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INSTRUCTIONS FOR USE

Reagent kit for isolation of viral, bacterial and fungal DNA and viral RNA from human clinical material with single wash "NA-Extra-SW"

TS 21.20.23-040-97638376-2021

Version 3 dated September 6, 2023

Content

Introduction

Target analyte

NA-Extra-SW reagent kit is used as an auxiliary instrument for in vitro diagnostics. NA-Extra-SW reagent kit is designed to isolate viral, bacterial and fungal DNA from human clinical material (nasopharyngeal swabs, oropharyngeal swabs, vaginal swabs, cervical smears, urethral smears, first-void urine, prostate secretions) and viral RNA from human clinical material (nasopharyngeal swabs, oropharyngeal swabs) for subsequent testing in clinical laboratory diagnostics by reverse transcription (RNA), polymerase chain reaction (DNA) in the diagnosis of respiratory and sexually transmitted infections.

Scientific validity

DNA/RNA isolation is an important step in sample preparation. Many techniques, such as amplification, reverse transcription, amplification products accumulation detection by real-time PCR, etc., cannot be performed directly on clinical samples without prior nucleic acid purification.

NA-Extra-SW reagent kit is used as an auxiliary instrument for in vitro diagnostics in performing genetic testing, which is used for medical purposes such as upper respiratory tract diseases diagnostics, viral and bacterial infectious diseases detection and sexually transmitted diseases diagnostics.

The scope of the reagent kit is clinical laboratory diagnostics. NA-Extra-SW reagent is as an auxiliary instrument for in vitro diagnostics.

Indications and contraindications for use

Indications for use: NA-Extra-SW reagent kit is recommended for use as an auxiliary instrument for in vitro diagnostics. Total isolated DNA/RNA is suitable for reverse transcription (RNA) and polymerase chain reaction (DNA) tests in the respiratory and sexually transmitted infections diagnostics.

Contraindications for use: no contraindications were identified if used by specially trained personnel and taking into account the intended use.

Population, demographic aspects of the medical device use: no population, demographic aspects of the NA-Extra-SW reagent kit use were identified.

Sterility: the product is not sterile.

1. Intended use

Intended use: NA-Extra-SW reagent kit is designed to isolate viral, bacterial and fungal DNA from human clinical material (nasopharyngeal swabs, oropharyngeal swabs, vaginal smears, cervical smears, urethral smears, first-void urine, prostate secretion) and viral RNA from human clinical material (nasopharyngeal swabs, oropharyngeal swabs) by a method based on the nucleic acids reversible binding to the magnetic bead surface with single wash, for subsequent testing in clinical laboratory diagnostics by reverse transcription (RNA), polymerase chain reaction (DNA) in the respiratory and sexually transmitted infections diagnostics. The reagent kit is as an auxiliary instrument for in vitro diagnostics.

Functional purpose: an auxiliary instrument for in vitro diagnostics. DNA/RNA isolated from human clinical material is not a basis for diagnosis, but it can be used for subsequent testing in clinical laboratory diagnostics by reverse transcription (RNA) and polymerase chain reaction (DNA), particularly for respiratory and sexually transmitted infections diagnostics.

For example, the following medical devices can be used together for subsequent isolated DNA/RNA testing:

- A reagent kit for qualitative detection of coronavirus RNA (SARS-CoV-*2*) by real-time RT-PCR method “CoV-2-Test” according to TS 21.20.23-015-97638376-2020, manufactured by TestGene LLC (registration certificate No. RZN 2020/10364 dated May 15, 2020);
- A reagent kit for RNA detection of SARS-CoV-2 virus, influenza A and B viruses by RT-PCR-RT “CoV-Influ-test”, manufactured by TestGene LLC (registration certificate No. RZN 2022/18297 dated September 23, 2022);
- A reagent kit for Mycoplasma pneumoniae and Chlamydophila pneumoniae DNA detection in biological material by polymerase chain reaction (PCR) with hybridization-fluorescence detection “AmpliSens® Mycoplasma pneumoniae/Chlamydophila pneumoniae-FL” according to TS 9398-176-01897593-2012, manufactured by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor (registration certificate no. FSR 2012/13957 dated February 27, 2019),

- Candida albicans Real-Time PCR Detection Kit according to TS 9398-002-46482062-2008, manufactured by NPO DNA Technology LLC (registration certificate No. FSR 2008/03847 dated December 29, 2017),

- A reagent kit for the qualitative and quantitative determination of human herpes virus type 6 (HHV6) DNA by polymerase chain reaction with real-time detection “HHV6-test” according to TS 21.20.23-043-97638376-2021, manufactured by TestGene LLC (registration certificate No. RZN 2023/19345 dated January 13, 2023),

- A reagent kit for qualitative DNA detection of Candida albicans, Chlamydia trachomatis, Gardnerella vaginalis, Mycoplasma genitalium, Mycoplasma hominis, Neisseria gonorrhoeae, Trichomonas vaginalis, Ureaplasma parvum, Ureaplasma urealyticum, CMV (Human betaherpesvirus 5), HSV1 (Human alphaherpesvirus 1), HSV2 (Human alphaherpesvirus 2) by multiplex PCR-RT "UROGEN" according to TS 21.20.23-045-97638376-2020, manufactured by TestGene LLC (registration certificate No. RZN 2023/21287 dated December 20, 2023).

Potential consumers of a medical device

The kit is intended for professional use in medical centers and clinical diagnostic laboratories. The professional level of potential users is a clinical laboratory diagnostics doctor, a medical laboratory technician.

2. Method principle

Test sample type

Material for DNA isolation is nasopharyngeal swabs, oropharyngeal swabs, vaginal swabs, cervical smears, urethral smears, first-void urine, prostate secretion**.**

Material for RNA isolation is nasopharyngeal swabs and oropharyngeal swabs.

Method principle

The method principle is based on reversible nucleic acid binding to magnetic beads surface. After a sample lysis, nucleic acids contained in it bind to magnetic beads. Then they should be washed with the Wash solution included in the kit. After washing cycles, dry the magnetic bead sediment, then the nucleic acids can be eluted. If more final material is required, the isolation protocol can be modified for scaling. To scale, change the Binding Buffer volume proportionally the clinical sample volume.

The kit functional capabilities allow using the kit for DNA/RNA isolation at automated nucleic acid isolation workstations.

Method limitations

The reagent kit cannot be used after the expiration date.

Do not use the reagent kit if the inner packaging is damaged, or the reagent appearance does not match the description.

A reagent kit transported or stored in violation of the temperature regime cannot be used.

Total DNA/RNA isolation time:

- manual isolation from 1 sample using the reagent kit, configuration form 1 (NA-Extra-SW-M) takes 35 minutes;
- isolation using automated sample preparation workstations Tecan Freedom EVO® or KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification from 96 samples at a time with the reagent kit, configuration form 1 (NA-Extra-SW-M) takes 23 minutes per cycle;
- automated isolation using KingFisher Flex magnetic bead processor for nucleic acids, cells, and proteins purification from 96 samples at a time using all configuration forms of the reagent kit takes 23 minutes per cycle.

3. Reagent kit components

The reagent kit is designed in two configuration forms:

1) Configuration form 1: NA-Extra-SW-M reagent kit for manual isolation and isolation using automated sample preparation workstations Tecan Freedom EVO® or KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification.

2) Configuration form 2: NA -Extra-SW-KF-u reagent kit for automated isolation using KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification.

