

Instructions for Use

RealLine Trichomonas vaginalis Fla-Format

ASSAY KIT FOR THE QUALITATIVE DETECTION OF *TRICHOMONAS VAGINALIS* DNA BY
REAL TIME PCR METHOD









In vitro Diagnostics



RealLine Trichomonas vaginalis (Fla-Format)	VBD2096	100 Tests
valid from	September 2019	

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Explanation of symbols used in labeling

	<i>In vitro</i> diagnostic medical device
	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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Trademarks:

Rotor-Gene® is a registered trademark of Qiagen Group, Germany.

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QUALITATIVE ASSAY KIT FOR THE DETECTION OF *TRICHOMONAS VAGINALIS* DNA BY REAL TIME PCR METHOD

In vitro Diagnostics

1. INTENDED USE

Clinical information:

Trichomonas vaginalis - a flagellate - causes Trichomoniasis, a disease that causes vaginal infections in women and urethritis in men. The transmission of Trichomoniasis runs through sexual intercourse and is one of the most common sexually transmitted diseases.

RealLine *Trichomonas vaginalis* (Fla-Format) assay kit is designed to detect DNA of *Trichomonas vaginalis* 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)

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), RealLine Cycler (BIORON Diagnostics GmbH), DT96 (DNA-Technology, Russia) and rotor type cyclers Rotor-Gene® 3000, 6000 and Q (Qiagen, Germany)

For the Eco48™ Realtime PCR System (PCRmax, UK) 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**

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

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2. KIT CONTENTS

Universal Positive Control Sample (PC)	1 tube, 1 ml;
Master Mix (MM), lyophilized	10 tubes (10 tests each);
Recovery Solution (RS)	2 vials, 2 ml each;

3. PRINCIPLE OF THE METHOD

The 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 cycles: 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. IC indicates if PCR inhibitors occur in the reaction mixture. 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.

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4. SPECIFICATIONS

4.1. Sensitivity:

Sensitivity of the detection of *Trichomonas vaginalis* DNA in Standard Reference Samples containing 100 copies of *Trichomonas vaginalis* DNA per sample – equals 100%.

4.2. Specificity:

Specificity of *Trichomonas vaginalis* DNA detection using Standard Reference Panel of negative samples (DNA extracts) - equals 100 %.

4.3. Diagnostic sensitivity:

Diagnostic sensitivity of *Trichomonas vaginalis* DNA detection: clinical trials conducted on 40 positive samples showed 100% sensitivity (interval 93% -100%, with a confidence level of 90%);

4.4. Diagnostic specificity

Diagnostic specificity of *Trichomonas vaginalis* DNA detection: clinical trials conducted on 40 negative samples showed 100% specificity (93% -100% interval, with a 90% confidence level).

Analysis by the CE-marked reference kit showed full match of results.

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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.
- ☞ 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 Real Time amplification reaction with 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 expiration date at the side label of the kit.

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, RealLine Extraction 100
- Internal Control reagent (VBC8881) and Negative Control Sample, 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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7. PREPARATION OF THE SPECIMEN

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

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.

After initial opening shelf life of Positive Control sample (PC) at (2 – 8) °C is no more than 1 month or in 50µl aliquots at (-18..-24)°C for no more than 3 month.

8. PROCEDURE

8.1. 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 no more than 3 month

To prepare recovered Master Mix, add **300 µl** of Recovery Solution (RS) to each tube with MM. Mix gently, hold at room temperature for 15 minutes, and then carefully re-mix.

Store recovered MM at (2 - 8) °C for not more than 7 days.

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

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8.2. Prepare an appropriate number of 0.2 ml tubes or a plate for PCR. Label each tube for each specimen and control.

Attention! Labels should be placed on the caps of tubes for Rotor-type cyclers and for block-type devices labels should be placed on the lateral side of the tubes.

8.3. Add **25 µl** of diluted Master Mix to each 0.2 ml tube.

8.4. 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 or the plate.

8.5. Place the tubes into the Real Time PCR system.

8.6. Program the real-time PCR cycler as follows:

For Rotor-Gene® 3000 (6000, Q):

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

* Measure the fluorescence at 60°C

For iQ™ iCycler, iQ5™ iCycler, CFX96™, DT-96 and RealLine Cycler:

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 data through **FAM** channel (iQ5 iCycler, CFX96, DT-96, Rotor-Gene 3000) and **Green** channel (Rotor-Gene 6000, Rotor-Gene Q) for the detection of amplification signal of IC DNA;
- Collect data through **ROX** channel (iQ5 iCycler, CFX96, DT-96, Rotor-Gene 3000), and **Orange** channel (Rotor-Gene 6000, Rotor-Gene Q) for the detection of amplification signal of *Trichomonas vaginalis* DNA;

8.8. Program the position of the tubes with the specimens, PC and NC according to the Instruction Manual for the cycler in use.

