Instructions for Use

RealLine Toxoplasma gondii
Str-Format

AN ASSAY KIT FOR THE QUALITATIVE DETECTION OF *TOXOPLASMA GONDII* DNA USING REAL TIME PCR METHOD

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

RealLine Toxoplasma gondii (Str-Format)	**VBD1798-R**	**48Tests**
valid from	**September 2019**	

RealLine Toxoplasma gondii Str-Format

Explanation of symbols used in labelling

RUO	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

BIORON Diagnostics GmbH

In den Rauhweiden 20
67354 Römerberg
Germany

Phone +49 6232 298 44 0
Fax: +49 6232 298 44 29
info@bioron.de

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

RealLine Toxoplasma gondii Str-Format

Table of content:

RealLine Toxoplasma gondii Str-Format

AN ASSAY KIT FOR THE QUALITATIVE DETECTION OF *TOXOPLASMA GONDII* DNA USING REAL TIME PCR METHOD

1. INTENDED USE

The protozoan *Toxoplasma gondii* cause a parasitic disease called Toxoplasmosis. It is estimated that between 30 % and 65 % of all people worldwide are infected with *T. gondii*. Early and definitive diagnosis is very important for recently infected pregnant women, to prevent infection of the fetus by appropriate treatment as well as for persons with severely weakened immune systems.

RealLine Toxoplasma gondii assay kit is designed to detect *Toxoplasma gondii* DNA isolated from specimens using extraction kits:
RealLine DNA-Extraction 2 (REF VBC8897)
RealLine DNA-Extraction 3 (REF VBC8889)
RealLine Extraction 100 (REF VBC8896)

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 validated for use with: iQ™5 iCycler (Bio-Rad, USA). The kit is compatible with real-time PCR systems such as iQ™ iCycler, CFX™96 (Bio-Rad, USA) and DT-96 (DNA-Technology, Russia) and RealLine Cycler (BIORON Diagnostics GmbH).

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.

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.

2. KIT CONTENTS

Positive Control sample *(*PC)	1 tube, 1 ml
Ready Master Mix for PCR (RMM), lyophilized	48 test-tubes (6 strips x 8 tubes));
The kit is additionally supplied with optical-transparent PCR-film.	

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-labelled DNA-probe, which specifically binds to the target region of pathogen DNA. Fluorescence 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.

RealLine Toxoplasma gondii Str-Format

4. SPECIFICATIONS

4.1. Sensitivity:

Sensitivity control was performed on 5 samples containing 100 *Toxoplasma gondii* DNA copies per sample, prepared from SRS (Standard Reference Sample containing *Toxoplasma gondii* DNA)
The sensitivity equals 100 %.

4.2. Specificity:

Specificity of *Toxoplasma gondii* DNA detection was determined using Standard Reference Panel of control sera samples not containing HBV DNA, *Borrelia burgdorferi sensu lato* DNA, *Toxoplasma gondii* DNA, *Helicobacter pylori* DNA, HCV RNA, HIV RNA, TBE RNA, *Rubella* RNA, Herpes virus infections DNA. Specificity of *Toxoplasma gondii* DNA detection equals 100 %.

4.3. Diagnostic evaluation:

Diagnostic evaluation was performed on 100 clinical samples:
40 samples, negative samples;
40 samples,positive samples containing *Toxoplasma gondii* DNA;
20 samples obtained from individuals infected with Varicella-zoster virus.

Determination of sensitivity was performed on 40 clinical samples obtained from the clinical material containing *Toxoplasma gondii* with a CE-marked Kit as reference kit. **RealLine Toxoplasma gondii (Fla-format)** kit determined all 40 samples as positive. Analysis by the reference kit proved all 40 samples containing *Toxoplasma gondii* to be positive.
Diagnostic sensitivity equals 100 %.

Determination of specificity was performed on 40 samples obtained from donors and 20 samples obtained from individuals infected with Varicella-zoster virus.
When studying clinical samples obtained from healthy donors by **RealLine Toxoplasma gondii (Fla-format)**, negative results were recorded for all 40 samples.
Analysis of similar samples by a CE-marked reference kit confirms the results obtained in all cases.

When studying clinical samples obtained from individuals infected with Varicella-zoster virus using **RealLine Toxoplasma gondii (Fla-format)** negative results were recorded for all 20 samples.
Analysis of similar samples by the reference kit confirms the results obtained in all cases.

Specificity equals 100 %.

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 box.

6. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- Real time PCR system, like described in paragraph 1
- DNA-Extraction Kit: **RealLine DNA-Extraction 3,** or **RealLine Extraction 100**
- Internal Control reagent (VBC8881) and Negative Control Sample , if the kit is used with the extraction kits of other suppliers
- Laminar safety box;
- Refrigerator;
- Microcentrifuge for 1.5 - 2 ml tubes;
- Vortex mixer with adjustable rotation speed;
- Half-automatic variable-volume single-channel pipettes with disposable tips;
- Disposable medical non-sterile powder-free gloves;
- Disposable pipette tips with filters;
- Biohazard waste container.
- Razor or scalpel.

RealLine Toxoplasma gondii Str-Format

7. PREPARATION OF THE ANALYSED 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 a kit of another supplier for the extraction of nucleic acids as recommended in p1, 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-Extraction 3, RealLine DNA-Extraction 2** 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 24 hours
After initial opening shelf life of Positive Control sample at (2 - 8) °C is 1 month or in 50 µl aliquots at minus (18 - 60) °C for up to 3 months.

8. PROCEDURE

8.1. Preparation of the reagents.

Prior the test take the kit out of the refrigerator and keep the Ready Master Mix (RMM) closed in the package at (18 – 25) °C for at least 30 minutes. Then open the package and cut the necessary number of tubes in strips with RMM (including prepared samples and controls) with the razor or scalpel. The cutted tubes hold 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 the shelf life of RMM at (2 - 8) °C is 3 months.

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

Attention! *Labels should be placed on the caps of tubes for rotor-type cyclers. For block-type cyclers labels should be placed on the lateral side of the tubes.*

8.3. Add **50 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 seal with the PCR transparent film.

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

8.5. Program real time PCR system.

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

* Measure the fluorescence at 60 °C

8.6. 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 ***Toxoplasma gondii*** **DNA.**

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

8.8. Run the program.

RealLine Toxoplasma gondii Str-Format

9. DATA ANALYSIS AND INTERPRETATION

9.1 The program should detect in **Positive Control** sample:

- Increase of the IC DNA amplification signal in channel **FAM** and determine the threshold cycle, IC **Ct**;
- Increase of the ***Toxoplasma*** DNA amplification signal in channel **ROX** and determine the **Ct** value;

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

9.3 For each sample the program should detect the increase of the amplification signal of IC DNA along channel **FAM** 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 cycles 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 *Toxoplasma* 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 required.

9.6 The sample is considered **positive** (containing *Toxoplasma* DNA) when **Ct** value via **ROX** channel for this sample is **less than or equals to 40.**

9.7 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.

10. STORAGE AND TRANSPORTATION

- Store the assay kit at (2 - 8) °C in the manufacturer's packing.
- Transport at (2 - 8) °C, 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 *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

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.

RealLine Toxoplasma gondii Str-Format

BIORON Diagnostics GmbH
info@bioron.de - www.bioron.de