

Instructions for Use

RealLine DNA - Express

EXTRACTION KIT FOR NUCLEIC ACIDS (DNA) FROM CLINICAL SPECIMENS LIKE EPITHELIAL CELLS, SALIVA, URINE AND SPERM










In vitro Diagnostics



RealLine DNA – Express	VBC8899	100 Tests
valid from:	September 2019	

RealLine DNA – Express

Explanation of symbols used in labeling

	<i>In vitro</i> diagnostic medical device
	Batch code
	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

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EXTRACTION KIT FOR NUCLEIC ACIDS (DNA) FROM CLINICAL SPECIMENS LIKE EPITHELIAL CELLS, SALIVA, URINE AND SPERM

In vitro Diagnostics

1. INTENDED USE

The **RealLine DNA-Express** kit is intended for transportation of specimens and DNA extraction from human semen, saliva, urine as well as from epithelial cells swabs of mucosa of the cervical canal, urethra, vagina, oropharynx and conjunctiva.

RealLine DNA-Express kit is intended for use in clinical practice in the process of diagnostics of different human infections using polymerase chain reaction (PCR) method.

The VBC8899 kit contains reagents required for DNA extraction from 100 specimens including controls.

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

2. KIT COMPONENTS

Transportation Solution	100 Tubes, 300 µl each;
Lysis Reagent with IC	100 Tubes, 500 µl each;
Negative Control sample (NC)	2 Vials, 1 ml each;

3. PRINCIPLE OF THE METHOD

RealLine DNA-Express kit contains the transport solution and the Lysis Reagent.

- The transport solution is designed for sampling and transportation of biological specimens.
- Lysis Reagent is a multicomponent solution containing the Internal Control sample (IC).

Once the biological specimen is in the transport solution and delivered to the laboratory, aliquots have to be transferred into a test tube with Lysis Reagent. Incubation of the samples at 98 °C in Lysis Reagent results in cell lysis and release of DNA into solution. Then the sample is ready for PCR test runs.

The extraction of nucleic acids from the specimens is performed together with preliminarily added **Internal Control sample (IC)** with subsequent amplification of selected DNA fragments and the detection of PCR products in real-time.

The use of **IC** prevents generation of false negative results associated with possible loss of DNA template during specimen preparation and indicates whether PCR inhibitors are present in the

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reaction mixture. IC should be added to each specimen (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.

The PCR assay kits of the “RealLine” series include a Positive Control sample (PC). PC must undergo the extraction procedure together with biological specimens and Negative Control sample (NC).

4. SPECIFICATIONS

- 4.1. The test for the absence of inhibition was performed on four negative samples from the Standard Reference Sample (SRS). The kit conformed to required quality specifications on criteria of lack of inhibition.
- 4.2. Efficiency of *Mycoplasma hominis* DNA extraction equals 100%. Efficiency is estimated as a percentage of samples identified by the kit as positive. Efficiency of extraction is determined on four samples containing 100 copies of *Mycoplasma hominis* DNA per sample, prepared from the positive sera of the Standard Reference Sample.
- 4.3. Efficiency of DNA extraction performed on 95 clinical specimens (epithelial cells swabs of the urogenital tract, oropharynx, conjunctiva, and specimens of semen, saliva and urine) equals 100% (range 97% -100%, with 90% confidence level).
- 4.4. Performance evaluation was performed on 40 clinical samples that were used for DNA extraction:
 - 20 epithelial cells samples from patients with diagnosed infection of *Ureaplasma urealyticum* or *Ureaplasma parvum*;
 - 20 samples from healthy individuals.

The test was performed on 20 clinical samples obtained from the epithelial cells material containing *Ureaplasma urealyticum* or *Ureaplasma parvum* with a CE-marked reference kit. The following PCR detection of extracted material was performed using a CE-marked kit for the detection of *Ureaplasma parvum/Ureaplasma urealyticum*.

The results confirmed that RealLine DNA-Express kit and the CE-marked reference kit show total agreement in 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 disposable latex gloves when working with the kit, since all human biological material should be treated as potentially infectious.
- ☞ Avoid microbial and nuclease contamination of reagents when removing aliquots from reagent vials. The use of sterile disposable pipettes and pipette tips is recommended.
- ☞ 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.
- ☞ Treat all disposable materials with disinfectant before utilization

6. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- Laminar safety box;
- Refrigerator;
- Eppendorf-type microcentrifuge with a maximum rotation speed of at least 8,000 rpm;
- Vortex-type tube shaker;
- Half-automatic variable-volume single-channel pipettes;
- Disposable medical non-sterile powder-free gloves;
- Disposable pipette tips with aerosol barrier;
- 1.5 ml tube racks;
- biohazard waste container

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7. PREPARATION OF SPECIMENS

7.1. Preparation of epithelial cell swabs

Transfer clinical material collected using disposable sterile probe to a tube with a transport solution. Mix thoroughly, collect residual liquid from the probe by pressing on tubes walls, remove the probe to the waste container and close the tube tightly. The specimen is ready for the extraction procedure (p. 9.1).

