5. **Seal the plate with a cover sheet**
6. **Spin briefly to eliminate bubbles and spin down the reaction mix**

Note: sample preparation can be done at room temperature.

8.2 Amplification protocol

Set the thermal cycler parameters (compatible with CFX96 Touch™ and Lightcycler® 480 instrument II) as follows

Step	Time	Temperature	Cycles	Scan
Enzyme activation	3 min	95°C		
Denaturation	15 secs	95°C	X 45	
Anneal/Elongation	30 secs	60°C		Scan all channels

The duration of the denaturation and annealing/elongation has been optimized taking into consideration the probes and primers.

Note: if you are using another PCR system, optimization should be needed.

9 INTERPRETATION OF RESULTS

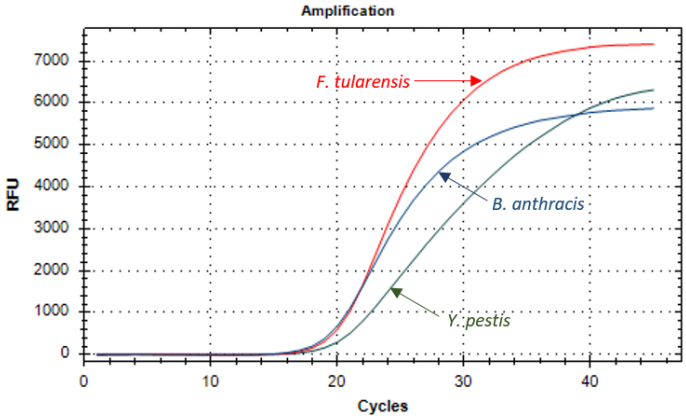
Pathogen specific amplification signals are detected via HEX channel for *B. anthracis*, FAM channel for *Y. pestis* and TexasRed® channel for *F. tularensis*.

The signal is considered positive when the amplification curve crosses the threshold line. The result is relevant provided both positive and negative controls give valid results.

Target	Negative Control	Positive Control	Interpretation
+	-	+	Valid, Positive
-	-	+	Valid, Negative
-	-	-	Invalid
+	+	+	Invalid

Positive control (5µl per well) is detected in FAM, HEX and TexasRed® channels. Cq values shown below are within normal range.

Target	Channel	Cq Value
B. anthracis	HEX	21±2
Y. pestis	FAM	22±2
F. tularensis	TexasRed	21±2



Example of curves obtained with 5µl of positive control

Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

10 ANNEXE: TROUBLESHOOTING

Problem	Possibility	Suggestion
Negative control/no template control gives positive result	Carry over contamination	Change nuclease-free water. Use fresh aliquots of reagents. Use filtered tips. Load positive control last.
No signal detected from positive control	Incorrect programming of instrument	Check program
	Reagent expired	Check the expiration date of reagents before repeat
	Storage condition not complying with instructions	Check storage condition properly and store at correct storage condition to avoid the degradation of reagents
	Pipetting error	Repeat the assay. Do a regular maintenance.

11 WARRANTY

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If you have questions about product specifications or performance, please call Bertin Bioreagent Technical Services (+33 (0)1 39 30 60 36)

12 SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate Material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.bertin-bioreagent.com where you can find, view, and print the MSDS for the kit and each kit component.

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