

Instructions for use

**RealLine Chlamydia trachomatis /  
Neisseria gonorrhoeae  
Fla-Format**

ASSAY KIT FOR THE QUALITATIVE DETECTION AND DIFFERENTIATION OF *CHLAMYDIA TRACHOMATIS* AND *NEISSERIA GONORRHOEAE* DNA BY REAL-TIME PCR METHOD IN ONE REACTION.

In vitro Diagnostics



<b>RealLine Chlamydia trachomatis / Neisseria gonorrhoeae (Fla-Format)</b>	<b>VBD0458</b>	<b>100 Tests</b>
<b>valid from</b>	<b>June 2016</b>	










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## RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

### Explanation of symbols used in labeling

	For in vitro diagnostic use
	Batch code
	Catalogue number
	Amount of tests
	Expiry Date
	Temperature limitation
	Consult instructions for use
	Keep out of sunlight
	Manufacturer



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## RealLine *Chlamydia trachomatis* / *Neisseria gonorrhoeae* Fla-Format

ASSAY KIT FOR THE QUALITATIVE DETECTION OF *CHLAMYDIA TRACHOMATIS* AND *NEISSERIA GONORRHOEAE* DNA BY REAL-TIME PCR METHOD IN ONE REACTION.

In vitro Diagnostics

### 1. INTRODUCTION

#### Clinical information

Untreated, Chlamydia infections by *Chlamydia trachomatis* can cause serious reproductive and other health problems with both short-term and long-term consequences. In both sexes Chlamydia infection can cause proctitis, trachoma and infertility as well as prostatitis, epididymitis in men and cervicitis, PID, ectopic pregnancy and acute/chronic pelvic pain, in women.

Chlamydia is known as the Silent Epidemic because in women it may not cause any symptoms in 75 % of cases, and can be latent for months or years before discovered.

*Neisseria gonorrhoeae* is a gram-negative motile bacteria and the causative agent of gonorrhoea (clap), one of the most common sexually transferred disease. In women gonorrhoea can lead to severe symptoms such as infertility and abortion. In men gonorrhoea can lead to painful urethritis.

**RealLine *Chlamydia trachomatis* / *Neisseria gonorrhoeae* (Fla-format)** assay kit is designed to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA isolated from clinical specimens using extraction kits

**RealLine DNA-Express (REF VBC8899)**

**RealLine DNA-Extraction 2 (REF VBC8897)**

**RealLine DNA-Extraction 3 (REF VBC8889)**

**RealLine Extraction 100 (REF VBC8896)**

**RealLine *Chlamydia trachomatis* / *Neisseria gonorrhoeae*** kit is designed for the analysis of clinical materials: saliva, urine, sperm, scrapings of the epithelial cells.

The assay is based on the real-time polymerase chain reaction (PCR) method with fluorescent detection of the amplified product.

The **Fla-format** Kit contains 10 vials with the lyophilized Mastermix, each vial with 10 reactions, for volume of 50 µl per reaction. The kit contains reagents required for 100 tests, including the positive control samples.

The kit is designed for use with block cyclers iQ™ iCycler, iQ5™ iCycler, CFX96™ (Bio-Rad, USA), DT96 (DNA-Technology Research and Production Company ZAO, Russia); and rotor type cyclers Rotor-Gene® 3000, 6000 and Rotor-Gene® Q (Qiagen, Germany).

## RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

For the Eco™ 48 Realtime PCR System (PCRmax, Great Britain) RealLine Fla-format kits can be recommended. The practice with this cycler to use 10 µl of the diluted Mastermix and 10 µl of extracted DNA, was validated. The protocol for using and cycling can be provided.

### 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.**

The detection of *Neisseria gonorrhoeae* with this kit has a high specificity, but a positive result is further recommended to confirm with the test using the RealLine Neisseria gonorrhoeae T2 (Str format) kit (REF VBD4494). These kits use different specific regions of the Neisseria gonorrhoeae genome as targets; therefore, a comprehensive study allows to confirm the presence of this pathogen.

