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Borrelia burgdorferi PCR Detection Kit — Product Insert

Product # 45200

Pathogen Information

Borrelia burgdorferi is a Gram-negative bacterium and is the predominant agent of Lyme disease in North America. Mice and other small rodents are the primary reservoir for the *B. burgdorferi* bacteria, and Ixodes ticks will then attach to these rodents and feed on their blood, thereby transmitting the bacteria to the tick larvae. Tick larvae become dormant in the winter months, however in the spring, the young ticks will attach to larger animals, including dogs and cats, resulting in transmission of the bacteria to the larger hosts and subsequently Lyme disease. Typically more dogs contract Lyme disease than cats, but both are susceptible. Symptoms of Lyme Disease may include loss of neuromuscular function, limping in hind leg(s), fatigue, loss of appetite, lethargy, paralysis, muscle and joint pain and fever. Serious cases of lyme disease may cause paralysis as well as muscle and heart tissue damage, possibly resulting in death. For the best possible treatment outcome early diagnosis of Lyme disease is crucial. Animals who display any symptoms which could possibly have a diagnosis of Lyme disease should be tested as soon as possible. Due to the seriousness of the disease a sensitive and specific diagnostic test is also necessary to avoid false negative results.

Principle of the Test

Norgen's *Borrelia burgdorferi* PCR Detection Kit constituents a ready-to-use system for the isolation and detection of *Borrelia burgdorferi* using end-point PCR. The kit first allows for the isolation of *Borrelia burgdorferi* DNA from urine samples using spin-column chromatography based on Norgen's proprietary resin. The *Borrelia burgdorferi* DNA is isolated free from inhibitors, and can then be used as the template in a PCR reaction for *Borrelia burgdorferi* detection using the provided *B. burgdorferi* Master Mix. The *B. burgdorferi* Mastermix contains reagents and enzymes for the specific amplification of a 277 bp region of the genome. In addition, Norgen's *Borrelia burgdorferi* PCR Detection contains a second Mastermix, the PCR Control Master Mix, which can be used to identify possible PCR inhibition and/or inadequate isolation via a separate PCR reaction with the use of the provided *PCR control (PCRC)* or *Isolation Control (IsoC),* respectively. This kit is designed to allow for the testing of 24 samples.

Kit Components:

Component	Contents
Binding Solution I	8 mL
Binding Solution II	4 mL
Wash Solution I	4 mL
Wash Solution II	12 mL
Elution Buffer	3 mL
Proteinase K in storage buffer	1 mL
Pronase in storage buffer	1 mL
Mini Spin Columns	24
Collection Tubes	24
Elution tubes (1.7 mL)	24
2x BORR Detection PCR Master Mix	0.35 mL
2x PCR Control Master Mix	0.35 mL
BORR Isolation Control (IsoC)[a]	0.3 mL
BORR Positive Control (PosC)[b]	0.1 mL
Nuclease Free-Water	1.25 mL
Norgen's DNA Marker	0.1 mL
Product Insert	1

[a] *The isolation control is a cloned PCR product.*
[b] *The positive control is a cloned PCR product of Borrelia burgdorferi*

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Micropipettors
- 96 – 100% ethanol
- 60°C water bath or incubator

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25°C). Buffers can be stored for up to 1 year without showing any reduction in performance.

Norgen's *Borrelia burgdorferi* PCR Detection Kit contains ready-to-use Proteinase K and Pronase solutions, which are dissolved in a specially prepared storage buffer. The Proteinase K and the Pronase are stable for up to 1 year after delivery when stored at room temperature. To prolong the lifetime of Proteinase K and Pronase, storage at 2–8°C is recommended.

The 2x BORR Detection PCR Master Mix, 2x PCR Control Master Mix, BORR Positive Control (*PosC*) and the BORR Isolation Control (*IsoC*) should be kept tightly sealed and stored at -20°C for up to 1 year without showing any reduction in performance. Repeated thawing and freezing (> 2 x) should be avoided, as this may reduce the sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots.

