Instructions for Use

RealLine Rickettsia species
Str-Format

KIT FOR THE QUALITATIVE DETECTION OF *RICKETTSIA SPECIES* DNA BY REAL-TIME PCR

For research use only. Not for use in diagnostic procedures.

RealLine Rickettsia species (Str-Format)	**VBD5391**	**48 Tests**
valid from	**October 2019**	

BIORON Diagnostics GmbH
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Explanation of symbols used in labeling

RUO	For research use only
LOT	Batch code
REF	Catalogue number
	Contains sufficient for <n> tests
	Use-by-date
	Temperature limit
	Consult instructions for use
	Keep away from sunlight
	Manufacturer

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1. INTRODUCTION AND INTENDED USE

Clinical information

Rickettsia are world-wide organisms, which are found in many ticks, fleas, mites and lice and serve as vectors. Endemic in Mediterranean countries, Eastern Europe, the tropics and North America In humans, they cause a whole range of diseases with different clinical pictures, which are grouped together medically to the group of Rickettsioses. Examples include typhus (syn. Typhus exanthematicus), rickettsialpox, Brill-Zinsser disease, Boutonneuse fever (Mediterranean tick-borne fever) and Rocky Mountain spotted fever. Like viruses, *Rickettsiae* thrive as intracellular parasites exclusively in living cells. In this way, they manage to escape the immune system of their hosts.

RealLine Rickettsia species assay kit is designed to detect *Rickettsia species* DNA isolated from samples using extraction kits:
RealLine RealLine DNA-Extraction 2 (REF VBC8897)
RealLine RealLine DNA-Extraction 3 (REF VBC8889)
RealLine RealLine Extraction 100 (REF VBC8896)
RealLine RealLine Extraction 1000 (REF VBC8895)
When using NA extraction kits of other manufacturers, it is highly recommended to use RealLine Internal Control sample (IC, VBC8881).

RealLine Rickettsia species reagents kit is intended for the detection of *Rickettsia species* DNA using the method of real-time polymerase chain reaction (PCR) with fluorescence detection of amplified product.

The **Str-Format Kit** contains 48 tubes (0.2 ml) in strips with lyophilized Mastermix. 50 µl of extracted DNA have to be pipetted into the tube and the ready mastermix is diluted. The kit contains reagents required for 48 tests, including control samples and the positive control sample.

The kit is intended for use with block-type cyclers: iQ™5 iCycler, iQ™ iCycler, CFX™96 (Bio-Rad, USA), RealLine Cyclers (BIORON Diagnostics GmbH) and DT-96 (DNA-Technology, Russia).

The use of:

- **Extraction Kits for nucleic acids from clinical specimen from other supplier**
- **other real-time PCR devices**
- **appropriate reaction volumes, other than 50 µl**

has to be validated in the lab by the user. The special notes regarding the internal control IC have to be strongly followed.

2. KIT CONTENTS

Ready Master Mix (RMM), lyophilized	48 test-tubes 6 strips x 8 tubes
Positive Control Sample (PC)	1 tube, 1 ml
Solution for Sample Preparation (SSP)	4 vials, 4 ml each
The kit is additionally supplied with optical-quality PCR-film	

3. PRINCIPLE OF THE METHOD

Real-time PCR is based on the detection of the fluorescence produced by a reporter molecule, which increases as the reaction proceeds. Reporter molecule is a dual-labeled DNA-probe that specifically binds to the target region of pathogens DNA. Fluorescence signal increases due to the separation of fluorescence dye and quencher by Taq DNA-polymerase exonuclease activity during amplification. PCR consists of repeated cycles: temperature denaturation of DNA, primer annealing and complementary chain synthesis.
Threshold cycle value (Ct) is a cycle number at which the fluorescence generated within a reaction crosses the threshold and the fluorescence signal rises significantly above the background. Increased signal is due to the use of a DNA hybridization probe that is specific for the given DNA sequence: it binds to one of the DNA strands in the course of reaction and provides additional specificity of the method. A DNA probe consists of a fluorescence dye at the 5'-end and a fluorescence quencher at the 3'-end that significantly reduces the fluorescence intensity. During the polymerase synthesis of the complementary strand, the probe is cleaved from the 5'-end due to the 5'-3' nuclease activity of Taq DNA polymerase, the quencher and the dye become separated, thus increasing the fluorescence signal due to accumulation of the reaction product. The detected fluorescence intensity depends on initial quantity of pathogens DNA template in the sample.
The use of **Internal Control sample (IC)** prevents generation of false negative results associated with possible loss of DNA template during sample preparation. IC indicates if PCR inhibitors occur in the reaction mixture. IC should be added to each sample (including control samples) prior to the DNA extraction procedure. Amplification and detection of IC influence neither sensitivity nor specificity of the target DNA PCR.