## 2. KIT CONTENTS

- Universal Positive Control Sample (PC) – 1 vial, 1 ml;  
based on the plasmid DNA with integrated DNA fragments of a number of herpes viruses and STD pathogens, including *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA
- Master Mix (MM), lyophilized – 10 tubes (10 tests each);
- Recovery Solution (RS) - 2 vials, 2ml each;

## RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

### 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 dual-labeled DNA-probe, which specifically binds to the target region of pathogen DNA. Fluorescent signal increases due to the fluorescent dye and quencher separating by Taq DNA-polymerase exonuclease activity during amplification. PCR process consists of repeated: temperature denaturation of DNA, primer annealing and complementary chain synthesis.

Threshold cycle value - Ct – is the cycle number at which the fluorescence generated within a reaction crosses the fluorescence threshold, a fluorescent signal rises significantly above the background fluorescence. Ct depends on initial quantity of pathogen DNA template.

The use of **Internal Control (IC)** prevents generation of false negative results associated with possible loss of DNA template during specimen preparation and DNA extraction. The IC indicates if PCR inhibitors occur in the reaction mixture and the IC template should be added in each single sample (including control samples) prior to DNA extraction procedure. The amplification and detection of IC does not influence the sensitivity or specificity of the target DNA PCR.

**Note:** IC is a component of the NA extraction kits of RealLine series. Internal Control is added to the sample during NA isolation step and is used throughout the whole process of NA extraction, amplification, detection.

## RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

### 4. SPECIFICATIONS

#### I. Sensitivity:

Sensitivity control was performed on five samples containing 100 copies of *Chlamydia trachomatis* DNA and *Neisseria gonorrhoeae* DNA per sample, prepared from Standard Reference Samples (*Chlamydia trachomatis* DNA SRS and *Neisseria gonorrhoeae* DNA SRS,).

The sensitivity equals 100%.

#### II. Specificity:

Specificity of RealLine Chlamydia trachomatis / Neisseria gonorrhoeae was ensured by specific primers and probes.

Specificity of *Chlamydia trachomatis* DNA and *Neisseria gonorrhoeae* DNA detection was also confirmed in laboratory tests using negative samples of the Standard Reference Panel consisting of 5 samples containing IC DNA and not containing DNA of STD agents. Specificity of *Chlamydia trachomatis* DNA and *Neisseria gonorrhoeae* DNA detection equals 100%.

#### III. Diagnostic evaluation:

##### **Diagnostic sensitivity of the *C. trachomatis* DNA detection:**

Diagnostic sensitivity was evaluated on a collection of 415 clinical samples: 200 urine samples (123 from men and 77 from women), 133 cervical scrapings and 82 urethra scrapings from patients with STI characteristic symptoms.

The presence of *C. trachomatis* DNA was confirmed in the samples by CE-marked reference kits. Diagnostic sensitivity equaled 96.6%.

##### **Diagnostic sensitivity of the *N. gonorrhoeae* DNA detection:**

Diagnostic sensitivity was evaluated on a collection of 228 clinical samples: 78 urine samples (55 from men and 23 from women), 129 cervical scrapings and 21 urethra scrapings from patients with STI characteristic symptoms.

The presence of *N. gonorrhoeae* DNA was confirmed in the samples by CE-marked reference kits. Diagnostic sensitivity equaled 98.4%.

All discordant results obtained concern samples with low content of detectable DNA fragment that falls outside the reliable detection limit of all the kits.

##### **Diagnostic specificity of the kit:**

Diagnostic specificity was estimated on a set of 200 urine samples and mucous scrapings from healthy people. 120 urine samples (30 from men and 90 from women), and 80 mucosal scrapings (11 from men and 69 from women) were analyzed. Obtained results were in full agreement with reference kits.

Diagnostic specificity equaled 100%.

## RealLine *Chlamydia trachomatis* / *Neisseria gonorrhoeae* Fla-Format

Carry-over tests were performed on 48 clinical samples of epithelial cells scrapings containing high concentration of *Chlamydia trachomatis* DNA and high concentration of *Neisseria gonorrhoeae* DNA as well as samples containing mix of *Chlamydia trachomatis* DNA and *Neisseria gonorrhoeae* DNA.