General Precautions

The user should exercise the following precautions when using the kit:

- Use sterile pipette tips with filters.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice.

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's *Borrelia burgdorferi* PCR Detection Kit, including the 2x BORR Detection PCR Master Mix, 2x PCR Control Master Mix, BORR Isolation Control and BORR Positive Control are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's *Borrelia burgdorferi* PCR Detection Kit is designed for research purposes only.

Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Disclaimers

The **Binding Solution I, Binding Solution II, Wash Solution I** and **Wash Solution II** contain guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions. If liquid containing these solutions is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Safety Information

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at www.norgenbiotek.com.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Protocol

Important Notes Prior to Beginning Protocol:

- A variable speed microcentrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- First time users should read the entire manual before proceeding with the protocol.
- Do not spin down or filter the urine sample before proceeding with the isolation, as this could decrease the DNA yield.
- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Always **vortex** both the **Proteinase K** and the **Pronase** before use.
- Prepare a working concentration of **Binding Solution II** and **Wash Solution I** by adding 11 mL of 96-100% ethanol (provided by the user) respectively to the supplied bottle containing the concentrated **Binding Solution II** and **Wash Solution I**. This will give a final volume of 15mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.
- Preheat an incubator or heating block to 60°C.
- ***Borrelia burgdorferi* Isolation Control (*IsoC*)**
    - A BORR Isolation Control (*IsoC*) is supplied. This allows the user to control the DNA isolation procedure. For this assay, add the BORR Isolation Control *(IsoC)* to the lysate during the isolation procedure
    - The BORR Isolation Control *(IsoC)* must not be added to the sample material directly.
    - Do not freeze and thaw the BORR Isolation Control *(IsoC)* more than 2 times.
    - The BORR Isolation Control *(IsoC)* must be kept on ice at all times during the isolation procedure.
- The PCR components of the *Borrelia burgdorferi* PCR Detection Kit should remain at -20°C until DNA is extracted and ready for PCR amplification.
- It is important to work quickly during this procedure.

A. Isolation of DNA

1. Add 250 µL of **Binding Buffer I** for every 1.75 mL urine sample. **Mix** well by inverting a few times.
2. Apply up to 650 µL of the urine sample onto a column and centrifuge for **1 minute at 10,000 RPM**. Discard the flowthrough and reassemble the spin column with its collection tube.

   Note: If the entire volume does not pass through into the collection tube the column can be spun for an additional minute. Repeat Step **2** until the entire urine sample has passed through the column.

3. Add 35 µL of **Proteinase K** and 35 µL of **Pronase** to each column and centrifuge for **1 minute at 10,000 RPM**. **Do Not Discard the Flowthrough,** as it contains the DNA.
4. Incubate the column and collection tube at **60°C for 20 minutes**.
5. After the 20 minute incubation, add 450 µL of **Binding Buffer II** and **10 µL of BORR Isolation Control (IsoC)** to the lysate in the collection tube, mix well by pipeting and then transfer the entire contents of the collection tube back onto the spin column.
6. Centrifuge for **1 minute at 10,000 RPM**. Discard the flow-through.
7. Apply 450 µL of **Wash Solution I** to the column and centrifuge for **1 minute at 14,000 RPM**. Discard the flowthrough and reassemble the spin column with its collection tube.
8. Apply 450 µL of **Wash Solution II** to the column and centrifuge for **1 minute at 14,000 RPM**. Discard the flow-through and reassemble the spin column with its collection tube.

9. Apply 450 µL of 96-100% ethanol (supplied by the user) to the column and centrifuge for **1 minute at 14,000 RPM**. Discard the flow-through and reassemble the spin column with its collection tube.
10. Repeat Step 9 a second time.
11. Spin the column, empty, for **1 minute at 14,000 RPM**. Discard the collection tube.
12. Incubate the column horizontally with the lid open for 3 minutes at 60°C.
13. Transfer the spin column to a fresh Elution Tube. Apply 75 µL of **Elution Buffer** to the column and centrifuge for **2 minutes at 2,000 RPM,** followed by **2 minutes at 14,000 RPM**.
14. The purified DNA sample could be used immediately for PCR as described below. It is recommended that samples be placed at -70°C for long term storage.