Test samples number

A reagent kit, configuration form 1 (NA-Extra-SW-M) is designed for multiple use, the number of reagents is intended to isolate DNA/RNA from 96 test samples.

A reagent kit, configuration form 2 (NA-Extra-SW-KF-u) is designed for single use and is intended to isolate DNA/RNA from 1-96 test samples using KingFisher Flex magnetic bead processor at a time per cycle.

Reagent kit components

Table 1 – NA-Extra-SW reagent kit (configuration form 1) components: NA-Extra-SW-M reagent kit for manual isolation and isolation using automated sample preparation stations Tecan Freedom EVO® or KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification

No.	Reagent name	Description	Quantity
1	Binding Buffer	Transparent, colorless liquid, may have a yellow or pink shade.	1 bottle (48 ml)
2	Magnetic beads	Brown suspension	1 tube (960 µl)
3	Wash solution	Transparent colorless liquid	1 bottle (68 ml)
4	Eluent	Transparent colorless liquid	1 bottle (10 ml)

Table 2 – NA-Extra-SW reagent kit (configuration form 2) components: NA-Extra-SW-KF-u reagent kit for automated isolation using KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification

No.	Reagent name	Description	Quantity
1	Binding Buffer	Transparent, colorless liquid, may have a yellow or pink shade.	1 bottle (48 ml)
2	Magnetic beads	Brown suspension	1 tube (960 µl)
3	Wash solution	Transparent colorless liquid	1 bottle (68 ml)
4	Eluent	Transparent colorless liquid	1 bottle (10 ml)
5	96 deep-well plate for KingFisher	Empty colorless 96 deep-well polypropylene 2200 µl plate	3 pcs.
6	Plate (200 µl) for KingFisher 96	Empty colorless 96-well polypropylene low-profile 200 µl plate	1 piece
7	KingFisher 96 Tip comb for DW magnets	Colorless polypropylene tip comb for DW magnets	1 piece

Binding Buffer is ready for use and includes: guanidine thiocyanate; tris hydrochloride; triton X; urea; sodium dodecyl sulfate.

Magnetic beads are ready for use and include: magnetic bead solution, deionized water.

Wash solution is ready for use and includes: guanidine thiocyanate; tris hydrochloride; isopropanol.

Eluent is ready for use and includes: tris hydrochloride; EDTA.

Calibrators and control materials are not used in the isolation kit.

The kit contains no products for medical use, substances of human or animal origin.

Note: The product does not contain any other ingredients that may affect the procedure.

4. Reagent kit characteristics
4.1 Technical and functional characteristics

Table 3 – NA-Extra-SW reagent kit technical and functional characteristics

Indicator	Characteristics and standards
1. Technical characteristics	
1.1. Appearance	
1.1.1. Configuration form 1: NA-Extra-SW-M reagent kit for manual isolation and isolation with automated sample preparation workstations Tecan Freedom EVO® or KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification	
Binding Buffer	Transparent, colorless liquid, may have a shade of yellow or pink.
Magnetic Beads	Brown suspension
Wash Solution	Transparent colorless liquid
Eluent	Transparent colorless liquid
1.1.2. Configuration form 2: NA-Extra-SW-KF-u reagent kit for automated isolation with KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification	
Binding Buffer	Transparent, colorless liquid, may have a shade of yellow or pink.
Magnetic Beads	Brown suspension
Wash Solution	Transparent colorless liquid
Eluent	Transparent colorless liquid
96 deep-well plate for KingFisher	Empty colorless 96 deep-well polypropylene 2200 µl plate
Plate (200 µl) for KingFisher 96	Empty colorless 96-well polypropylene low-profile 200 µl plate
Tip comb for DW magnets for KingFisher 96	Colorless polypropylene tip comb for DW magnets
1.2. Physical and chemical parameters	
Hydrogen ion concentration indicators, pH	
Binding Buffer	min 6.0 pH, max 8.0 pH
Wash Solution	min 6.0 pH, max 8.0 pH
1.3. Completeness	According to clause 1.4 TS 21.20.23-040-97638376-2021
1.4. Labelling	According to clause 4 TS 21.20.23-040-97638376-2021
1.5. Packaging	According to clause 5 TS 21.20.23-040-97638376-2021
2. Functional characteristics	

2.1 DNA/RNA isolation purity, A260/280, at least	1.7
2.2. No kit components contamination with extraneous DNA/RNA	Negative result with NC in a control PCR via FAM and HEX channels
2.3. DNA/RNA suitability for PCR/RT-PCR	When testing a control sample (CS) that has passed the DNA/RNA isolation stage using NA-Extra-SW kit, the Ct value in FAM and HEX channels does not exceed 30 cycles, and the Δ Ct value is between the CS and PC (included in the standard enterprise sample ESS-Extra-control kit) does not exceed 2 cycles in FAM and HEX channels.

Note: control PCR is carried out using standard enterprise sample (ESS) SOP-Extra-control kit.

During a control PCR, deionized sterile water DNase/RNase free is used as a negative control sample (NC).

A plasmid and RNA mixture in a bacteriophage capsid is used as a control sample (CS).

4.2 Analytical efficiency characteristics

4.2.1 Precision under repeatability conditions

Repeatability data is obtained inside a laboratory for specific equipment and within a specific reagent kit batch.

The repeatability of the results obtained using a reagent kit was evaluated by conducting consequential assays of a control sample, which is an internal control sample (ICS) included in ESS-Extra-control kit (manufactured by TestGene LLC, Russia; quality certificate No. 124, lot: 202111-124 expiration date: 2022-11). Internal control sample (ICS) is E. coli K-12 strain at 100 mk/µl concentration, transduced by the MS2 bacteriophage (single-stranded RNA virus) and transformed by the pUC19 plasmid containing an insertion in the form of a detectable COMT gene in human genomic DNA in a TE buffer.

DNA/RNA isolation was performed according to the instructions for the kit from 100 µl control sample.

After DNA/RNA isolation DNA/RNA isolation purity was evaluated (expressed in the optical densities ratio of the nucleic acid preparation, A260/280).

To evaluate precision under repeatability conditions, the arithmetic mean of the sample, variance, standard deviation, and coefficient of variation were calculated based on the obtained values of DNA/RNA isolation purity in control sample repeats.

The assay results showed that the coefficient of variation under the kit repeatability conditions is up to 3%.

4.2.2 Precision under reproducibility conditions

Reproducibility evaluation was carried out in a similar way to precision calculation under repeatability conditions, however, two different batches of reagent kits were used for testing, the assays were carried out in two different laboratories, by different operators, on different days (Reproducibility Unit 1, Reproducibility Unit 2).

DNA/RNA isolation was performed according to the instructions for the kit from 100 µl control sample.

After DNA/RNA isolation DNA/RNA isolation purity was evaluated (expressed in the optical densities ration of the nucleic acid preparation, A260/280).

The assay results showed that the coefficient of variation under the kit reproducibility conditions is up to 4%.

4.2.3 Interfering substances effect evaluation

The potentially interfering substances effect on NA-Extra-SW reagent kit performance was studied for potentially interfering substances that may occur during the clinical material sampling and during DNA/RNA isolation from clinical material.

The following substances are classified as PCR inhibitors that may occur during DNA/RNA isolation, according to the results of risk analysis and R&D: sodium dodecyl sulfate and urea (included in the Binding Buffer), as well as isopropanol (included in the Wash Solution), which may be present in DNA/RNA eluate as a result of incomplete removal during DNA/RNA isolation.