No carry-over effect was observed.

### 5. WARNING AND PRECAUTIONS

- ☞ For in vitro use only.
- ☞ The kits must be used by skilled personnel only.
- ☞ 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 nuclease 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.
- ☞ The use of sterile disposable pipettes and pipette tips is recommended.
- ☞ 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 expiration date.

### 6. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- real time PCR system, like described in p.1
- DNA-Extraction Kit: **RealLine DNA - Express**, **RealLine DNA - Extraction 2**, **RealLine DNA - Extraction 3** or **RealLine Extraction 100** Internal Control reagent (VBC8881) and Negative Control Sample or H<sub>2</sub>O (molecular biology grade), if the kit is used with the extraction kits of other supplier.
- Plates or Tubes suitable for the used device with caps or a sealing foil for PCR
- laminar safety box;
- refrigerator;
- half-automatic variable-volume single-channel pipettes;
- disposable medical non-sterile powder-free gloves;
- disposable pipette tips with aerosol barrier;
- biohazard waste container.

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## RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

### 7. PREPARATION OF THE ANALYSED SAMPLES AND REAGENTS

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 or H<sub>2</sub>O (molecular biology grade).
- For the PC use **70 µl** of Negative Control Sample or H<sub>2</sub>O (molecular biology grade) and **30 µl** of Positive Control to the tube marked PC.

#### 7.1. Sample preparation

Prepare the samples for the assay using **RealLine DNA - Express**, **RealLine DNA - Extraction 2**, **RealLine DNA - Extraction 3** or **RealLine Extraction 100** extraction kits according their instruction manuals.

If samples of isolated DNA were stored frozen prior the assay, thaw them and keep at least 30 minutes at a temperature of (18 – 25) °C.

*The isolated DNA can be stored at (2 – 8) °C for 2 days.*

*After initial opening shelf life of Positive Control sample is 1 month at (2 – 8) °C*

#### 7.2. Preparation of the reagents.

Prior the test take the kit out of the refrigerator and keep the **Master Mix (MM)** closed in the package at (18 – 25) °C for at least 30 minutes. Then open the package and take the necessary number of tubes with MM (*including prepared samples and controls: 1 NC and 1 PC*). Each tube is intended for 10 tests.

Put the remaining tubes immediately back into the foil pouch, squeeze the air out and tightly close with the clip.

*After initial opening store MM at (2 – 8) °C for the entire shelf life of the kit.*

To prepare diluted Master Mix, add **300 µl** of **Recovery Solution (RS)** to each tube with MM. Mix gently, store at room temperature for 15 minutes, and then carefully remix.

*Store diluted MM at (2 – 8) °C for 2 weeks.*

*After initial opening shelf life of Recovery Solution is 3 months at (2 – 8) °C.*

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### 8. PROCEDURE

8.1. Prepare an appropriate number of 0.2 ml tubes or the plate. Label each tube for each specimen and control.

**Attention!** Labels should be placed on the caps of tubes for Rotor-Gene® 3000/6000/Q devices. For iQ™ iCycler, iQ™5 iCycler, CFX™96, DT-96 PCR devices labels should be placed on the lateral side of the tubes.

8.2. Add **25 µl** of prepared Master Mix to each 0.2 ml tube.

8.3. Add **25 µl** of corresponding isolated DNA solution to each tube using a separate pipette tip with filter. Do not touch the pellet! Tightly close the tubes with caps or sealings.

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

8.5. Program real time PCR system.

**For Rotor-Gene® 3000 (6000, Q):**

- Click **New** button.
- Select a Dual labeled Probe template from the Advanced tab of the New Run wizard. Click **New** button.
- Select 36-Well Rotor type, check that no Domed 0.2 ml Tubes are used. Click **Next** button.
- In the new window determine Reaction volume as 50 µl. Click **Next** button.
- The Temperature profile of real time PCR should be set. Click **Edit Profile** button.
- 

Stage 1:	50°C	2min	50 cycles
Stage 2:	95°C	2min	
Stage 3:	94°C	10 sec	
	60°C*	40 sec	