B. *Borrelia burgdorferi* PCR Assay Preparation

Notes:

- Before use, suitable amounts of all PCR components should be completely thawed at room temperature, vortexed and centrifuged briefly.
- The amount of 2X BORR Detection PCR Master Mix and 2X PCR Control Master Mix provided is enough for up to 32 PCR reactions (24 sample PCR, 4 positive control PCR and 4 no template control PCR).
- For each sample, one PCR reaction using the **2X BORR Detection PCR Mastermix** and one PCR reaction using **2X PCR Control Mastermix** should be set up in order to have a proper interpretation of the result.
- For every PCR run, one reaction containing *BORR* Positive Control (*PosC*) and one reaction as no template control must be included for proper interpretation of results.
- The recommended minimum number of DNA samples tested per PCR run is 6.
- Using a lower volume from the sample than recommended may affect the sensitivity of *Borrelia burgdorferi* Limit of Detection.

1. Prepare the PCR for sample detection (Set #1, using **2X BORR Detection PCR Mastermix**) and control detection (Set #2, using **2X PCR Conrtol Mastermix**) as shown in Table 1 below. The recommended amount of sample DNA to be used is 2.5 µL. However, a volume between 1 and 5 µL of sample DNA may be used as template. Ensure that one BORR detection reaction and one control reaction is prepared for each DNA sample. Adjust the final volume of the PCR reaction to 20 µL using the Nuclease-Free Water provided.

Table 1. PCR Assay Preparation

PCR Components	Volume Per PCR Reaction
2X BORR Detection PCR Master Mix Or 2X PCR Control Master Mix	**10 µL**
Sample DNA	**2.5 µL**
Nuclease-Free Water	**7.5 µL**
Total Volume	**20 µL**

2. For every PCR set, prepare **one** positive control PCR as shown in Table 2 below:

Table 2. PCR Positive Control Preparation

PCR Components	Volume Per PCR Reaction
2X BORR Detection PCR Master Mix Or 2X PCR Control Master Mix	**10 µL**
BORR *Positive Control (PosC)*	**10 µL**
Total Volume	**20 µL**

3. For every PCR set, prepare **one** no template control PCR as shown in Table 3 below:

Table 3. PCR Negative Control Preparation

PCR Components	Volume Per PCR Reaction
2X BORR Detection PCR Master Mix Or 2X PCR Control Master Mix	**10 µL**
Nuclease-Free Water	**10 µL**
Total Volume	**20 µL**

C. *Borrelia burgdorferi* PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 4 below.
2. Run one step PCR.

Table 4. *Borrelia burgdorferi* Assay Program

PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**95°C**	**3 min**
Cycle 2 (35x)	**Step 1**	**94°C**	**15 sec**
	Step 2	**58°C**	**15 sec**
	Step 3	**72°C**	**30 sec**
Cycle 3	**Step 1**	**72°C**	**5 min**
Cycle 4	**Step 1**	**4°C**	**∞**

D. *Borrelia burgdorferi* PCR Assay Results Interpretation

1. For the analysis of the PCR data, the entire 15-20 µL PCR Reaction should be loaded on a 1X TAE 1.7% Agarose DNA gel along with 10 µL of Norgen's DNA Marker (provided).
2. The PCR products should be resolved on the 1X TAE 1.5% Agarose gel at 150V for 30 minutes (Gel running time will be vary depending on an electrophoresis apparatus).
3. Sample results are provided below:

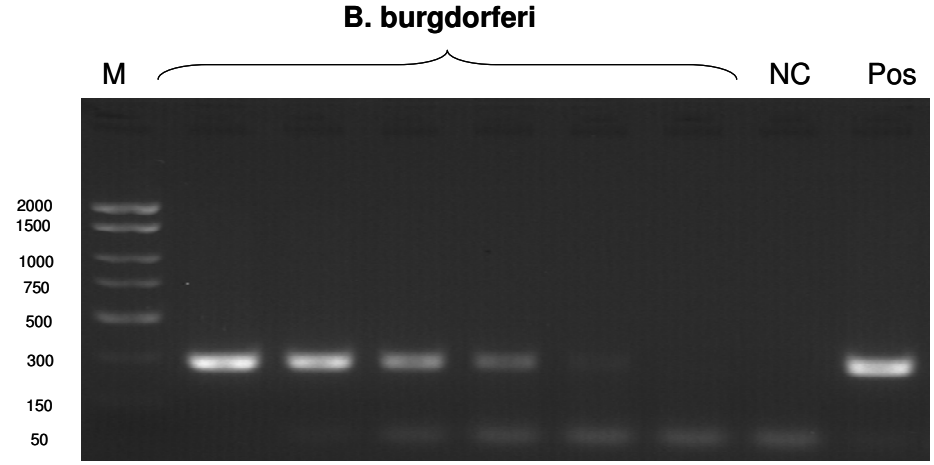

Figure 1: A representative 1.5X TAE 1.7% agarose gel showing the amplification of *B. burgdorferi* under different concentrations using the 2X *B. burgdorferi* Detection PCR Mastermix. The size of the *B. burgdorferi* target amplicon corresponds to 277 bp as represented by the provided DNA Marker (M). NC = Negative Control

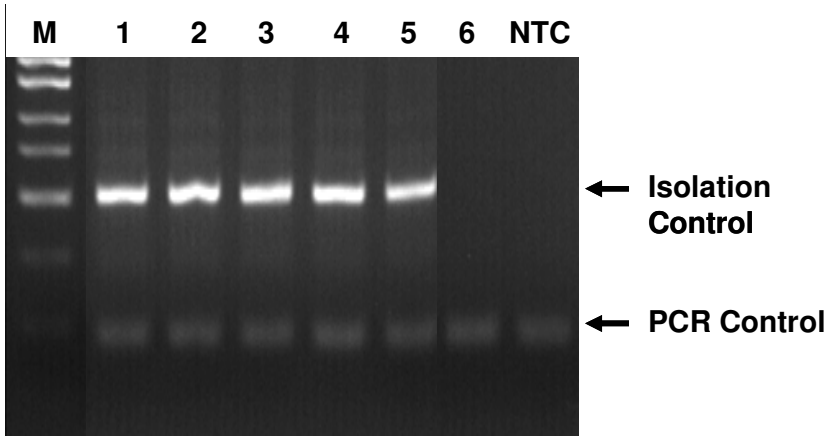

Figure 2: A representative 1X TAE 1.7% agarose gel showing the amplification of **Isolation Control** and **PCR Control** under different conditions using the **2X PCR Control Mastermix**. The size of the Isolation Control amplicon and PCR Control amplicon correspond to 499 bp and 150 bp, respectively, as represented by the provided DNA Marker (M). Lanes 1 to 5 showed detection of both Isolation Control and PCR Control, suggesting that the DNA isolation as well as the PCR reaction was successful. Lane 6 showed only the detection of PCR Control suggesting that while the PCR was successful, the isolation failed to recover even the spiked-in Isolation control. **NTC** = Negative Control.

Table 5. Interpretation of PCR Assay Results

Input Type	Target reaction	Control Reaction		Interpretation
	B. burgdorferi **Target Band (350 bp)**	***IsoC*** **Band (499 bp)**	***PCRC*** **Band (150 bp)**	
Positive Control	X	X	X	Valid
Negative Control			X	Valid
Sample	X	X	X	Positive
Sample		X	X	Negative
Sample			X	Re-test
Sample				Re-test
Sample		X		Negative
Sample	X		X	Positive
Sample	X	X		Positive
Sample	X			Re-test

****** For results obtained that are not covered in Table 5 above, please refer to the Troubleshooting Section.

E. *Borrelia burgdorferi* PCR Assay Specificity and Sensitivity

- The specificity of Norgen's *Borrelia burgdorferi* PCR Detection Kit is first and foremost ensured by the selection of the *Borrelia burgdorferi* specific primers, as well as the selection of stringent reaction conditions. The *Borrelia burgdorferi* specific primers were checked for possible homologies to GenBank published sequences by sequence comparison analysis and published strains.