The maximum concentrations of interfering substances that can occur during DNA/RNA isolation from clinical material, which had no effect on the laboratory control sample amplification, are: sodium

dodecyl sulfate – 0.007 µg/ml of cDNA sample, urea – 20 mM/ml of cDNA sample, isopropanol – 5 µl/ml of cDNA sample.

Potentially interfering substances and their concentrations are shown in Table 4.

Table 4 - Potentially interfering substances and their concentrations

Interfering substances	Interfering substance concentration	Clinical material type
Endogenous interfering substances		
Hemoglobin	≥ 1 mg/ml	nasopharyngeal swabs, oropharyngeal swabs, vaginal swabs, cervical smears, urethral smears, first-void urine, prostate secretion
Mucin	5%	nasopharyngeal swabs, oropharyngeal swabs, vaginal swabs, cervical smears, urethral smears
Exogenous interfering substances		
Anti-inflammatory medicines		
Acetaminophen	200 mcmol	nasopharyngeal swabs, oropharyngeal swabs
Acetylsalicylic acid	3.7 mM	nasopharyngeal swabs, oropharyngeal swabs
Ibuprofen	2.5 mM	nasopharyngeal swabs, oropharyngeal swabs
Antibiotics		
Erythromycin	81.6 mcmol	nasopharyngeal swabs, oropharyngeal swabs, vaginal swabs, cervical smears, urethral smears, first-void urine, prostate secretion
Ciprofloxacin	31 mcmol	nasopharyngeal swabs, oropharyngeal swabs, vaginal swabs, cervical smears, urethral smears, first-void urine, prostate secretion
Tobramycin	5 µg/ml	nasopharyngeal swabs, oropharyngeal swabs, vaginal swabs, cervical smears, urethral smears, first-void urine, prostate secretion
Nasal spray and drops		
Neosinephrine (Phenylephrine)	10%	nasopharyngeal swabs, oropharyngeal swabs
Afrin (Oxymetazoline)	10%	nasopharyngeal swabs, oropharyngeal swabs
Saline Nasal Spray	10%	nasopharyngeal swabs, oropharyngeal swabs

Medications for oral administration		
Ambrobene (ambroxol hydrochloride)	0.003 mg/ml	nasopharyngeal swabs, oropharyngeal swabs
Bromhexine (bromhexine)	0.016 mg/ml	nasopharyngeal swabs, oropharyngeal swabs
Kaletra (lopinavir, ritonavir)	0.02 mg/ml	nasopharyngeal swabs, oropharyngeal swabs
Interferon (interferon alpha)	0.2 units /ml	nasopharyngeal swabs, oropharyngeal swabs
Theraflu (paracetamol, phenyramine, phenylephrine)	0.071 mg/ml	nasopharyngeal swabs, oropharyngeal swabs
Vaginal medications		
Polygynax (neomycin, nystatin, polymyxin B)	34 IU/ml	vaginal swabs, cervical smear, urethral smear, first-void urine
Ginalgin (metronidazole, chlorquinaldol)	0.07 mg/ml	vaginal swabs, cervical smear, urethral smear, first-void urine
Cicloprox (ciclopirox)	0.02 mg/ml	vaginal swabs, cervical smear, urethral smear, first-void urine
Clotrimazole (clotrimazole)	0.02 mg / ml	vaginal swabs, cervical smear, urethral smear, first-void urine
Fluomizin (dequalinium chloride)	0.002 mg/ml	vaginal swabs, cervical smear, urethral smear, first-void urine
Elzhina (ornidazole, neomycin, prednisolone, econazole)	0.12 mg/ml	vaginal swabs, cervical smear, urethral smear, first-void urine
Substances included in NA-Extra-SW kit		
sodium dodecyl sulfate	0.007 µg/ml of cDNA sample	nasopharyngeal swabs, oropharyngeal swabs, vaginal swabs, cervical smears, urethral smears, first-void urine, prostate secretion
urea	20 mM/ml of cDNA sample	nasopharyngeal swabs, oropharyngeal swabs, vaginal swabs, cervical smears, urethral smears, first-void urine, prostate secretion

isopropanol	5 µl/ml of cDNA sample	nasopharyngeal swabs, oropharyngeal swabs, vaginal swabs, cervical smears, urethral smears, first-void urine, prostate secretion

Based on the results of PCR series with nucleic acids isolated from the control sample and solutions with the potential interfering substances added into the control sample at a concentration that is expected to occur during NA-Extra-SW reagent kit normal use, the above mentioned substances do not have an interfering effect.

To reduce PCR inhibitors number, it is required to follow the clinical material sampling instructions.

Limitations on the test material use:

- start the subsequent analysis right after DNA/RNA isolation procedure completion;
- do not use the test material in case of storage and transportation conditions violation (temperature, duration, multiple freezing and thawing);
- it is not allowed to use samples contaminated with extraneous biological material.

4.3 Clinical efficiency characteristics

The tested medical device effectiveness if used in accordance with its intended use specified in the manufacturer's documentation was confirmed by conducting clinical and laboratory studies using reverse transcription (RNA), polymerase chain reaction (DNA) with nucleic acid samples isolated from 212 samples of clinical material (nasopharyngeal swabs, oropharyngeal swabs, vaginal swabs, cervical smears, urethral smears, first-void urine, prostate secretion) using registered in vitro diagnostic medical devices:

- A reagent kit for qualitative detection of coronavirus RNA (SARS-CoV-2) by real-time RT-PCR method “CoV-2-Test” according to TS 21.20.23-015-97638376-2020, manufactured by TestGene LLC (registration certificate No. RZN 2020/10364 dated May 15, 2020);

- A reagent kit for RNA detection of SARS-CoV-2 virus, influenza A and B viruses by RT-PCR-RT “CoV-Influ-test”, manufactured by

TestGene LLC (registration certificate No. RZN 2022/18297 dated September 23, 2022);

- A reagent kit for Mycoplasma pneumoniae and Chlamydophila pneumoniae DNA detection in biological material by polymerase chain reaction (PCR) with hybridization-fluorescence detection "AmpliSens® Mycoplasma pneumoniae/Chlamydophila pneumoniae-FL" according to TS 9398-176-01897593-2012, manufactured by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor (registration certificate no. FSR 2012/13957 dated February 27, 2019),

-Candida albicans Real-Time PCR Detection Kit according to TS 9398-002-46482062-2008, manufactured by NPO DNA Technology LLC (registration certificate No. FSR 2008/03847 dated December 29, 2017),

- A reagent kit for the qualitative and quantitative determination of human herpes virus type 6 (HHV6) DNA by polymerase chain reaction with real-time detection “HHV6-test” according to TS 21.20.23-043-97638376-2021, manufactured by TestGene LLC (registration certificate No. RZN 2023/19345 dated January 13, 2023),

- A reagent kit for qualitative DNA detection of Candida albicans, Chlamydia trachomatis, Gardnerella vaginalis, Mycoplasma genitalium, Mycoplasma hominis, Neisseria gonorrhoeae, Trichomonas vaginalis, Ureaplasma parvum, Ureaplasma urealyticum, CMV (Human betaherpesvirus 5), HSV1 (Human alphaherpesvirus 1), HSV2 (Human alphaherpesvirus 2) by multiplex PCR-RT "UROGEN" according to TS 21.20.23-045-97638376-2020, manufactured by TestGene LLC (registration certificate No. RZN 2023/21287 dated December 20, 2023).