\* Measure the fluorescence at 60°C

- Then temperature profile is set, click **OK** button.
- In the **New Run Wizard** window click **Calibrate (Gain optimisation)** button. The window **Auto Gain Calibration Setup** opens.  
In the line **Channel Settings** choose **ROX (Orange)**, click **Add**. Set **Tube Position 1, Min Reading 5, Max Reading 10**, click **OK**.  
In the line **Channel Settings** choose **FAM (Green)**, click **Add**. Set **Tube Position 1, Min Reading 5, Max Reading 10**, click **OK**.  
In the line **Channel Settings** choose **JOE (Yellow)**, click **Add**. Set **Tube Position 1, Min Reading 5, Max Reading 10**, click **OK**.
- Tick off **Perform Calibration Before 1st Acquisition**. Click **Close** button.
- Click **Next** button, start the amplification process by clicking **Start Run**.
- Save a file in the Rotor-Gene®/templates folder, named RealLine with \*.ret extension. In subsequent work RealLine template would be presented in New run wizard.
- Save reaction result file with Rotor-Gene® Run File \*.rex extension.

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## RealLine *Chlamydia trachomatis* / *Neisseria gonorrhoeae* Fla-Format

- Record the positions of the controls and specimens according to the instruction manual of the operating device. Click **Start run** button.

### 8.6. For iQ™ iCycler, iQ™5 iCycler, CFX™96, DT-96:

Program real time PCR device according the instruction manual as follows:

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

\* Measure the fluorescence at 60°C

### 8.7. 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 **HEX** channel for detection of amplification of *Chlamydia trachomatis* DNA.
- Collect real-time PCR data through the **ROX** channel for detection of amplification of *Neisseria gonorrhoeae* DNA.

### 8.8. 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.

### 8.9. Run the program.

## RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

### 9. DATA ANALYSIS AND INTERPRETATION

#### For Rotor-Gene® 3000 (6000, Q):

##### 9.1. Results for Internal Control DNA amplification

- Click **Analysis** button, choose **Quantitation** from the list, choose **Cycling A. FAM** (*Cycling A. Green*), click **Show** button.
- Click **OK** button, and cancel automatic **Threshold** determination.
- Click **Linear scale** button.
- In the **Quantitation analysis** menu buttons **Dynamic tube** and **Slope Correct** should be pressed.
- Click **More Settings** (*Outlier Removal*) button, determine **NTC threshold** value as **5%**.
- In the column **CT Calculation** (*right part of the window*) determine **Threshold** value as **0,04**.
- In the result table (*Quant. Results window*) **Ct** will be displayed.

##### 9.2. Results for Chlamydia trachomatis DNA amplification

- Click **Analysis** button, choose **Quantitation** from the list, choose **Cycling A. JOE** (*Cycling A. Orange*) click, **Show** button.
- Click **OK** button, and cancel automatic **Threshold** determination.
- Click **Linear scale** button. Settings should change to **Log. scale**.
- In the **Quantitation analysis** menu buttons **Dynamic tube** and **Slope Correct** should be pressed.
- Click **More Settings** (*Outlier Removal*) button, determine **NTC threshold** value as **10%**.
- In the column **CT Calculation** (*right part of the window*) determine **Threshold** value as **0,04**.
- In the result table (*Quant. Results window*) **Ct** will be displayed.

##### 9.3. Results for Neisseria gonorrhoeae DNA amplification

- Click **Analysis** button, choose **Quantitation** from the list, choose **Cycling A. ROX** (*Cycling A. Orange*) click, **Show** button.
- Click **OK** button, and cancel automatic **Threshold** determination.
- Click **Linear scale** button. Settings should change to **Log. scale**.
- In the **Quantitation analysis** menu buttons **Dynamic tube** and **Slope Correct** should be pressed.
- Click **More Settings** (*Outlier Removal*) button, determine **NTC threshold** value as **5%**.
- In the column **CT Calculation** (*right part of the window*) determine **Threshold** value as **0,04**.
- In the result table (*Quant. Results window*) **Ct** will be displayed.

