RealLine Prep NA for SARS-CoV-2

Instructions for Use

RealLine Prep NA for SARS-CoV-2

EXTRACTION OF RNA AND DNA FROM BIOLOGICAL MATERIAL

Research use only

RealLine Prep NA for SARS-CoV-2	**BI1010**	**100 Tests**
valid from	**April 2020**	

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

RealLine Prep NA for SARS-CoV-2

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

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

RealLine Prep NA for SARS-CoV-2

TABLE OF CONTENT:

1. INTENDED USE 4
2. KIT COMPONENTS 4
3. WARNINGS AND SAFETY PRECAUTIONS 5
4. MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED 6
5. SAMPLE COLLECTION AND PREPARATION OF SPECIMENS 7
6. NUCLEIC ACID EXTRACTION 10
7. SHIPPING, STORAGE AND HANDLING REQUIREMENTS 11

RealLine Prep NA for SARS-CoV-2

EXTRACTION KIT FOR DNA AND RNA FROM BIOLOGICAL MATERIAL

1. INTENDED USE

The **RealLine Prep NA** kit is intended for **DNA** and **RNA** purification from biological material:

- Epithelial scrapes from: posterior pharyngeal wall, urethra, cervical canal posterior vaginal vault
- Swabs and washouts from nasal and oropharyngeal cavities
- Material from dead and sick animals(swabs and washouts from trachea, nasal cavity, pharyngeal cavity, cloaca, faeces, internal organs) etc
- Blood plasma,
- Saliva,
- Phlegm,
- Milk,
- Urine,
- Sperm,
- Prostate fluid,
- Cerebrospinal fluid,

The **RealLine Prep NA** kit is intended to use with RealLine SARS-CoV-2 kit which is using the method of reverse transcription with subsequent polymerase chain reaction (RT-PCR).

The kit is designed for nucleic acids purification from 100 samples including controls.

In the **RealLine Prep NA** kit the total volume of purified DNA/RNA is 50 µl or 16.5 µl in case of only RNA detection.

2. KIT COMPONENTS

Lysis Buffer	1 vial, 30 ml
Precipitation buffer	1 vial, 40 ml
Washout Solution No.1	1 vial, 50 ml
Washout Solution No.2	1 vial, 30 ml
Dilution buffer	4 tubes, 1.25 ml each
Negative Control (NC)	2 tubes, 1.5 ml each

3. WARNINGS AND SAFETY PRECAUTIONS

- The laboratory makeup should comply the requirements regulating work with microorganisms of I-IV classes of pathogenicity.
- Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Any material coming in contact with the biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 ºC before disposal.
- Molecular biology procedures, such as nucleic acids extraction, reverse transcription, amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.
- Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapour/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Do not use the kit after the expiry date provided. 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.
- Significant health effects are NOT anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

RealLine Prep NA for SARS-CoV-2

4. MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- Laminar safety box;
- Refrigerator;
- Microcentrifuge;
- Vortex mixer;
- Thermal shaker (65 °C to 98 °C)
- 1.5 ml plastic tubes and racks
- Semi-automatic variable-volume single-channel pipettes: 20-200 µl and 200-1000 µl;
- Disposable filter tips for automatic pipettes labelled “RNase-free, DNase-free”;
- Disposable medical non-sterile powder-free gloves;
- Container for the discharge of waste tips, tubes and other consumables;;
- Biohazard waste container
- optional: pump with a trapping flask for supernatant removal;
- sterile saline (0.9% NaCl).

If isolating NA from phlegm:

Method 1:	Method 2:
- 10% trisodium phosphate x 12 H_2O;	mucolysin.
- 1M HCl solution;	
- 5% chloramines solution;	
- distilled water	

5. SAMPLE COLLECTION AND PREPARATION OF SPECIMENS

5.1. Blood plasma

Blood should be taken into 2.0 or 4.0 ml vacuum blood collection tubes (Vacuette for example), containing EDTA (ethylenediaminetetraacetic acid disodium salt) in final concentration of 2.0 mg/ml. The use of sodium citrate anticoagulant is allowed. Invert the tube 2-3 times to mix the blood with anticoagulant. The use of heparin anticoagulant is not allowed.