Deliver unfrozen samples to the laboratory for testing in adapted container within 3 to 4 hours. Samples must be transported following local and national instructions for the transport of pathogen material.

Transportation and storage of specimens:

- At (18 - 25) °C – for no more than 48 hours;
- At (2 - 8) °C – for no more than 2 weeks;
- Frozen at - (18 - 60) °C – for no more than 2 months.

Do not freeze – thaw samples repeatedly!

7.2. Preparation of semen and saliva specimens:

Place 300 µl of investigated material (semen or saliva) into the tube with transport solution, mix thoroughly on vortex for 10 sec. The sample is ready for the extraction process (p.9.1).

Transportation and storage of specimens:

- At (18 - 25) °C – for no more than 48 hours;
- At (2 - 8) °C – for no more than 2 weeks;
- Frozen at - (18 - 60) °C – for no more than 2 months.

Do not freeze – thaw samples repeatedly!

7.3. Preparation of urine samples

Collect the first portion of morning urine in a clean disposal collection cup with leakproof lid.

- 7.3.1. Take the necessary number of tubes with transport and Lysis solutions (according the number of urine samples). Mark each tube with transport solution.
- 7.3.2. Gently mix the urine in the containers.
- 7.3.3. Transfer 1.0 ml of urine into a tube with transport solution using disposable tips with filters for each specimen.
- 7.3.4. Close the tubes tightly and vortex for 10 sec.
- 7.3.5. Centrifuge at 8000 rpm for 5 min at (18 – 25) °C
- 7.3.6. Carefully remove the supernatant without touching the pellet.

The pellet can be stored like described below or directly used as follows. After storage start here with the next step 7.3.7.

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Note: *If a large salt precipitate was obtained, it is recommended to repeat the washing procedure with 500 µl of RealLine Transportation Solution (VBC8885), also repeat the centrifugation and remove the supernatant; procedures as described above.*

7.3.7. Add 500 µl of Lysis reagent to the resulting cell pellet.

7.3.8. Vortex thoroughly for 10 sec. The sample is ready for thermal treatment, see p.9.7.

Transportation and storage of samples of urine cell pellet:

- up to 2 hours at (18 - 25) °C;
- up to 24 hours at (2 - 8) °C; *
- up to 2 weeks at - (18 - 60) °C.

* optional urine can be stored in the Transport Solution up to 1 week at (2 - 8) °C, this is also recommended. But inhibitors of the PCR can be active. After storage at (2 - 8) °C or less than 0 °C the precipitation of salts can be formed and this should be washed out like described to optimize the PCR reaction.

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8. PREPARATION OF REAGENTS

- 8.1** Prior to work, take the kit out of the refrigerator and keep at (18 – 25) °C for at least 30 min
- 8.2** Prepare a vial with Positive Control (PC) sample (a component of the RealLine PCR kit) according to PCR kit instruction manual.

Note: Kits for the detection of DNA from STI pathogens or Herpes virus infections contain the **Universal Positive Control Sample**. This control contains a plasmid with all corresponding DNA sequences. If different pathogens are detected in parallel, only one **PC** has to be created. Please note the label of the positive control tubes. If you have any questions, please contact techsupport@bioron.de

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9. EXTRACTION PROTOCOL

Attention! For urine samples start the extraction protocol at p.9.7.

- 9.1 Prepare and label the appropriate number of test tubes with Lysis Reagent needed for patient specimen and control samples.
- 9.2 Spin the tubes with specimen briefly to collect any drops
- 9.3 Resuspend cell pellet formed by centrifugation. Add **100 µl** of each specimen into a tube with Lysis Reagent using disposable tips with aerosol barrier.
- 9.4 Add 100 µl of Negative Control to the tube labeled NC.
- 9.5 Add 100 µl of Positive Control to the tube labeled PC.
- 9.6 Close the tubes tightly. Vortex for 10 sec.
- 9.7 Place the tubes into a pre-heated thermal shaker or thermal block and incubate for **15 minutes at 98 °C**.
- 9.8 After heating cool the tubes down to room temperature, then centrifuge at 8000 rpm at (15 – 25)°C for 5 min.
- 9.9 Use the resulting supernatant as a DNA-containing sample suitable for further PCR analysis.

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

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10. STORAGE AND TRANSPORTATION

- Store the kit at (2 - 8) °C in the manufacturer's packing.
- Transport the kit 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.
- The kit is intended for use with PCR kits of RealLine series only!
- Do not use kits with damaged inner packages and get in contact with BIORON Diagnostics GmbH.

For questions: techsupport@bioron.de

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