To evaluate inter-series convergence DNA/RNA isolation from clinical material samples was performed in two series of each configuration form of the test medical device. For configuration form 1DNA/RNA isolation was carried out in four ways, as specified by the manufacturer in the operational documentation:

- manually using a magnetic rack,
- manually using a centrifuge,
- using Freedom EVO® Tecan automated sample preparation workstation;
- using KingFisher Flex magnetic bead processor for nucleic acids, cells, and proteins purification.

For configuration form 2, DNA/RNA isolation was performed using KingFisher Flex magnetic bead processor for nucleic acids, cells, and proteins purification.

Clopper-Pearson Confidence Interval; Clopper, C., & Pearson, E. (1934) method was used for clinical and laboratory test data statistical processing. The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial. Biometrika, 26(4), 404-413. doi:10.2307/2331986).

The diagnostic characteristics study results for all configuration forms of the tested medical device based on the clinical material samples are shown in Table 5. The confidence interval lower limit was determined by the Klopper and Pearson method.

Table 5 – Clinical study results

Test material	Configuration form	DNA/RNA isolation method	Diagnostic characteristics	Number of samples	Number of observations	**CI** with 95% confidence probability
DNA						
Nasopharyngeal swabs	Configuration form 1	Manually using a magnetic rack	100%	25	50	100% (95% CI:92.89%-100%)
		Manually using a centrifuge				
		Tecan Freedom EVO®				
		KingFisher Flex				
	Configuration form 2	KingFisher Flex	100%	25	50	100% (95% CI:92.89%-100%)
Oropharyngeal swabs	Configuration form 1	Manually using a magnetic rack	100%	25	50	100% (95% CI:92.89%-100%)
		Manually using a centrifuge				
		Tecan Freedom EVO®				
		KingFisher Flex				
	Configuration form 2	KingFisher Flex	100%	25	50	100% (95% CI:92.89%-100%)
Vaginal swabs	Configuration form 1	Manually using a magnetic rack	100%	25	50	100% (95% CI:92.89%-100%)
		Manually using a centrifuge				
		Tecan Freedom EVO®				
		KingFisher Flex				
	Configuration form 2	KingFisher Flex	100%	25	50	100% (95% CI:92.89%-100%)

Urethral smears	Configura-tion form 1	Manually using a magnetic rack	100%	22	44	100% (95% CI:92.89%-100%)
		Manually using a centrifuge				
		Tecan Freedom EVO®				
		KingFisher Flex				
	Configura-tion form 2	KingFisher Flex	100%	22	44	100% (95% CI:92.89%-100%)
Cervical smears	Configura-tion form 1	Manually using a magnetic rack	100%	25	50	100% (95% CI:92.89%-100%)
		Manually using a centrifuge				
		Tecan Freedom EVO®				
		KingFisher Flex				
	Configura-tion form 2	KingFisher Flex	100%	25	50	100% (95% CI:92.89%-100%)
First-void urine	Configura-tion form 1	Manually using a magnetic rack	100%	21	42	100% (95% CI:91.59%-100%)
		Manually using a centrifuge				
		Tecan Freedom EVO®				
		KingFisher Flex				
	Configura-tion form 2	KingFisher Flex	100%	21	42	100% (95% CI:91.59%-100%)
Prostatic secretion	Configura-tion form 1	Manually using a magnetic rack	100%	19	38	100% (95% CI:90.75%-100%)
		Manually using a centrifuge				
		Tecan Freedom EVO®				
		KingFisher Flex				
	Configura-tion form 2	KingFisher Flex	100%	19	38	100% (95% CI:90.75%-100%)
RNA						
Nasopharyn-geal swabs	Configura-tion form 1	Manually using a magnetic rack	100%	25	50	100% (95% CI:92.89%-100%)
		Manually using a centrifuge				
		Tecan Freedom EVO®				
		KingFisher Flex				
	Configura-tion form 2	KingFisher Flex	100%	25	50	100% (95% CI:92.89%-100%)
Oropharyn-geal swabs	Configura-tion form 1	Manually using a magnetic rack	100%	25	50	100% (95% CI:92.89%-100%)

		Manually using a centrifuge				
		Tecan Freedom EVO®				
		KingFisher Flex				
	Configura-tion form 2	KingFisher Flex	100%	25	50	100% (95% CI:92.89%-100%)

5. Risks associated with NA-Extra-SW reagent kit use

The border risk zone includes the following hazards:

- loss of functional properties of the reagents included in the kit due to transportation, storage or operation under inappropriate conditions,
- contaminants in the obtained isolated DNA/RNA,
- carrying out the DNA/RNA isolation procedure from an insufficient amount of clinical material,
- failure to comply with the sample preparation, testing and disposal requirements due to the unqualified personnel work,
- use of an unusable kit (use after the expiration date or in case of packaging damage).

No risks were identified in the unacceptable risk zone.

Cumulative residual risk of using a medical device “Reagent kit for isolation of viral, bacterial and fungal DNA and viral RNA from human clinical material “NA-Extra-SW” according to TS 21.20.23-040-97638376-2021, produced by TestGene LLC, is acceptable, the benefits of its use exceed the risk.

6. Safety precautions

Potential risk class - 2a - in accordance with the Nomenclature Classification of Medical Devices approved by the Order of the Ministry of Health of the Russian Federation No. 4n dated 06.06.2012.

The work should be carried out in a laboratory performing molecular biological (PCR) assays of clinical material, in compliance with the sanitary and epidemiological rules of SP 3.3686-21 "Sanitary and epidemiological requirements for the prevention of infectious diseases", MU 1.3.2569-09 "Organization of work of laboratories using nucleic acid amplification methods when working with material containing microorganisms of pathogenicity groups I– IV".

The following requirements should always be met when working:

▪ the test samples should be considered as infectious and dangerous, and work and storage should be organized in accordance with SanPiN 3.3686-21 "Sanitary and epidemiological requirements for the prevention of infectious diseases";

▪ clean and disinfect spilled samples or reagents using disinfectants in accordance with SanPiN 3.3686-21 "Sanitary and epidemiological requirements for the infectious diseases prevention";

▪ the laboratory process should be unidirectional. The analysis is carried out in separate rooms (zones). Work should begin in the Isolation Area and continue in the Amplification and Detection Area. Do not return samples, equipment and reagents to the area where the previous process stage was carried out;

▪ unused reagents, expired reagents, as well as used reagents should be disposed of in accordance with the requirements of SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial and public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures";

▪ use and change disposable filter tips for automatic dispensers during each operation. Disposable plastic containers must be disposed of in a special container with a disinfectant that can be used to disinfect medical waste;

▪ table surfaces, as well as rooms in which PCR is performed, must be exposed to ultraviolet radiation before and after work completion in accordance with accepted standards;

▪ use the kit strictly for its intended use, according to these instructions;

▪ only specially trained personnel is allowed to work with the kit;

▪ do not use the reagent kit after the expiration date;

▪ do not use the reagent kit if the inner packaging is damaged, or the reagent appearance does not match the description;

▪ use disposable gloves, lab coats, protect your eyes while working with samples and reagents, and wash your hands thoroughly after finishing work;

▪ all kit components of are non-toxic to humans in the stated concentrations. In case of kit components contact with the skin or mucous membranes, rinse the affected area with plenty of water.