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- 9.4. The program should detect in **Positive Control** sample:
- increase of the IC DNA amplification signal along channel **FAM** (*Green*) and determine the threshold cycle, IC **Ct**;
  - increase of the *Chlamydia trachomatis* DNA amplification signal along channel **JOE** (*Yellow*) and determine the **Ct** value;
  - increase of the *Neisseria gonorrhoeae* DNA amplification signal along channel **ROX** (*Orange*) and determine the **Ct** value;

- 9.5. For **NC** the program should detect the increase of the amplification signal of IC DNA along channel **FAM** (*Green*) and determine the threshold cycle, IC **Ct**. No significant **ROX** (*Orange*) and **JOE** (*Yellow*) fluorescent increase should appear (*no Chlamydia trachomatis and Neisseria gonorrhoeae DNA amplification*).

When **Ct** value for NC through **ROX** (*Orange*) **JOE** (*Yellow*) channel is **less than or equal to 40**, this indicates the presence of contamination (see paragraph 9.10.).

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

- 9.7. Calculate (IC **Ct**)<sub>av</sub> 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**)<sub>av</sub> should be ignored. Recalculate the (IC **Ct**)<sub>av</sub> for the remaining values after the screening.

- 9.8. The sample is considered **negative** (not containing *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA), if **Ct** value via **JOE** (*Yellow*) **and ROX** (*Orange*) channels for this sample is **above 40** or is not determined.

When IC **Ct** value for such sample differs from the (IC **Ct**)<sub>av</sub> 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.

- 9.9. The sample is considered **positive** (containing *Chlamydia trachomatis* DNA) when **Ct** value via **JOE** (*Yellow*) channel for this sample is **less than or equals to 40**.

The sample is considered **positive**, i.e. contains *Neisseria gonorrhoeae* DNA, when **Ct** value via **ROX** (*Orange*) channel for this sample is **less than or equals to 40**.

- 9.10. 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.

## RealLine *Chlamydia trachomatis* / *Neisseria gonorrhoeae* Fla-Format

For iQ™ iCycler, iQ™5 iCycler, CFX™96, DT-96:

9.11. For **PC** the program should detect:

- increase of the IC DNA amplification signal (channel **FAM**) and determine the threshold cycle, IC **Ct**;
- increase of the *Neisseria gonorrhoeae* DNA amplification signal (channel **ROX**) and determine the PC **Ct** value;
- increase of the *Chlamydia trachomatis* DNA amplification signal (channel **HEX** ) and determine the threshold cycle, PC **Ct**.

9.12. 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 **ROX** or **HEX** fluorescent increase should appear (*no Neisseria gonorrhoeae or Chlamydia trachomatis DNA amplification*).

When **Ct** value for NC through the **ROX channel is less than or equal to 40**, this indicates the presence of contamination (see paragraph 8.17).

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

9.14. Calculate (IC **Ct**)<sub>av</sub> 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**)<sub>av</sub> should be ignored. Recalculate the (IC **Ct**)<sub>av</sub> for the remaining values after the screening.

9.15. The sample is considered **negative** (not containing *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA), if **Ct** value via **ROX** channel for this sample is **above 40** or is not determined.

When IC **Ct** value for such sample differs from the (IC **Ct**)<sub>av</sub> 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.

9.16. The sample is considered **positive**, i.e. containing *Chlamydia trachomatis* DNA, when **Ct** value via **HEX** channel for this sample is **less than or equals to 40**.

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

9.17. In case of contamination all positive results of this individual PCR run are considered unreliable. 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.

## RealLine Chlamydia trachomatis / Neisseria gonorrhoeae Fla-Format

### 10. STORAGE AND TRANSPORTATION

- Store the assay kit at (2 – 8) °C in the manufacturer's packing.
- Transportation at 25 °C for up to 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 sample: 1 month at (2 – 8) °C.
  - Ready Master Mix (MM): unused MM at (2 – 8) °C for the entire shelf life of the kit.
  - Diluted MM: at (2 – 8) °C for 2 weeks.
  - Recovery Solution: at (2 – 8) °C for 3 months.

**For questions please contact: [techsupport@bioron.de](mailto:techsupport@bioron.de)**