RealLine Prep NA-S
DNA/RNA Extraction Kit

Instructions for Use

RealLine Prep NA-S

DNA/RNA EXTRACTION KIT FROM BIOLOGICAL MATERIAL

Research use only

RealLine Prep NA-S	**BI1007**	**100 Tests**
valid from	**July 2021**	

RealLine Prep NA-S DNA/RNA Extraction Kit

Explanation of symbols used in labeling:

RUO	For 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

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RealLine Prep NA-S DNA/RNA Extraction Kit

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EXTRACTION KIT FOR DNA AND RNA FROM BIOLOGICAL MATERIAL

1. INTENDED USE

The **RealLine Prep NA-S DNA/RNA Extraction Kit** is intended for a fast **NA** extraction from biological materials for further analysis by RT-PCR (PCR with Reverse Transcription)/PCR (polymerase chain reaction).

The **RealLine Prep NA-S Kit** is designed to extract NA from biological material nasopharyngeal and oropharyngeal swabs.

Indications for the use: the kit is an auxiliary agent for in vitro diagnostics (nucleic acids extraction for further RT-PCR/PCR analysis) in clinical and diagnostic laboratory.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use the **RealLine Prep NA-S** DNA/RNA Extraction Kit.

The **RealLine Prep NA-S** DNA/RNA Extraction Kit can be used in clinical and diagnostic laboratories of medical institutions and research practice.

Potential users: personnel qualified in molecular diagnostics methods and working in the clinical and diagnostic laboratory.

It is necessary to apply the kit only as directed in this user manual.

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2. KIT COMPONENTS

Reagent	Description	Amount
Lysis Buffer	Slightly foamy light blue or colorless transparent liquid	1 vial, 30 ml
Precipitation buffer	Colorless transparent liquid	1 vial, 40 ml
Washout Solution	Colorless transparent liquid	1 vial, 50 ml
Dilution buffer	Colorless transparent liquid	1 vial, 5 ml
Negative Control NC	Colorless transparent liquid	2 vials, 1.5 ml

All components are ready to use and do not require additional preparation for operation.

The kit is intended for single use and designed for DNA/RNA extraction from 100 analyzed samples (including negative controls).

3. METHOD

The **RealLine Prep-NA-S DNA/RNA Extraction Kit** offers a fast DNA/RNA extraction method. The method is based on the release of nucleic acids under the action of a chaotropic agent (guanidine thiocyanate) with subsequent precipitation and clearing from impurities.

The RealLine Prep-NA-S DNA/RNA Extraction Kit can be used in conjunction with medical devices designed for the analysis of nucleic acids by RT-PCR/PCR. The obtaining NA preparation is ready for further analysis with RT-PCR/PCR.

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4. WARNINGS AND SAFETY PRECAUTIONS

Only personnel trained in the methods of molecular diagnostics and the rules of work in the clinical and diagnostic laboratory are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Use powder-free surgical gloves. Use protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121ºC before disposal.

All the liquid solutions are designed for single use and cannot be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work. Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

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Emergency actions

- **Eye Contact:** If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, of I-IV classes of pathogenicity, obtain medical attention.
- **Skin Contact:** If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.
- **Ingestion:** If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

Do not use the kit:

- If the transportation and storage conditions are breached;
- If the reagents' appearance does not respond to the kit passport;
- If the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are NOT anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

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5. MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

Specimen collection

- Specimen collection: sterile single use swabs, cotton swabs etc. for sampling of biomaterial;
- Use specimen transport medium: RealLine Transport Solution or equivalent for the transportation of the sample.

NA extraction

- Biological safety cabinet class II-III;
- Vortex mixer;
- Refrigerator;
- High speed centrifuge (RCF 16000[*] x g);
- Solid-state thermostat that supports temperatures from 65 °C;
- Single channel pipettes (dispensers covering 20-1000 µl volume range);
- RNase and DNase free filtered pipette tips (volume 20 µl, 200 µl, 1000 µl);
- Electric pump with a trapping flask for supernatant removal;
- RNase and DNase free non-filtered pipette tips for pump with a trapping flask;
- 1.5 ml RNase and DNase free micro-centrifuge tubes (Eppendorf Safe-Lock Tubes are recommended);
- PCR tube rack for 1.5 ml tubes;
- Powder-free surgical gloves;
- Disinfectant solution;
- Container for used pipette tips, tubes and other consumables;
- Transport media for samples;
- Physiological saline solution 0.9 % NaCl Sterile.