The necessary precautions regarding the effects of magnetic fields, external electrical influences, electrostatic discharges, pressure or pressure changes, overload or sources of thermal ignition are not provided.

The kit contains no substances of human or animal origin with a potential infectious nature, therefore, precautions against any special, unusual risks during the product use or sale are not provided.

7. Equipment and materials

Equipment:

1. Class II sterile laminar biological (microbiological) box (for example, Sterile laminar cabinet, manufactured by AMS AO, Russia, No. FSR 2011/10419 dated October 6, 2022 or a box for clean operations DNA/RNA UV-Cleaner Box UVC/T-M-AR, Biosan, Latvia, RC No. RZN 2023/19369 dated January 18, 2023);
2. Thermostat for Eppendorf type tubes, maintaining temperatures from +25°C to +100°C (for example, TT-2 Termit, NPO DNA Technology LLC, Russia, RC No. FSR 2012/14090 dated November 23, 2012);
3. Vortex (for example, Vortex V-3, manufactured by ELMI SIA, Latvia, RC No. RZN 2017/5466 dated May 31, 2023 or centrifuge-mixer CM-70M, manufactured by ELMI SIA, Latvia, RC No. RZN 2016/4616 dated May 31, 2023);
4. Separate set of automated variable volume dispensers that allow to select liquid volumes of 100-1000 µl (for example, Eppendorf Research Plus, Germany, RC No. FSZ 2011/11028 dated November 15, 2011 or Biohit, Finland, RC No. FSZ 2012/12201 dated May 18, 2012);
5. Refrigerator from +2°C to +8°C (for example, POZIS, Russia, No. RZN 2015/3531 dated July 15, 2019),
6. Freezer from -2°C to -40°C (for example, POZIS, Russia, No. RZN 2016/4688 dated February 1, 2023);

7. Magnetic rack for 1.5/2 ml Eppendorf-type tubes (InterLabService, Russia, RC No. RZN 2022/16917 dated July 19, 2022);
8. Centrifuge for 1.5/2 ml Eppendorf type tubes (for example, ROTOFIX 32 centrifuge, RC No. FSZ 2011/09920 dated May 18, 2012 or MiniSpin centrifuge, RC No. FSZ 2012/13316 dated May 12, 2012);
9. When using any kit configuration forms (NA-Extra-SW-M or NA-Extra-SW-KF-u): KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification, (Thermo Fisher Scientific, Finland), RC No. FSZ 2009/05562 dated March 16, 2022;
10. When using the configuration form 1 (NA-Extra-SW-M), it is possible to use Tecan Freedom EVO® automated sample preparation workstations (TECAN, Switzerland, Austria), RC No. FSZ 2008/03047 dated July 4, 2016;
11. Multi-channel automated variable volume dispensers (for example, Eppendorf, Germany, RC No. FSZ 2011/11028 dated November 15, 2011) or Tecan Freedom EVO® automated sample preparation workstation (TECAN, Switzerland, Austria, RC No. FSZ 2008/03047 dated July 4, 2016) can be used to introduce reagents into deep 96 well plates for KingFisher;
12. 100 ml reagent reservoirs can be used to add reagents into 2200 µl deep plates (for example, GUANGZHOU JET BIOFILTRATION CO., China, RC No. FSZ 2012/12495 dated June 2, 2020),

Additionally, the following can be used:

13. Aspirator with a trap flask (Biosan, Latvia, No. FSZ 2011/09791 dated May 25, 2011);

Materials and reagents not included in the device:

1. Disposable polypropylene screw-on or tightly closed 1.5 ml microtubes, extraneous DNA/RNA and DNase/RNase free (Axygen, USA, RC No. FSZ 2012/11892 dated August 26, 2014);
2. When using the configuration form 1 in conjunction with KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification the following is required:

- 96-well deep 2200 µl plates extraneous DNA/RNA and DNase/RNase free, (Thermo Fisher Scientific, Finland, RC No. FSZ 2009/05562 dated March 16, 2022) – 3 pcs.;
- 96-well low-profile 200 µl plate extraneous DNA/RNA and DNase/RNase free (Thermo Fisher Scientific, Finland, RC No. FSZ 2009/05562 dated March 16, 2022) – 1 pc.
- Tip comb for KingFisher 96 DW magnets, (Thermo Fisher Scientific, Finland, RC No. FSZ 2009/05562 dated March 16, 2022) – 1 pc.;

3. Racks for 1.5 ml tubes and tips (Axygen, USA, No. FSZ 2012/11892 dated August 26, 2014);
4. Magnetic separation rack for 1.5-2 ml Eppendorf type tubes (InterLabService, Russia, RC No. RZN 2022/16917 dated July 19, 2022);
5. Disposable tips for variable volume dispensers with aerosol barrier of 100 µl, 1000 µl and 5 µl extraneous DNA/RNA and DNase/RNase free (Axygen, USA, RC No. FSZ 2012/12077 dated February 27, 2014);
6. Disposable tips for 100 µl and 1000 µl variable volume dispensers, extraneous DNA/RNA and DNase/RNase free (Axygen, USA, RC No. FSZ 2012/12077 dated February 27, 2014);
7. Disposable or separate lab coats and disposable gloves;
8. Containers with disinfectant solution for used tips, tubes and other consumables disposal.

Additionally, the following can be used:

9. Sterile saline solution (0.9% NaCl),
10. Deionized sterile DNase/RNase-free water,
11. As a transport medium for adding to the urine cellular sediment, it is recommended to use "Reagent for clinical material transportation and storage "Transport medium with mucolytic (TMM)" according to TS 9398-098-01897593-2009", manufactured by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia (registration certificate No. FSR 2009/05514 dated March 13, 2019).
12. A reagent kit for RNA stabilization in biosamples STOR-M according to TS 9398-099-46482062-2017 manufactured by

DNA-Technology TS LLC (Registration certificate No. RZN 2019/9453 dated December 24, 2018) is recommended for use as a solution for RNA stabilization and preservation in urine cellular sediment.

Do not use other materials and reagents not included in the kit.

Measuring equipment is not required during the kit operation.

8. Test samples

Before starting work, review the guidelines "Sampling, transportation and storage of clinical material for PCR diagnostics" developed by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Moscow, 2012.

Initial clinical material transportation and storage conditions.

Table 6 – Clinical material storage and transportation conditions

Sample type	Material collection requirements	Material storage and transportation conditions
Nasopharyn-geal swabs	Smears (mucus) are taken with dry sterile cotton swabs on a plastic rod. Insert a swab with a light movement along the outer wall of the nose to 2-3 cm depth to the lower concha. Then lower slightly the swab, insert into the lower nasal passage under the lower concha, make a rotational movement and remove along the outer wall of the nose. After taking the material, place the swab (the applied part of the probe with a cotton swab) in a sterile disposable tube with special transport medium (or 500 ml of sterile saline solution) and carefully break off the plastic rod at a distance up to 0.5 cm from the applied part, leaving the applied part of the probe with the material in the transport medium. Close the tube tightly with a lid.	- at room temperature – up to 6 hours; - at 2-8°C – up to 3 days; - at -20°C – up to 1 month; - at -70°C – for a long time. It is allowed to freeze and thaw the material only once.
Oropharyn-geal swabs	Swabs are taken with sterile cotton swabs on a plastic rod by	- at room temperature – for 6 hours; - at 2-8°C – up to 3 days;