8.9. Run the program.

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9. DATA ANALYSIS AND INTERPRETATION

- 9.1. 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 *Trichomonas vaginalis* DNA amplification signal along channel **ROX** (*Orange*) and determine the **Ct** value;
- 9.2. 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*) fluorescent increase should appear (*no Trichomonas vaginalis DNA amplification*).

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

- 9.3. For each sample the program should detect the increase of the amplification signal of IC DNA along channel **FAM** (*Green*) and determine IC **Ct**.
- 9.4. 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.
- 9.5. The sample is considered **negative** (not containing *Trichomonas vaginalis* DNA), if **Ct** value via **ROX** (*Orange*) 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.

- 9.6. The sample is considered **positive** (containing *Trichomonas vaginalis* DNA) when **Ct** value via **ROX** (*Orange*) channel for this sample is **less than or equals to 40**.
- 9.7. If **Ct** value for NC through **ROX** (*Orange*) channels is **less than or equal to 40**, this indicates the presence of contamination. In this case, 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 determined as positive. Samples that showed negative results in this run should be considered as negative.

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10. STORAGE AND TRANSPORTATION

- Store and transport the assay kit at (2 – 8) °C in the manufacturer’s packing.
- Transport at (2 – 8) °C . Transportation at up to 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, or in aliquots at (-18...-24) °C for 3 month.
 - Ready Master Mix (MM): unused MM at (2 – 8) °C for no more than 3 month
 - Diluted MM: at (2 – 8) °C for 7days.
 - Recovery Solution: at (2 – 8) °C for 3 months.

Technical support: techsupport@bioron.de

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.

ANNEX II: Programming the device and analysis of results using Rotor-Gene cyclers:

Rotor-Gene 3000, Rotor-Gene 6000 (Corbett research, Australia), Rotor-Gene Q (Qiagen, Germany)

Hereinafter, detection channels and terms corresponding to different versions of devices and software are listed in the following order: Rotor-Gene 3000 (Rotor-Gene 6000, Rotor-Gene Q).

Program Real Time PCR system as follows:

1. Click **“New”** button.
2. Select an **“Advanced”** template from the tab of the New Run wizard. Click **“New”** button.
3. Select **36-Well Rotor** type, check that No Domed 0.2 ml Tubes are used. Click **“Next”** button.
4. In the new window, determine Reaction volume as **50 µl**. Click **“Next”** button.
5. The temperature profile of real time PCR should be set. Click **“Edit Profile”** button.

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

* Measure the fluorescence at 60°C

6. Then temperature profile is set, click **“OK”** button.

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7. In the “**New Run Wizard**” window click “**Calibrate**” (“**Gain optimization**”) 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**”.
8. Tick off “**Perform Calibration Before 1st Acquisition**”. Click “**Close**” button.
9. Click “**Next**” button, start the amplification process by clicking “**Start Run**” button.
10. Save a file in the Rotor-Gene/templates folder, named RealLine with *.ret extension. In subsequent work RealBest template would be presented in New run wizard.
11. Save reaction result file with Rotor-Gene Run File *.rex extension.
12. Record the positions of the controls and specimens according to the instruction manual of the operating device. Click “**Start run**” button.

Results for Internal Control DNA amplification

1. Click **Analysis** button, choose **Quantitation** from the list, choose **Cycling A. FAM** (*Cycling A. Green*), click **Show** button.
2. Click **OK** button, and cancel automatic **Threshold** determination.
3. Click **Linear scale** button. Settings should change to **Log. scale**.
4. In the **Quantitation analysis** menu buttons **Dynamic tube** and **Slope Correct** should be pressed.
5. Click **More Settings** (*Outlier Removal*) button, determine **NTC threshold** value as 5 %.
6. In the column **CT Calculation** (*right part of the window*) determine **Threshold** value as **0.04**.
7. In the result table (**Quant. Results window**) **Ct** will be displayed.

Results for *Trichomonas vaginalis* DNA amplification

1. Click **Analysis** button, choose **Quantitation** from the list, choose **Cycling A. ROX** (*Cycling A. Orange*) click, **Show** button.
2. Click **OK** button, and cancel automatic **Threshold** determination.
3. Click **Linear scale** button. Settings should change to **Log. scale**.
4. In the **Quantitation analysis** menu buttons **Dynamic tube** and **Slope Correct** should be pressed.
5. Click **More Settings** (*Outlier Removal*) button, determine **NTC threshold** value as 5 %.
6. In the column **CT Calculation** (*right part of the window*) determine **Threshold** value as **0.04**.
7. In the result table (**Quant. Results window**) **Ct** will be displayed.

