Instructions for Use

RealLine DNA – Extraction 2

KIT FOR THE EXTRACTION OF DNA FROM CLINICAL SPECIMENS

In vitro diagnostics

CE

RealLine DNA-Extraction 2	**VBC8897**	**96 Tests**
valid from:	**November 2020**	

BIORON Diagnostics GmbH
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RealLine DNA - Extraction 2

Explanation of symbols used in labeling

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

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1. INTENDED USE

The **RealLine DNA-Extraction 2** kit is intended for DNA extraction from clinical specimens:

- blood serum (plasma),
- biopsy material,
- cerebrospinal fluid,
- urine,
- epithelial cells swabs.

The kit (VBC8897) contains reagents for the DNA extraction from 96 samples including control samples. Up to 4 independent extraction procedures can be performed using this kit, each for the extraction of DNA from 24 samples, including control samples.

The kit is validated for use with PCR kits of RealLine series designed for the detection of DNA of different infectious agents.

2. KIT COMPONENTS

Specimen preparation reagents:	
Lysis Reagent	4 vials, 8 ml each
Solution for NA Precipitation	4 vials 12 ml each
Wash Solution № 1	4 vials, 13 ml each
Wash Solution № 2	4 vials, 8 ml each
Specimen Diluent	4 vials, 15 ml each
Sorbent (suspension of magnetic particles)	2 vials, 1 ml each
Control samples:	
Recovery Solution for Controls (RSC)	2 vial, 4 ml each
Negative Control sample (NC)	2 vials, 2 ml each
Internal Control sample (IC), lyophilized	4 vials
The kit also includes plastic caps for vials with IC	4 caps

3. PRINCIPLE OF THE METHOD

The method of DNA extraction consists in the temperature treatment of specimens with pre-heated multicomponent Lysis Reagent followed by precipitation of nucleic acids to silica gel-covered magnetic particles, two washings, and elution. The samples are then ready for PCR.

Internal Control sample (IC) is used to monitor the possibility of false-negative results (due to DNA loss during extraction procedure or inhibitory effects of sample components). IC should be added to each specimen and control sample prior to the DNA extraction procedure.

RealLine PCR assay kits include **Positive Control sample (PC)**. PC must undergo the extraction procedure together with clinical specimens and Negative Control sample (NC).

4. SPECIFICATIONS

4.1. Inhibition control and extraction efficiency tests

Test for the absence of inhibition and efficiency of extraction is performed on four samples prepared from the positive sample of the Standard Reference Sample (SRS). The positive sample included in the SRS represents a lyophilized mix of epithelial cells material with a high concentration of *Chlamydia trachomatis* DNA diluted in a pool of epithelial cells swabs not containing *Chlamydia trachomatis* DNA.

The kit conforms to the required quality control specifications regarding lack of inhibition (IC is detected in all four samples, i.e. **IC Ct** through the “FAM” channel is less than or equal to 40).
The efficiency of *Chlamydia trachomatis* DNA extraction is determined on four samples prepared from the positive sample of the SRS as the percentage of samples determined using RMM (Ready Master Mix for PCR) for *Chlamydia trachomatis* DNA detection as positive. Efficiency equals 100 %.

4.2. Performance evaluation

Performance evaluation was carried out on 44 clinical specimens that were used for DNA extraction: 21 epithelial cells swabs from patients with laboratory signs of mycoplasmosis and 23 epithelial cells swabs from healthy persons.
DNA was extracted from the specimens using **RealLine DNA-Extraction 2** kit and a CE-marked reference extraction kit. The following detection of *Mycoplasma hominis* DNA in the extracted material was performed using a CE-marked PCR assay kit.

DNA extraction efficiency equals 100 % (interval 93.4 % – 100 %, with a confidence level of 90 %). **RealLine DNA-extraction 2** kit and the reference CE-marked extraction kit showed a full match of results.

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5. SAFETY 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
- Wear protective disposable gloves, laboratory coats, and eye protection when handling specimens and kit components
- 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.
- Use only disposable pipette tips with filters.
- Never use the same pipette tip for different samples.
- Do not pool reagents from different lots or from different vials of the same lot.
- Do not use the components from the kits of different lots in one experiment.
- Dispose of 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.
- To obtain reliable results, strictly follow this Instruction Manual provided with the kit.

6. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- Laminar safety box;
- Refrigerator;
- Eppendorf-type microcentrifuge with a maximum rotation speed of at least 13,000 rpm;
- Vortex-type tube shaker;
- Thermoshaker;
- Half-automatic variable-volume single-channel pipettes;
- Disposable medical non-sterile powder-free gloves;
- Disposable pipette tips with aerosol barrier;
- 2.0 ml polypropylene tubes, sterile;
- Magnetic rack for nucleic acids isolation;
- Biohazard waste container.

7. PREPARATION OF SPECIMENS

7.1. Preparation of blood serum and plasma specimens

Attention! *Do not use heparinized plasma for the analysis!*

Transfer serum (plasma) to a sterile polypropylene tube within 6 hours after blood collection.

Transportation and storage of specimens:

- At (18 – 26) °C – for no more than 6 hours;
- At (2 – 8) °C – for no more than 5 days;
- At minus 18 °C and below – for a long time.

Only one freeze-thaw cycle is allowed!

Prior to use, centrifuge serum (plasma) specimens at 13,000 rpm and (18–26) °C for 5 min.

7.2. Preparation of biopsy material

Add 250 µl of RealLineTransport solution (BIORON Diagnostics GmbH) to the tubes with biopsy material and perform the homogenizing procedure.

Transportation and storage of specimens:

- At (18 – 26) °C – for no more than 6 hours;
- At (2 – 8) °C – for no more than 3 days;
- At minus 20 °C – for no more than 1 week;
- At minus 70 °C – for a long time.