5.1.1. Centrifuge the tubes with blood (3000 rpm) for 20 min at the room temperature (18 – 25) °C.

5.1.2. Take the upper fraction (plasma) with a semi-automatic pipettes and put it into the new 1.5 ml tube.

Note: Time from peripheral blood sampling to obtaining plasma must not exceed 6 hours. Plasma storage at minus 20 °C for not longer than 3 months is accepted.

Mix plasma just before NA extraction.

5.2. Phlegm

5.2.1 Method 1

- Put approximately 500 µl of biological sample into sterile container and close it tightly.
- Add to the sample an equal volume of 10 % triple-substituted sodium phosphate x12 H_2O and mix intensively.
- Incubate the mixture for 18 – 24 hours at 37 °C, then neutralize with 1M HCl (down to pH 6.8 – 7.4).
- Centrifuge for 20 min at 1000 rpm.
- Take out the supernatant into the 5 % solution of chloramine for disinfection.
- Add 500 µl of distilled water to precipitate, mix by pipetting and put to the new 1.5 ml tube.
- Centrifuge the tube at 13000 rpm for 10 min.
- Remove the supernatant, leaving approximately 100 µl (precipitate+liquid fraction) in the tube.

5.2.2 Method 2

- Add mucolysin to the sampling container in the 5:1 ratio (5 parts of mucolysin to 1 part of phlegm), referring to container calibrations.
- Close the lid of the container, mix the content and incubate for 20 – 30 min at room temperature, shake the container every 2 - 3 min.

Storage of processed phlegm in a container is accepted for one day from (2 – 8) °C or for a long time at not above (- 16) °C (in case of repeated RNA/DNA extraction necessity).

RealLine Prep NA for SARS-CoV-2

5.3. Epithelial scrapes

5.3.1 Put an epithelial scrape (posterior pharyngeal wall, urethra, cervical canal, posterior vaginal vault) using sterile sample brush into the plastic 1.5 ml tube with transport medium (or alternatively with 500 µl of sterile buffered saline), mix thoroughly.

5.3.2 Remove the sample brush, pressing it to the tube wall and squeezing the excess of liquid. Close the tube tightly.

5.3.3 Centrifuge the tube at 13000 rpm for 10 min.

5.3.4 Remove the supernatant, leaving approximately 100 µl (precipitate+liquid fraction) in the tube.

Remove mucus with sterile cotton wool swab before taking scrape from cervical channel.

5.4. Urine

5.4.1 Take the portion (approximately 50 ml) of the first-void urine to sterile container and close it tightly.

5.4.2 Transfer 1.0 ml of the sample to the 1.5 ml tube.

5.4.3 Centrifuge the tube at 13000 rpm for 10 min.

5.4.4 Remove the supernatant completely.

5.4.5 Add 1.0 ml of sterile buffered saline to the precipitate.

5.4.6 Centrifuge the tube at 13000 rpm for 10 min.

5.4.7 Remove the supernatant, leaving approximately 100 µl (precipitate + liquid fraction) in the tube.

5.5. Saliva, cerebrospinal fluid, synovial fluid

5.5.1 Collect the saliva, cerebrospinal fluid, synovial fluid (approximately 500 µl) to the sterile container and close it tightly.

5.5.2 Transfer 500 µl of the sample to the 1.5 ml tube.

5.5.3 Centrifuge the tube at 13000 rpm for 10 min.

5.5.4 Remove the supernatant, leaving approximately 50 µl (precipitate + liquid fraction).

5.5.5 Add 500 µl of sterile buffered saline to the precipitate.

5.5.6 Centrifuge the tube at 13000 rpm for 10 min.

5.5.7 Remove the supernatant, leaving approximately 100 µl (precipitate + liquid fraction).

5.6. Sperm, prostate fluid

5.6.1 Put 20–30 µl of the liquid sample into the 1.5 ml tube with transport medium (or alternatively with 500 µl of sterile buffered saline), vortex the tubes for 5-10 sec.

5.6.2 Centrifuge the tube at 13000 rpm for 10 min.

5.6.3 Remove the supernatant, leaving approximately 100 µl (precipitate + liquid fraction) in the tube.

5.7. Milk

5.7.1 Collect the sample into the sterile container and close it tightly.

5.7.2 Mix thoroughly and put 1.0 ml of the sample into the 1.5 ml tube.

Milk collection period must not exceed 24 hours. Keep from (2 – 8) °C for the whole collection period.