*[*1] if used in conjunction with the **RealLine SARS-CoV-2 Detection Kit (REF BI1019-96, BI1020-96)** and **RealLine SARS-CoV-2 / Influenza A+B (REF BI1029-96, BI1030-96) from BIORON Diagnostics GmbH** a centrifuge with RCF no less than 12 000 x g is allowed*

6. SAMPLE COLLECTION AND PREPARATION OF SPECIMENS

The **RealLine Prep NA-S DNA/RNA Extraction Kit** is designed to extract DNA/RNA from nasopharyngeal and oropharyngeal swabs.

General recommendations

- Use DNAse and RNAse free filter tips;
- Do not touch the tube walls while adding a liquid in the tube. If touching the wall, change the tip. Change the tip each time while removing a liquid from the tube;
- Open only the cap of the tube which is in work, then close the tube before proceeding to the next tube to prevent contamination.

Sample collection

Swabs from the nasal cavity sampling

Take the swab (mucus) using dry sterile disposable cotton sponge with plastic basis. Insert the sponge carefully along the outer wall of the nose to a depth of 2-3 cm to the lower shell. Then lower the sponge down slightly, insert into the lower nasal passage under the lower nasal conch, after a rotational movement remove along the outer wall of the nose. Transfer the sponge with the biomaterial (work part of the probe with cotton sponge) into a disposable sterile test tube with transport medium, rinse the sponge thoroughly, avoiding splashing of the liquid. Then remove the sponge from the solution and, by rotating it against the wall of the test tube above the level of the solution, squeeze out the excess liquid. Dispose the used sponge, close the test tube and mark.

Swabs from the oropharynx sampling

Take the swab using dry sterile disposable cotton sponge with plastic basis by rotate moving from tonsils surface, faucial pillars and back oropharyngeal wall. Transfer the sponge with the biomaterial (work part of the probe with cotton sponge) into a disposable sterile test tube with transport medium, rinse the sponge thoroughly, avoiding splashing of the liquid. Then remove the sponge from the solution and, by rotating it against the wall of the test tube above the level of the solution, squeeze out the excess liquid. Dispose the used sponge, close the test tube and mark.

Transportation and storage of the samples

Samples may be transported and stored in physiological saline at temperatures from (2 – 8) °C for no more than 24 hours prior to analysis. When it is impossible to deliver the material in the laboratory during the day, a one-time freezing of the material is allowed. The frozen material is allowed to be stored at temperatures from -18 to -22 °C for no longer than 3 months.

In case of usage transport media biological material samples are transported and stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

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7. PROCEDURE

- The lysis buffer can form the precipitate. Dissolve it at 65 °C for 10 min prior to use.
- Always open the tube that you are working with, and close it after handling. It is not allowed to work simultaneously with several tubes with open caps.
- When performing centrifugation, always place the tubes in the centrifuge in the same orientation for easy visualization of the precipitate.
- After centrifugation, remove supernatant by gradually dipping the tip into the liquid (i.e. lowering it as the liquid is removed). To avoid loss of precipitate with NA, it is necessary to remove supernatant, leaning the tip to the opposite wall of the tube from the precipitate.
- When using a solid-state thermostat with a pressure lid during incubation of tubes, the thermostat lid must be open to avoid overheating of the tubes and their spontaneous opening when removing the tubes from the thermostat.
- Simultaneously with the extraction of NA, a negative control sample should go through all stages of NA extraction.
- For sample preparation and extraction use DNAse and RNAse free filter tips (without filter for electric pump).
- Change the tip each time while removing, transferring or adding liquid into the tube. Do not touch the tube walls while adding liquid in the tube. If touching the wall, change the tip.
- Proceed the tubes with samples and negative control equally.

7.1 Mark the required number of 1.5 ml tubes considering the number of samples to be tested and negative control (NC).

When used in conjunction with the kits for the analysis of nucleic acids by RT-PCR/PCR, for which the use of internal control sample is provided, at this stage it should be added into the tubes (in the amount according to the instructions for use for the corresponding kit).

If used in conjunction with the **RealLine SARS-CoV-2 Kit** and **RealLine SARS-CoV-2/ Influenza A+B kit (BIORON Diagnostics GmbH)**, add 10 µl of the corresponding pre-vortexed internal control (RNA-IC) from the respective kits into the each tube.