Only one freeze-thaw cycle is allowed!

7.3. Preparation of cerebrospinal fluid specimens

Cerebrospinal fluid specimens are ready for NA extraction.

Transportation and storage of specimens:

- At (18 – 26) °C – for no more than 2 hours;
- At (2 – 8) °C – for no more than 24 hours;
- At minus (18–60) °C – for no more than 2 weeks.

Only one freeze-thaw cycle is allowed!

7.4. Preparation of urine specimens

Collect the first portion of morning urine into a clean collection cup with a leakproof lid. Transfer 1.5 – 2 ml of urine into a new 2 ml tube, centrifuge at 3,000 rpm for 3 min. Carefully remove the supernatant without disturbing the pellet. Use the obtained urine cell pellet for NA extraction.

Transportation and storage of specimens:

- At (18 – 26) °C – for no more than 2 hours;
- At (2 – 8) °C – for no more than 24 hours;
- At minus 20 °C – for no more than 2 weeks.
- *Only one freeze-thaw cycle is allowed!*

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7.5. Preparation of epithelial cells swabs

Transfer the clinical material (epithelial cells swabs from the cervical canal, urethra, vagina, etc.) collected using disposable sterile probes into the tube with RealLine Transport solution (BIORON Diagnostics GmbH). Mix thoroughly, collect the residual liquid from the probe by pressing it to the tube walls. Discard the probe and tightly close the tube. Spin the tubes briefly to collect drops.

Transportation and storage of specimens:

- At (18 – 26) °C – for no more than 48 hours;
- At (2 – 8) °C – for no more than 2 weeks;
- At minus (18 – 60) °C – for no more than 2 months.

Only one freeze-thaw cycle is allowed!

8. PREPARATION OF KIT COMPONENTS

8.1. Prior to use, take the kit out of the refrigerator, open the package, and keep at (18 – 26) °C for at least 30 min.

8.2. Take the tube with PC out of the PCR assay kit of **RealLine** series.

8.3. Negative Control (NC) sample is ready to use.
After initial opened, NC should be stored at (2 - 8) °C for no more than 1 month.

8.4. Prior to use, warm Lysis Reagent at (50 - 60) °C to dissolve the precipitated material and mix the solution.

8.5. Mix the vial with Sorbent on vortex to a homogeneous suspension. Add **260 µl Sorbent suspension** into one vial with Lysis Reagent. Mix carefully.

8.6. Open a vial with Internal Control sample (IC) by removing the cap and rubber stopper. Place the cap and stopper in the disinfecting solution. Add **1 ml of Recovery Solution for Control samples (RSC)** to the vial with IC, tightly close the vial with a screw cap included in the kit. Carefully mix the contents of the vial, keep at (18 – 26) °C for 15 min, and thoroughly mix once again.
Store diluted IC at (2 – 8) °C for no more than 1 month.

8.7. Add **750 µl IC** solution into the vial with Lysis Reagent and Sorbent, mix thoroughly.

Attention! Once opened, any unused portion of Lysis Reagent should be discarded.

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9. EXTRACTION PROTOCOL

9.1. Prepare an appropriate number of reaction tubes needed for patient specimen, **PC** and **NC**.

9.2. Add **100 µl** of Negative Control sample to the tube labeled as **NC**.

9.3. Add **70 µl** of Negative Control sample and **30** µl of Positive Control sample (a component of the PCR assay kit) to the tube labeled as **PC.**

9.4. Add **100 µl** of each specimen to the remaining tubes using a new tip with filter for each specimen.

9.5. Add **300 µl** of Lysis Reagent with Sorbent and IC to each tube. Vortex the tubes for 10–15 sec. Incubate in a thermal shaker at 65 °C and 1,300 rpm for 5 min. Spin briefly to collect drops.

9.6. Add **400 µl** of Solution for NA Precipitation to each tube. Vortex the tubes for 10–15 sec. Centrifuge at 13,000 rpm for 5 min.

9.7. Without stirring the pellet, place the tubes into the magnetic rack. Using a new tip for each sample, carefully remove the supernatant without disturbing the pellet.

9.8. Add **500 µl** of Wash Solution No. 1 to each tube. Vortex the tubes for 10–15 sec. Centrifuge at 13,000 rpm for 2 min.

9.9. Without stirring the pellet, place the tubes into the magnetic rack. Using a new tip for each sample, carefully remove the supernatant without disturbing the pellet.

9.10. Add **300 µl** of Wash Solution No. 2 to each tube. Vortex the tubes for 10–15 sec. Centrifuge at 13,000 rpm for 2 min.

9.11. Without stirring the pellet, place the tubes into the magnetic rack. Using a new tip for each sample, carefully remove the supernatant without disturbing the pellets.

9.12. Dry the pellets in open tubes for 2–3 min at (18–26) °C.

9.13. Add **600 µl** of Specimen Diluent to each tube. Vortex thoroughly to resuspend the pellet. Incubate in a thermal shaker at 65 °C and 1,300 rpm for 5 min. Centrifuge at 13,000 rpm for 1 min.

The samples are ready for PCR.

Attention! *Store the extracted DNA at (2 – 8) °C for no more than 24 hours*.

10. STORAGE AND TRANSPORTATION

- Transport and store the kit at (2 - 8) °C in the manufacturer's packing.
- Transportation up to 26 °C for up to 10 days is allowed.
- Do not freeze the kit!
- Note the expiry date on the labels.
- 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.

- **After first usage please be aware of the expiry date of the single components of the kit:**
  Store diluted IC at (2 - 8) °C up to 1 month.
  Once opened, NC should be stored at (2 - 8) °C and used within 1 month.
  Once opened and prepared the Lysis buffer must be discarded.

Technical Support: techsupport@bioron.de

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