5.8. Smear and washout

5.8.1 Centrifuge the tube with analyzed material at 13000 RPM for 10 min.

5.8.2 Remove supernatant leaving approximately 100 µl (pellet + liquid fraction).

5.9. Faeces

5.9.1 Put approximately 250 mg (µl) of faeces into the 1.5 ml tube with 1.0 ml of sterile buffered saline.

5.9.2 Vortex the tube for 5-10 sec.

5.9.3 Centrifuge the tube at 1000 rpm for 2-3 min.

5.9.4 Transfer 800–1000 µl liquid material to 1.5 ml plastic tube, centrifuge the tube at 13000 RPM for 10 min.

5.9.5 Remove the supernatant, leaving approximately 100 µl (precipitate+liquid fraction) in the tube.

5.10. Animal internal organs

5.10.1 Transfer ~250 mg analyzed material to 1.5 ml plastic tubes.

5.10.2 Add 1.0 ml sterile saline.

5.10.3 Vortex the tube for 3–5 sec, spin at 1000 RPM for 3–5 sec.

5.10.4 Remove supernatant.

5.10.5 The sample is ready for DNA/RNA extraction.

5.10.6 Transportation and storage of material being tested

Biological material samples must be transported and stored from (2 – 8) °C for not longer than 24 hours.

Obtained material storage at minus 20 °C for 3 months is accepted.

RealLine Prep NA for SARS-CoV-2

6. NUCLEIC ACID EXTRACTION

The lysis buffer can form the precipitate. Dissolve it at 65 °C for 10 min prior to use.

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

For pre-processed samples with obtaining pellet and supernatant (see p. 5: Phegm Method 1, Saliva, Cerebrospinal fluid, Urine, Sperm, Prostatic fluid, Smears and Washouts, Faeces) tubes with 100 µl of material prepared for testing must be marked.

6.2. Add internal control to each tube, if the use of ICs at the sample preparation stage is necessary and described in the IFUs of subsequent PCR detection

Add **300 µl of the lysis buffer** into the each tube avoiding contact of the pipette tip with an edge of the tube.

6.3. Add 100 µl of the sample into the marked tubes (except for sample tubes passed pre-processing to obtain a precipitate, see and NC tube).

6.4. Add 100 µl negative control NC to the tube marked NC. Close lids tightly, vortex for 3-5 sec.

6.5. Incubate the tubes for 15 min at 65 °C, spin down the drops at 13000 rpm for 30 sec.

6.6. Add 400 µl of the precipitation buffer. Close the tubes tightly and vortex them for 3–5 sec.

6.7. Centrifuge the tubes at 13000 rpm for 15 min.

6.8. Remove supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

6.9. Add **500 µl of the Washout solution №1** to the precipitate and mix by inverting the tube 3-5 times. Centrifuge the tubes at 13000 rpm for 5 min.

6.10. Remove supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

6.11. Add **300 µl of the Washout solution №2** to the precipitate and mix by inverting the tube 3-5 times.Centrifuge the tubes at 13000 rpm for 5 min.

6.12. Remove supernatant completely avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

6.13. Open the tubes and dry the precipitate at 65 °C for 5 min.

6.14. Add 50 µl dilution buffer or 16.5 µl dilution buffer for the use with the subsequent RealLine SARS-CoV-2 detection to pellet.

6.15. Spin the drops down by centrifuging for 1-3 sec.

6.16. Incubate the tubes for 10 min at 65 °C.

6.17. Centrifuge the tubes at 13000 RPM for 30 sec.

The DNA/RNA preparation is ready for reverse transcription or PCR.

The obtaining RNA preparation must be used immediately for reverse transcription reaction and cannot be stored. DNA preparation can be stored at minus 20 °C for 1 month or at minus 70 °C for not more than 1 year.

7. SHIPPING, STORAGE AND HANDLING REQUIREMENTS

- The reagent kit must be stored at temperatures between (2 – 8) °C during the storage period.
- Transportation of DNA/RNA Extraction Kit can be held by all types of roofed transport at the temperatures between (2 – 8) °C during the entire shelf-life.
- Shelf-life – 12 months from the date of Quality Control Department approval in compliance with all transportation, storage and operation conditions.

Technical support: techsupport@bioron.de

RealLine Prep NA for SARS-CoV-2

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