7.2 Add **300 µl** of L**ysis Buffer** in each tube without touching the tube's walls. Close the tubes.

7.3 Add **100 µl** of prepared samples into the marked tubes. Do not add samples to the Positive Control PC tube.

7.4 Add **100 µl of transport medium** or sterile physiological saline solution in the PC- tube. Close the tubes tightly, vortex on vortex-microcentrifuge for 3-5 sec.

7.5 Incubate the tubes for 5 min at 65 °C.

7.6 Spin down the drops by centrifuging on vortex-microcentrifuge for 1-3 sec.

7.7 Add **400 µl Precipitation Buffer** into the each tube.

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7.8 Vortex the tubes on vortex-microcentrifuge for 3–5 sec.

7.9 Centrifuge the tubes at 12000** - 16000 x g for 5 min.

7.10 Remove the supernatant fully. Use a new tip for each tube. Avoid touching the precipitate.

7.11 Add **500 µl Washout Solution** to the precipitate.

The washout solution should be added into the tube slowly, avoiding splashing of liquid inside the tube.

7.12 Mix by inverting the tubes 3-5 times.

It is allowed that the liquid remains in the cone of the tube (the lower part of the tube with NA, visually - the precipitate), since the tube is inverted to remove the remnants of the previous solutions from the walls and the cap of the tube. If the precipitate has moved away from the wall/bottom of the tube, make sure that it is in the liquid before the next step.

7.13 Centrifuge the tubes at 12000**- 16000 x g for 1 min.

7.14 Remove the supernatant fully. Use a new tip for each tube. Avoid touching the precipitate.

Try to remove the liquid as completely as possible avoiding contact with the precipitate. It is allowed to leave a small amount of liquid in the tube (5.0-10 µl), if there is a danger of contact with the precipitate. At this stage, the precipitate can be on the back wall of the test tube, as well as on the bottom.

7.15 Add **50 µl Dilution Buffer** to the precipitate.

7.16 Spin down the drops by centrifuging on vortex-microcentrifuge for 1-3 sec.

7.17 Incubate the tubes at 65 °C for 5 min.

7.18 Vortex the tubes on vortex-microcentrifuge for 5-7 sec to distribute the nucleic acids evenly (visually - precipitate) in the liquid.

7.19 Centrifuge the tubes at 12000 - 16000 x g for 30 sec.

The NA preparation is ready for RT-PCR/PCR.

The resulting NA preparation must be used immediately for RT-PCR/PCR. If it is needed, the resulting RNA preparation can be stored at temperatures from (2 – 8) °C for no longer than 30 min, the resulting DNA preparation can be stored at temperatures from (2 – 8) °C for no longer than one day.

The resulting NA preparation must be stored at temperatures not above minus 18 °C for no longer than one month or at temperatures not above minus 68 °C for no longer than one year with a single defrost before RT-PCR/PCR.

*** if used in conjunction with the RealLine SARS-CoV-2 or RealLine SARS-CoV-2 / Influenza A+B kits (BIORON Diagnostics GmbH)*

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8. SPECIFICATIONS

a) The minimum amount of biomaterial for nucleic acids extraction is 100 µl.

b) Interfering substances

The maximum concentration of interfering substances that may be in biomaterial samples (nasopharyngeal and oropharyngeal swabs), which do not affect the reverse transcription and polymerase chain reaction: whole blood - 5% v/v, chlorhexidine (0.05% water solution) – 10% v/v, xylometazoline hydrochloride 0.1% - 10% v/v.

9. SHIPPING, STORAGE AND HANDLING REQUIREMENTS

- Expiry date – 12 months from the date of production.
- All components of the **RealLine Prep-NA-S DNA/RNA Extraction Kit** must be stored at temperatures from (2 – 8) °C and out of light over the storage period.
- The kit can be transported by all types of roofed transport at temperatures corresponding to storage conditions over the storage period or in thermal containers with ice packs at temperatures from (2 – 25) °C for no more than 5 days.
- Shelf-life of the kit following the first opening of the primary container: the components of the kit should be stored at temperatures from (2 – 8) °C and out of light during the storage period.
- The kit stored in under undue regime should not be used.
- An expired RealLine Prep-NA-S DNA/RNA Extraction Kit should not be used.
- We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.
- Do not use kits with damaged inner and outer packaging and get in contact with BIORON Diagnostics GmbH.

The conformity of the **RealLine Prep-NA-S DNA/RNA Extraction Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

If you face to any undescribed issues contact customer service department regarding quality issues with the kit:

techsupport@bioron.de

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SPACE FOR YOUR NOTES:

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