QUICK USER GUIDE

BIOTOXIS qPCR DETECTION KIT

Bacillus anthracis, Yersinia pestis, Francisella tularensis

/ PRODUCT DESCRIPTION

The Biotoxis qPCR detection kit provides a simple, reliable and rapid method for the detection of three pathogens in air, water, and any biological samples. This kit has been developed for the in-vitro detection of *B. anthracis*, *Y. pestis* and *F. tularensis*. The use of this kit has been validated with 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. 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 also contains a positive control with known quantification that can be used for standard curve construction. Extra care must be taken to avoid cross-contamination.

/ KIT CONTENTS AND STORAGE CONDITIONS

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

/ SAMPLE PREPARATION

- **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.

Reaction Mix Preparation:

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

- **Pipette 20 µl of the reaction mix (previously prepared) into individual wells according to your qPCR experimental plate set up.**
- **Pipette 5 µl of DNA template into each well, according to your experimental plate set up.**
- **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.**
- **Seal the plate with a cover sheet.**
- **Spin briefly to eliminate bubbles and spin down the reaction mix.**

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/ 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

/ INTERPRETATION OF RESULTS

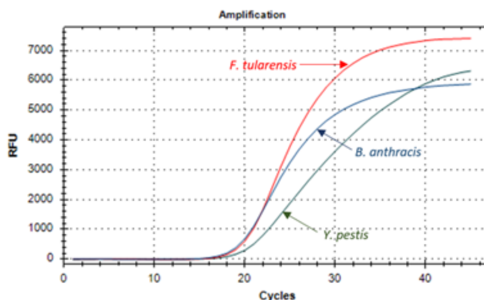
Pathogen-specific amplification signals are detected via HEX channel for *B. anthracis*, FAM channel for *Y. pestis* and TexasRed® channel for *F. tularensis*.

The signal is positive if 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 through 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