Note: Internal Control sample is added during NA extraction step and is used throughout the whole process of NA extraction, amplification, detection**.**

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4. SPECIFICATIONS

4.1. Analytical specificity

of *Rickettsia species* DNA detection is determined on four samples prepared from the Standard Reference Panel of control sera as percentage of samples detected by the kit as negative. Specificity equals 100 %.

4.2. Analytical sensitivity

of *Rickettsia species* DNA detection is determined on four samples containing 100 copies of *Rickettsia species* DNA per sample and prepared from *Rickettsia species* DNA Standard Reference Sample, as percentage of samples detected by the kit as positive. Sensitivity equals 100%.

5. PRODUCT USE LIMITATIONS

☝ **For Research Use Only.** Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

6. WARNING AND PRECAUTIONS

- The kits must be used by skilled personnel only.
- To obtain reliable results, strictly follow this Instruction Manual provided with the kit.
- When handling the kit, follow the national safety requirements for working with pathogens.
- To prevent contamination, the stages of DNA isolation and PCR test run must be spatially separated.
- Avoid microbial and ribonuclease contamination of reagents when removing aliquots from reagent vials.
- Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents.
- Every workplace must be provided with its own set of variable-volume pipettes, necessary auxiliary materials and equipment. It is prohibited to relocate them to other workplaces.
- To conduct amplification reaction with real-time PCR products detection, use only disposable tips with filters.
- Never use the same tips for different samples.
- Do not pool reagents from different lots or from different vials of the same lot.
- Dispose unused reagents and waste in accordance with country, federal, state and local regulations.
- Do not use the kit after the total expiration date at the side label of the box.

7. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- Real time PCR system, like described in p.1
- DNA-Extraction Kit: **RealLine DNA-Extraction 2, RealLine DNA-Extraction 3, RealLine Extraction 100 or RealLine Extraction 1000**
- Internal Control reagent (VBC8881), if the kit is used with the extraction kits of other supplier.
- Laminar safety box;
- Refrigerator;
- Half-automatic variable-volume single-channel pipettes;
- Disposable medical non-sterile powder-free gloves;
- Disposable pipette tips with aerosol barrier;
- Racks for 2.0 ml and 0.2 ml tubes
- Biohazard waste container;
- Razor or scalpel

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8. PREPARATION OF SAMPLES

*Each group of samples undergoing the procedure of DNA isolation must include a **Positive Control sample (PC)** from this kit and a **Negative Control sample (NC)** which is a component of the DNA extraction kit.*

We strongly recommend the implementation of the Internal Control IC, the Negative Control NC and Positive Control PC samples to the extraction procedure.
When using kits of another supplier for the extraction of nucleic acids as recommended in chapter 1: add **20 µl** of **IC (VBC8881)** to each tube.

- For the NC use **100 µl** of the Negative Control Sample
- For the PC use **70 µl** of Negative Control Sample and **30 µl** of Positive Control to the tube marked PC.

8.1 EDTA-treated blood plasma, leukocyte blood fraction, biopsy materials

The assay is performed on extracted DNA sample obtained using one of the in chapter 1 listed DNA extraction kits according to the Instruction Manual to the kit. If an extraction kit with magnetic particles is used, keep the tubes with extracted NA in a magnetic rack.

8.2 Whole blood

The reagents kit **RealLine Hemolytic** can be used for preliminary treatment of whole blood in order to obtain blood samples suitable for DNA extraction, according to the Instruction Manual to the kit.

8.3 Inoculation eschar swabs

Prepare 1.5 ml Eppendorf-type tube with 300 µl of Solution for Sample Preparation (SSP).
Use a cotton swab stick to soak the skin crust at the inoculation eschar surface with saline during 1-2 min. Remove the remaining skin. Sample ulcerated area under the skin with cotton swab and collect the contents while passing over the eschar surface 5-7 times, gently penetrating inside the lesion with the tip. Squeeze the liquid from the swab against the tube wall. Repeat the procedure. Place the swab at the bottom of the tube, mix the content thoroughly, squeeze the liquid from the swab against the tube wall, remove the stick to the disinfectant solution. Tightly close the tube. Vortex the tube (20 - 30 sec).
Use 100 µl of obtained sample for DNA extraction.

8.4 Tick suspensions

To prepare tick suspension the reagents kit **RealLine Extraction 100** can be used, the procedure is described in the Instruction Manual to the kit. Solution for Sample Preparation (SSP) is used to prepare tick suspension.

Store the extracted DNA at (2 – 8) °C for no more than 24 hours.
After initial opening of the tube, store PC at (2 – 8) °C for no more than 1 month or in 50 µl aliquots at minus (18 – 24) °C during 3 months.
After initial opening of the vial, store SSP at (2 – 8) °C for no more than 3 months.