	rotational movements from the surface of the tonsils, faucial pillars and the posterior pharyngeal wall. After taking the material, place the swab (the applied part of the probe with a cotton swab) in a sterile disposable tube with special transport medium (or 500 ml of sterile saline solution) and carefully break off the plastic rod at a distance up to 0.5 cm from the applied part, leaving the applied part of the probe with the material in the transport medium. Close the tube tightly with a lid.	- at -20°C – up to 1 month; - at -70°C – for a long time. It is allowed to freeze and thaw the material only once.
Vaginal swabs	Material should be taken prior to manual examination. The speculum before manipulation can be moistened with hot water, it is not allowed to use antiseptics for speculum treatment. The swab is taken from the posterolateral vaginal wall. In girls, the material is taken from the mucous membrane of the vaginal vestibule, and in some cases - from the posterior vaginal wall through the hymenal rings.	**Transportation:** at +2 to +8°C - 24 hours. If it is impossible to deliver the material to a laboratory during a day only single material freezing is allowed. **Storage conditions:** - at +2 ... +8°C – up to 24 hours. - at -18 ... -22°C – up to 1 month.
Cervical smears	Before taking the material, remove mucus with a cotton swab and then treat the cervix with sterile saline solution. Insert the probe into the cervical canal at 0.5-1.5 cm depth. When removing the probe, it is not allowed to touch the vaginal walls.	**Transportation:** at +2 to +8°C - 24 hours. If it is impossible to deliver the material to a laboratory during a day only single material freezing is allowed. **Storage conditions:** - at +2 ... +8°C – up to 24 hours. - at -18 ... -22°C – up to 1 month.
Urethral smears	Women should not wash their genital area or do douching on the examination eve. Before taking clinical material it is recommended to refrain from	**Transportation:** at +2 to +8°C - 24 hours. If it is impossible to deliver the material to a laboratory during a day only single material freezing is allowed.

	urinating for 1.5-2 hours. Right before taking clinical material, treat the external urethral orifice with a tampon, which can be moistened with sterile saline solution. If there is purulent discharge it is recommended to take a smear 15-20 minutes after urination; if there is no discharge it is necessary to massage the urethra with a probe to take clinical material. Insert the probe in urethra at 1.0-1.5 cm depth in women, in children take material for testing only from the urethra external opening.	**Storage conditions:** - at +2 ... +8°C – up to 24 hours. - at -18 ... -22°C – up to 1 month.
First-void urine	For testing collect at least 20-30 ml of the first-void morning urine in a special dry sterile 50 ml container. Sample pretreatment. Shake the urine container. Transfer 1 ml of urine using a filter tip into sterile 1.5 ml tubes and centrifugate for 5 minutes at 10,000 g, if there is a large quantity of salts, resuspend only the top layer of the salt sediment in 1 ml volume and then concentrate again. Remove the supernatant completely using a vacuum aspirator with a trap flask without taking the sediment. Add the transport medium to the sediment to increase the final volume up to 0.2 ml, mix thoroughly the contents on a vortex. As a transport medium for adding to the urine cellular sediment, it is recommended to use the "Reagent for clinical material transportation and	**Transportation:** Transport whole urine samples and cellular sediment obtained from it without a RNA stabilization and preservation solution at +2...+8 °C for up to 3 days. **Storage conditions:** - Without a RNA stabilization and preservation solution at +2...+8°C - up to 3 days. Freezing of whole urine samples is strictly prohibited. - Urine sediment in a RNA stabilization and preservation solution STOR-M (DNA Technology TS LLC, Russia, RU No. RZN 2019/9453 dated December 24, 2018) - at +2...+8°C - up to 10 days. - Long-term sample storage (from 10 days to a year) is recommended at a temperature below -18°C in RNA stabilization and preservation solution.

	storage "Transport medium with mucolytic (TMM)" according to TS 9398-098-01897593-2009", manufactured by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia (registration certificate No. FSR 2009/05514 dated March 13, 2019).	
Prostate secretion	Before taking prostate secretion, treat the glans penis with a sterile cotton swab moistened with saline solution. Take prostate secretion after a preliminary prostate massage through the rectum. A doctor massages with a few forceful movements from the base to the top. Then the prostatic secretion is squeezed from the spongy urethra and collected in a sterile container (wide-mouth bottles, tubes).	- at room temperature – up to 6 hours; - at +2...+8°C – up to 1 day; - at -20°C – up to 1 week; - at -70°C – for a long time.

Clinical material preparation procedure for the nucleic acid isolation.

8.1. Nasopharyngeal swabs, oropharyngeal swabs.

8.1.1. Centrifuge the tube containing the test material at 13,000 g for 10 minutes.

8.1.2. Remove the supernatant, leaving about 100 µl (sediment + liquid fraction) in the tube.

8.2. Vaginal swabs, cervical smears, urethral smears.

8.2.1. Transfer epithelial cell scrapes using disposable sterile probes into 1.5 ml plastic tubes containing 500 µl of saline solution (if isolating DNA).

8.2.2. Remove the probe by pressing it against the tube wall and squeezing out the excess liquid. Close the tube tightly. The obtained material is ready for DNA isolation.

Note. Before taking cervical smear, remove the mucus with a sterile cotton swab.

8.3. Urine.

8.3.1. Collect a urine portion (approximately 50 ml) in a sterile container and close tightly with a lid.

8.3.2. Fill sterile 50, 15, 10 or 2 ml RNase-free labeled plastic centrifuge tubes with taken urine samples using separate sterile tips.

8.3.3. Centrifuge 50, 15 or 10 ml tubes at 3,000 g for 20 minutes or at 5,000 g for 15 minutes; centrifuge 2 ml tubes at 10,000 g for 5 minutes.

8.3.4. Remove the supernatant with separate sterile tips not touching the sediments.

8.3.5. When using 15, 10 or 2 ml tubes, layer carefully the next urine portions of the same samples over the sediments with separate sterile tips, preventing cross-contamination, repeat steps 2-4 until the urine is used fully.

8.3.6. When centrifuging samples in 50, 15, or 10 ml tubes, transfer the obtained suspensions into 1.5-2 ml labeled sterile plastic tubes with separate sterile tips.

8.3.7. Place the tubes in a refrigerator +2... +8 °C (do not freeze).

8.4. Prostate secretion.

8.4.1. Transfer 20-30 µl of liquid material with a pipette into a 1.5 ml plastic tube with a preservative (or 500 µl of sterile saline solution).

8.4.2. Centrifuge the tube at 16,000 g for 10 minutes.

8.4.3. Remove the supernatant, leaving in the tube approximately 50 µl (sediment + liquid fraction).

Isolated DNA storage conditions:

- at +4°C – up to 1 day;
- at -18 ... -22°C – up to 1 month;
- at - 80°C – for a long time.

Isolated RNA storage conditions:

- at +2 ...+8°C – up to 4 hours (recommended);
- at -18... -22°C – up to 1 week;
- at a temperature of below -80°C – up to 1 year.

9. Components preparation for testing

It is not required to install, assemble, adjust, calibrate the medical device for commissioning.

When using the configuration form 1 for manual isolation:

1) Layering or precipitate separation does not affect the solution quality. If there is a sediment or component layering in one of the bottles, it is necessary to heat it at 70°C and mix it until the sediment dissolves completely and the solutions homogenize.

2) Before starting work and before each manipulation with the magnetic bead solution, resuspend it completely using a vortex or by pipetting, since the magnetic bead suspension is two-phase, it forms clearly separable phases easily and quickly.

