EURx® MOLECULAR BIOLOGY PRODUCTS

Borrelia qPCR Detection Kit

Kit Components

Component	Cat. No. E0450-01 50 reactions of 20 µl	Cat. No. E0450-02 100 reactions of 20 µl
Borrelia qPCR Master Mix (2x)	3 x 180 µl	6 x 180 µl
PC—positive control	1 x 100 µl	1 x 200 µl
Water, nuclease free	3 x 500 µl	6 x 500 µl

Borrelia qPCR Detection Kit, plus ROX Solution

Kit Components

Component	Cat. No. E0451-01 50 reactions of 20 µl	Cat. No. E0451-02 100 reactions of 20 µl
Borrelia qPCR Master Mix (2x)	3 x 180 µl	6 x 180 µl
ROX Solution, 25 µM	25 µl	50 µl
PC—positive control	1 x 100 µl	1 x 200 µl
Water, nuclease free	3 x 500 µl	6 x 500 µl

Storage

Store the components at -20°C in the dark.
Repeated thawing and freezing (> 2x) should be avoided, as this may reduce the sensitivity.

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Description

- Borrelia qPCR Detection Kit is a ready-to-use system for the detection of *Borrelia burgdorferi* DNA in animal tissue samples (tick, mammal).
- The kit contains reagents and primers for the amplification of a 153 bp region of the Borrelia genome. The detection of the Borrelia amplicon is based on using the specific FAM-labelled probe.
- In addition, the kit contains a second heterologous amplification system to monitor PCR reaction and identify possible PCR inhibition. The inner control consists of exogenous DNA, primers and the HEX-labelled probe. The amplification of the inner control does not interfere with the amplification of the specific Borrelia DNA and confirms PCR reaction correctness.
- The specificity of Borrelia qPCR Detection Kit has been confirmed by PCR using purified DNA (source: DSMZ) from: *Borrelia burgdorferi*, *Borrelia garinii*, *Borrelia afzelii*, *Borrelia valaisiana* and *Borrelia hermsii* species.
- Borrelia qPCR Master Mix (2x) contains onTaq DNA Polymerase, optimized reaction buffer, dNTPs (dTTP is partially replaced with dUTP) and uracil-N-glycosylase (UNG) to prevent carryover contamination between reactions. UNG removes uracil from any dU-containing contaminating amplicons, leaving a basic sites and making DNA molecules susceptible to hydrolysis during the initial denaturation step.
- onTaq DNA Polymerase is a modified "hot start" enzyme that is blocked at moderate temperatures and allows room temperature reaction setup.
- The polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 95°C for 15 minutes.
- There are two variants of the kit: without ROX and with ROX Solution provided separately. The use of ROX passive reference dye is necessary for all real-time PCR cyclers from Applied Biosystems and optional for cyclers from Stratagene. ROX compensates for variations of fluorescent signal between wells due to slight differences in reaction volume and fluorescence fluctuations. ROX is not involved in PCR reaction and does not interfere with real-time PCR on any instrument. Refer to the table below to determine the recommended amount of ROX (25 µM) required for a specific PCR cycler.
- DNA isolation from animal tissues should be performed with silica membrane-based commercial kits.

Recommended amounts of ROX for a specific real-time PCR cycler

Instrument	Amount of ROX per 20 µl reaction	Final ROX concentration
Applied Biosystems: 7300, 7900HT, StepOne, StepOnePlus, ABI PRISM 7000 and 7700	0.4 µl	500 nM
Applied Biosystems: 7500, ViiA 7, Stratagene: Mx3000P, Mx3005P, Mx4000	0.4 µl 10 x diluted (in water)	50 nM
PCR machines from other manufacturers: Bio-Rad, Roche, Corbett, Eppendorf, Cepheid, etc.	Not required	-

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Protocol

Preparation of PCR Reaction:

Component	Negative Control 1 rxn	DNA Samples 1 rxn	Positive Control 1 rxn	Final concentration
Borrelia qPCR Master Mix (2x)	10 µl	10 µl	10 µl	1 x
PC—positive control	-	-	1 µl	
Sample DNA	-	≤10 µl or ≤9.6 µl (if ROX Solution used)	-	≤500 ng
Optional: ROX Solution, 25 µM	0.4 µl or 0.4 µl 10 x diluted	0.4 µl or 0.4 µl 10 x diluted	0.4 µl or 0.4 µl 10 x diluted	500 nM 50 nM
Water, nuclease free	To 20 µl	To 20 µl	To 20 µl	-
Total volume	20 µl	20 µl	20 µl	-

Notes:

1. A reaction volume of 20 µl should be used with most real-time cyclers. Other reaction volumes may be used if recommended for a specific instrument.
2. Thaw, gently vortex and briefly centrifuge all solutions. Keep the kit components on ice. Avoid multiple thawing and freezing (> 2x) of Borrelia qPCR Master Mix (2x), as this may reduce the sensitivity. Protect Borrelia qPCR Master Mix (2x) from light exposure.
3. Set up PCR reactions at room temperature. Use of Borrelia qPCR Master Mix (2x) allows room temperature reaction setup.
4. Prepare a reaction master mix by adding all the reaction components except template DNA.
6. Mix the reaction mix thoroughly and dispense appropriate volumes into PCR tubes or plates.
7. Add template DNA (≤500 ng/reaction) to the individual PCR tubes or wells containing the reaction mix. Optimal DNA quantity is 100-200 ng.
8. For every PCR run prepare 2 additional reactions: negative and positive controls. The negative control should be prepared first and the positive control should be prepared last.
9. Centrifuge briefly to settle down the reaction components and remove bubbles. Bubbles interfere with fluorescent detection.
10. Place the samples in the cycler and start the program.
11. Perform data analysis.

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Thermal Cycling Conditions:

Step	Temperature	Time	Number of Cycles
UNG pre-treatment	37°C	2 min	1
Initial Denaturation	95°C	15 min	1
Denaturation	95°C	10 s	45
Annealing/Extension	60°C	60 s	
Cooling	4°C	Indefinite	1

Notes:

1. The UNG pre-treatment step is used to prevent carryover contamination. UNG degrades any dUMP- containing PCR products.
2. During the initial denaturation step onTaq DNA Polymerase is activated. onTaq DNA polymerase requires 15 min incubation at 95°C to restore activity.
3. Fluorescence detection should be set on the end of the extension step and executed using FAM and HEX channels. FAM channel detects Borrelia DNA and HEX channel detects the inner control.

Results Interpretation:

Sample type	FAM channel	HEX channel	Result
Negative control	-	+	correct
Positive control	+	+	correct
Sample 1	-	+	Negative (Borrelia$^-$)
Sample 2	+	+	Positive (Borrelia$^+$)

Thermal Cycling Conditions:

Notes:

1. A negative fluorescence signal in the HEX channel for the negative and positive controls means that Borrelia qPCR Detection Kit is of poor quality.
2. A positive fluorescence signal in the HEX channel for the negative and positive controls and at the same time a negative fluorescence signal in the HEX channel for DNA samples means that purified DNA is of poor quality.

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