9. PROCEDURE

9.1. Preparation of the reagents.

Prior to the test take the kit out of the refrigerator and keep the **Ready Master Mix for PCR (RMM)** closed in the package at (18 – 25) °C for at least 30 minutes. Open the package and cut off the necessary number of tubes with RMM (including the test and control samples: 1 NC and 1 PC) with the razor or scalpel. Cut the tubes together with the covering film.
Put the remaining strips immediately back into the foil pouch, squeeze the air out and tightly close with the clip.
After initial opening store RMM at (2 – 8) °C for no more than 3 months.

9.2. **Label** the tubes with RMM for each test and control sample.
Attention! *Labels should be placed on the lateral side of the tubes.*

9.3. Add **50 µl** of corresponding isolated DNA solution to each tube using a separate pipette tip with filter. Tightly close the tubes with caps or seal with the PCR transparent film.

9.4. Place the tubes into the real-time PCR system.

9.5. Program real time PCR system as follows:

Step 1:	50°C	2min	
Step 2:	95°C	2min	
Step 3:	94°C	10 sec	50 cycles
	60°C*	20 sec	

* Measure the fluorescence at 60°C

9.6. Select the amplification detection channels:

- Collect real-time PCR data through the **FAM** channel for detection of amplification of IC DNA.
- Collect real-time PCR data through the **ROX** channel for detection of amplification of ***Rickettsia species*** DNA.

9.7. Program the positions of test tubes with samples, positive and negative controls according to the instruction manual for the real time PCR system in use.

9.8. Run the program.

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10. DATA ANALYSIS AND INTERPRETATION

10.1 For **Positive Control** the program should detect:

- Increase of the IC DNA amplification signal (channel **FAM**) and determine the threshold cycle, IC **Ct**;
- Increase of the ***Rickettsia species*** DNA amplification signal (channel **ROX**) and determine the PC **Ct** value.

10.2 For **NC** the program should detect the increase of the amplification signal of IC DNA (channel **FAM**) and determine the threshold cycle, IC **Ct**. No significant **ROX** fluorescent increase should appear (*no* ***Rickettsia species*** DNA amplification).

If **Ct** value for NC through **ROX** channel **is less than or equal to 40,** this indicates the presence of contamination (see paragraph 9.7).

10.3 For each sample the program should detect the increase of the amplification signal of IC DNA (channel **FAM**) and determine IC **Ct**.

10.4 Calculate (IC **Ct**)$_{av}$ as an average IC **Ct** of all analyzed samples (including PC and NC). IC **Ct** values that differ by more than 2 from the (IC **Ct**)$_{av}$ should be ignored. Recalculate the (IC **Ct**)$_{av}$ for the remaining values after the screening.

10.5 The sample is considered **positive**, i.e. contains ***Rickettsia species*** DNA, when **Ct** value via **ROX** channel for this sample is **less than or equals to 40.**

10.6 The sample is considered **negative** (not containing ***Rickettsia species*** DNA), if **Ct** value via **ROX** channels for this sample is **above 40** or is not determined.

If IC **Ct** value for such sample differs from the (IC **Ct**)$_{av}$ value by more than 2, the result is regarded as equivocal. A repeated analysis of the sample, starting with the DNA isolation step is necessary.

10.7 In case of contamination all positive results of this individual PCR run are considered equivocal. Actions are required to identify and eliminate the source of contamination, and repeat the analysis of all samples of this run that were identified as positive. Samples that showed negative results in this run should be considered as negative.

11. STORAGE AND TRANSPORTATION

- Store the assay kit at (2 - 8) °C in the manufacturer's packing for the entire shelf life.
- Transport at (2 – 8) °C; transportation for up to 25 °C for no more than 10 days is allowed.
- Do not freeze the kit!
- Do not pool reagents from different lots or from different vials of the same lot.
- Strictly follow the Instruction manual for reliable results.
- Do not use kits with damaged inner packages and get in contact with BIORON Diagnostics GmbH.

- **Storage and shelf life of solutions and components of the kit after initial opening:**
  Positive Control and Negative Control sample: 1 month at (2 - 8) °C *or in 50 µl aliquots at minus (18 - 60) °C for up to 3 months*.
  Ready Master Mix (RMM): 3 months at (2 – 8) °C

ANNEX I: Settings for RealLine Cycler and DT96:
for these cyclers the measurement exposure must be adjusted. Choose the **Operation with the device** mode in the **Settings** menu, select the item **Measurement exposition:**

- **FAM** to **250**
- **HEX** and **ROX** to **1000**

Confirm that the current exposure value is saved by pressing **YES**
Attention! The specified exposure values are applicable only for RealLine kits and, if necessary, must be changed for other purposes.

Technical Support: techsupport@bioron.de

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