3) Mix thoroughly all kit components before starting work.

4) If there is a large number of test samples, it is allowed to transfer the entire contents of the tube with magnetic beads (960 µl) into a bottle with the Binding buffer. This mixture can be stored for 7 days. Carefully mix the prepared suspension of magnetic beads in the Binding buffer before each use.

When using the configuration form 1 in conjunction with Tecan Freedom EVO® automated sample preparation workstation:

1) Layering or precipitate separation does not affect the solution quality. If there is a sediment or component layering in the plate wells, it is necessary to heat it at 70°C and mix it until the precipitate dissolves completely and the solutions homogenize.

2) Mix all kit components before use;

3) Transfer the entire contents of the tube with magnetic beads (960 µl) into the bottle with the Binding buffer. This mixture can be stored for 7 days. Carefully mix the prepared suspension of magnetic beads in the Binding buffer before each use.

4) Prepare Tecan Freedom EVO® automated sample preparation workstation in accordance with the Instructions for Use.

5) Pour the prepared solutions from the bottles into the cuvettes and place them into the workstation in the order described in the workstation operation protocol.

6) Load special tips, tubes for DNA/RNA isolation reactions, and tubes for isolated DNA/RNA into the workstation in the order described in the workstation operation protocol.

When using the configuration form 1 in conjunction with KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification

1) Layering or precipitate separation does not affect the solution quality. If there is a sediment or component layering in the plate wells, it is necessary to heat it at 70°C and mix it until the precipitate dissolves completely and the solutions homogenize.

2) Mix all kit components before use;

3) Transfer the entire contents of the tube with magnetic beads (960 µl) into the bottle with the Binding Buffer. This mixture can be stored for 7 days. Carefully mix the prepared suspension of magnetic beads in the Binding Buffer before each use.

4) Before carrying out DNA/RNA isolation, prepare:

- 96-well deep 2200 µl plates extraneous DNA/RNA and DNase/RNase free, (Thermo Fisher Scientific, Finland, RC No. FSZ 2009/05562 dated March 16, 2022) – 3 pcs.;
- 96-well low-profile 200 µl plate extraneous DNA/RNA and DNase/RNase free (Thermo Fisher Scientific, Finland, RC No. FSZ 2009/05562 dated March 16, 2022) – 1 pc.
- Tip comb for DW magnets for KingFisher 96, (Thermo Fisher Scientific, Finland, RC No. FSZ 2009/05562 dated March 16, 2022) – 1 pc.

5) Place the tip comb in a free plate (200 µl) for KingFisher 96 according to the Instructions for KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification;

6) Label 96-well plates for KingFisher and add reagents into them according to the scheme (Table 7):

Table 7 – Reagent introduction scheme into plates when using a kit for automated isolation and KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification

Plate number	**Plate type**	**Added component**	**Amount added into each plate well**
Plate 1	Plate (200 µl) for KingFisher 96	Tip Comb for DW magnets for KingFisher 96	-

Plate number	Plate type	Added component	Amount added into each plate well
Plate 2	96-well deep plate (2200 µl) for KingFisher	Mixture of Magnetic Beads and a Binding Buffer	510 µl
Plate 3	96-weel deep plate for KingFisher	Wash solution	700 µl
Plate 4	96-weel deep plate for KingFisher	Eluent	100 µl

A single-channel or multi-channel variable-volume automatic dispenser can be used to introduce reagents. Attention: add each reagent with a separate tip, the reagent remains must not get into the other.

7) Prepare the equipment according to the Instructions for Use;

8) Upload the protocol from the KingFisher Flex - NA-Extra-SW file to the device (attached file), corresponding to the diagram below;

- Tip comb for magnets selection (Tips) (Plate 1)
- lysis (Plate 2): duration – 5 minutes, heating – fast, temperature – 80°C, medium intensity mixing;
- isolation (Plate 2): duration – 6 minutes, temperature – 25°C, medium intensity mixing, magnetic bead collection – 15 seconds;
- washing 2 (Plate 3): duration - 2 minutes, temperature – 25°C, mixing, magnetic beads collection – 15 seconds,
- magnetic beads drying – 2 minutes;
- elution (Plate 4): duration – 6 minutes, temperature – 80°C, mixing – slow, magnetic bead collection – 15 seconds.

When using the configuration form 2 in conjunction with KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification

1) Layering or precipitate separation does not affect the solution quality. If there is a sediment or component layering in the plate wells, it is necessary to heat it at 70°C and mix it until the precipitate dissolves completely and the solutions homogenize.

2) Transfer the entire contents of the tube with magnetic beads (960 µl) into the bottle with the Binding buffer. This mixture can be stored for

7 days. Carefully mix the prepared suspension of magnetic beads in the Binding buffer before each use.

5) Place the tip comb in a free 96 plate (200 µl) for KingFisher according to the Instructions for Use for KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification;

5) Label 96-well plates for KingFisher and add reagents into them according to the scheme (Table 8):

Table 8 – Reagent introduction scheme into plates when using configuration form 2 for automated isolation using KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification

Plate number	Plate type	Added component	Amount added into each plate well
Plate 1	Plate (200 µl) for KingFisher 96	Tip comb for DW magnets for KingFisher 96	-
Plate 2	96-well deep plate (2200 µl) for KingFisher	Mixture of magnetic beads and a binding buffer	510 µl
Plate 3	96-weel deep plate for KingFisher	Wash solution	700 µl
Plate 4	96-weel deep plate for KingFisher	Eluent	100 µl

A single-channel or multi-channel variable-volume automatic dispenser can be used to add reagents. Attention: add each reagent with a separate tip, the reagent remains must not get into the other.

5) Prepare the equipment according to the Instructions for Use;

6) Upload the protocol from the KingFisher Flex - NA-Extra-SW file to the device (attached file), corresponding to the diagram below:

- Tip comb for magnets selection (Tips) (Plate 1)
- lysis (Plate 2): duration – 5 minutes, heating – fast, temperature – 80°C, medium intensity mixing;
- isolation (Plate 2): duration – 6 minutes, temperature – 25°C, medium intensity mixing, magnetic bead collection – 15 seconds;
- washing 2 (Plate 3): duration - 2 minutes, temperature – 25°C, mixing, collecting magnetic beads – 15 seconds,
- magnetic beads drying – 2 minutes;

- elution (Plate 4): duration – 6 minutes, temperature – 80°C, mixing – slow, magnetic bead collection – 15 seconds.

10. Testing procedure

Only specially trained personnel with PCR analysis skills can work with the kit.

DNA / RNA isolation procedure using NA-Extra-SW reagent kit, configuration form 1, when manually isolated using a magnetic rack.

Prepare and label one 1.5-2.0 ml tube for each test sample.

1. Add 500 µl of Binding Buffer and 10 µl of Magnetic Bead solution and ICS solution (if provided for this analysis) into each tube. If the magnetic beads are premixed with Binding Buffer, transfer 510 µl of the magnetic beads and binding buffer mixture and 10 µl of ICS (if provided for this analysis) into each tube.
2. Add 100 ml of clinical material sample into each tube, mix on a vortex for 3-5 seconds.
3. For lysis, incubate the tubes at 70°C for 5 minutes, mixing the solution every 2 minutes using vortex.
4. Upon lysis completion, transfer the tubes to a laboratory rack and incubate DNA/RNA binding mixture at room temperature for 10 minutes, mixing the solution every 3 minutes during incubation by turning the tubes over.
5. Upon incubation completion, remove drops by short centrifugation, then place the tubes in a magnetic separation rack and wait until the beads collect completely on the tube wall (usually it takes 1-2 minutes) and remove carefully the supernatant using a separate tip for each sample.
6. Add 700 µl of Wash solution into the tubes, close the lids tightly, resuspend magnetic beads using vortex, and remove drops by short centrifugation.
7. Place the tubes in a magnetic separation rack, wait until the beads collect completely on the tube wall and remove carefully the supernatant similar to step 5.