F. Linear Range

- The linear range of Norgen's *Borrelia burgdorferi* PCR Detection Kit was determined by analysing a dilution series of a *Borrelia burgdorferi* quantification standards ranging from 1pg to 10 ng.
- Each dilution has been tested in replicates (n = 4) using Norgen's *Borrelia burgdorferi* PCR Detection Kit on a 1X TAE 1.7% agarose gel.
- The linear range of Norgen's *Borrelia burgdorferi* PCR Detection Kit has been determined to cover concentrations from 1pg to 10 ng.
- Under the conditions of the Norgen's *Borrelia burgdorferi* DNA Isolation procedure, Norgen's *Borrelia burgdorferi* PCR Detection Kit covers a linear range from 100 copies to 1 x 10^6 copies.

Frequently Asked Questions

1. How many samples should be included per PCR run?

- Norgen's *Borrelia burgdorferi* PCR Detection Kit is designed to test 24 samples. For every 6 samples, a non-template control (Nuclease Free Water) and a Positive Control must be included. It is preferable to pool and test 6 samples at a time. If not, the provided Positive Control is enough to run 3 samples at a time.

2. How can I interpret my results if neither the *B. burgdorferi* PCR control nor the *B. burgdorferi* Isolation Control (*IsoC*) amplifies?

- If neither the *B. burgdorferi* PCR control nor the *B. burgdorferi* Isolation Control (*IsoC*) amplifies, the sample must be re-tested. If the positive control showed amplification, then the problem occurred during the isolation, where as if the Positive control did not amplify, therefore the problem has occurred during the setup of the PCR assay reaction.

3. How should it be interpreted if only the *B. burgdorferi* PCR control showed amplification but neither the *B. burgdorferi* target nor the *B. burgdorferi* Isolation control amplified for a sample?

- This indicates a poor isolation. The isolation procedure must be repeated.

4. How should it be interpreted if only the *B. burgdorferi* Isolation Control (*IsoC*) was amplified in a sample?

- The sample tested can be considered as *B. burgdorferi* negative.

5. How should it be interpreted if the *B. burgdorferi* PCR control and the *B. burgdorferi* target showed amplification in a sample?

- The sample tested can be considered positive. It could happen when too much template was added to the reaction.

6. How should it be interpreted if only the *B. burgdorferi* target and the *B. burgdorferi* PCR control were amplified in a sample?

- The sample tested can be considered as *B. burgdorferi* positive.

7. How should it be interpreted if only the *B. burgdorferi* target was amplified in a sample?

- It is recommended that the isolation is repeated.

8. How should it be interpreted if only the *B. burgdorferi* PCR control and the *B. burgdorferi* Isolation control showed amplification in a sample?

- The sample tested can be considered negative

9. What if I forgot to do a dry spin after my third wash?

- Your first DNA elution will be contaminated with the Wash Solution. This may dilute the DNA yield in your first elution and it may interfere with the PCR detection, as ethanol is known to be a PCR inhibitor.

10. What if I forgot to add the *B. burgdorferi* Isolation Control (*IsoC*) during the isolation?

- It is recommended that the isolation is repeated.

11. What if I forgot to run the Control PCR for the sample and I only ran the Detection PCR and I obtained a positive result?

- The result can be considered positive. However, any negative result must be verified by running the associated control PCR to ensure that it is a true negative and not a false negative due to problems with the DNA isolation or the PCR reactions.

Related Products	Product #
Dirofilaria immitis PCR Detection Kit	44500
Toxoplasma gondii PCR Detection Kit	44700

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Norgen's *B. burgdorferi* PCR Detection Kit) or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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