ATTENTION! Residual amounts of supernatant in tubes during steps 5 and 7 may have a negative effect on PCR. The supernatant must be carefully removed.

8. Place the tubes with the lids open in a thermostat and incubate at 70°C for 5 minutes to dry the magnetic beads and remove residual alcohol.

9. Add 50 µl of eluent into the tubes using a separate filter tip. Carefully resuspend the magnetic beads by pipetting, and close the lids tightly.

10. Incubate the tubes at 70°C for 10 minutes. During incubation, mix the tube contents 2-3 times gently shaking the sediment.

11. Place the tubes in a magnetic separation rack and wait until the beads collect completely on the tube wall.

12. Transfer the supernatant containing the isolated DNA/RNA into a new tube.

ATTENTION! The purified DNA/RNA is isolated without removing the tubes from the magnetic separation rack.

If the isolation purpose was to obtain RNA, it is recommended to use it immediately for the reverse transcription reaction.

DNA/RNA isolation procedure using NA-Extra-SW reagent kit, configuration form 1, when manually isolated using a centrifuge.

For each test sample, prepare and label one 1.5-2.0 ml tube.

1. Add 500 µl of Binding Buffer and 10 µl of magnetic bead solution and ICS solution (if provided for this analysis) into each tube. If the magnetic beads are premixed with Binding Buffer, transfer 510 µl of the magnetic beads and binding buffer mixture and 10 µl of ICS (if provided for this analysis) into each tube.

2. Add 100 µl of clinical material sample into each tube, mix using vortex for 3-5 seconds.

3. For lysis, incubate the tubes at 70°C for 5 minutes, mixing the solution every 2 minutes using vortex.

4. Upon lysis completion, transfer the tubes to a laboratory rack and incubate DNA/RNA binding mixture at room temperature for 10 minutes, mixing the solution every 3 minutes during incubation by turning the tubes over.

5. Upon incubation completion, centrifuge the tubes at 10,000 g for 1 min.

6. Carefully, without touching the sediment on the inner wall of the tube, take the supernatant with a dispenser or aspirator using a separate tip for each sample.

7. Add 700 µl of Wash solution into the tubes, close the lids tightly, resuspend magnetic beads on the vortex, and remove drops by short centrifugation.

8. Centrifugate the tubes at 10,000 g for 1 min.

9. Carefully remove the supernatant similar to step 6.

10. Place the tubes with the lids open in a thermostat and incubate at 70°C for 5 minutes to dry the magnetic beads.

11. Add 50 µl of eluent into the tubes using a separate filter tip. Carefully resuspend the magnetic beads by pipetting, and close the lids tightly.

12. Incubate the tubes at 70°C for 10 minutes. During incubation, mix the tube contents 2-3 times gently shaking the sediment.

13. Centrifugate the tubes at 10,000 g for 1 min.

14. Using a dispenser, carefully transfer the supernatant containing the isolated DNA/RNA into new tubes without touching the sediment with the tip.

ATTENTION! If the sample is not transferred into a separate tube within 3 minutes after centrifugation, it is required to perform a second centrifugation.

If the isolation purpose was to obtain RNA, it is recommended to use it immediately for the reverse transcription reaction.

DNA/RNA isolation procedure using NA-Extra-SW reagent kit, configuration form 1, in conjunction with Tecan Freedom EVO® automated sample preparation workstation:

1. Upload the appropriate isolation protocol for work with NA-Extra SW kit in the TECAN Freedom EVO® workstation software.

2. Start the workstation;

3. Upon DNA/RNA isolation completion, remove the tubes with the isolated DNA/RNA from the workstation.

4. If the isolation purpose was to obtain RNA, it is recommended to use it immediately for the reverse transcription reaction.

5. Remove used consumables from the workstation and clean according to the Instructions for Use.

DNA/RNA isolation procedure using NA-Extra-SW reagent kit, configuration form 1, in conjunction with KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification.

1. Add 100 ml of the clinical material test sample into the wells of plate 2, mix by pipetting.
2. Place the plates according to the device Instructions for Use and start DNA/RNA isolation according to the uploaded protocol of KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification;
3. Upon the device operation completion, a supernatant containing isolated DNA/RNA is in the wells of the plate 4.

DNA/RNA isolation procedure using NA-Extra-SW reagent kit, configuration form 2, together with KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification.

1. Add 100 ml of the clinical material test sample into the wells of plate 2, mix by pipetting.
2. Place the plates according to the device Instructions for Use and start DNA/RNA isolation according to the uploaded protocol of KingFisher Flex magnetic bead processor for nucleic acids, cells and proteins purification;
3. Upon the device operation completion, a supernatant containing isolated DNA/RNA is in the wells of the plate 4.

11. Possible problems and their solutions

1. Low DNA/RNA yield, cause and possible solution:

▪ sample condition (the sample contains insufficient DNA / RNA; the sample was stored for a long time, or improperly stored, or was frozen-thawed several times) – possible solutions: take more initial material or elute in less amount of buffer; recollect the material;

▪ incomplete beads drying before Eluent addition - increase the drying time (incubation at 70°C) after the Wash solution removal;

▪ incomplete lysis – after applying the lysing solution, suspend the sample as thoroughly as possible (for lysis, place tubes with open lids in a thermostat and incubate at 70°C for 5 minutes to dry the magnetic beads);

2. Protein impurities – it is required to achieve the most thorough magnetic bead suspension.

3. Possible DNA/RNA degradation, reason and possible solution – an old sample, or the sample was frozen/thawed - it is required to recollect the material. Do not freeze the sample during transportation and storage.

12. Kit storage, transportation and operation conditions

Storage. Store a reagent kit in the manufacturer's packaging at +2°C... +30°C. Atmospheric pressure is not subject to control because it does not affect the product quality.

A reagent kit stored in violation of the regulated regime cannot be used.

The shelf life of the opened kit components – 12 months from the acceptance date of the manufacturer's QCD, if stored at +2°C... +30°C. The shelf life of the Magnetic Beads and the Binding Buffer mixture – up to 7 days.

A reagent kit stored in violation of the regulated regime cannot be used.

Transportation. Transport NA-Extra-SW reagent kit by all transport types in covered vehicles in accordance with the transportation rules applicable to this transport type. Transport the reagent kit at +2°C... +30°C. Atmospheric pressure is not subject to control because it does not affect the product quality. Reagent kits transported in violation of the temperature regime cannot be used.

Shelf life. NA-Extra-SW kit shelf life is 12 months from the acceptance date of the manufacturer's QCD, if all transportation, storage and operation conditions are met. A reagent kit with expired shelf life cannot be used.

13. Disposal

Reagent kits that have become unusable, including due to expiration dates, must be disposed of in accordance with the requirements of SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for

the maintenance of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial and public premises, organization and conduct of sanitary and anti-epidemic (preventive) measures".

According to the medical waste classification, the kits belong to Class A (epidemiologically safe waste, similar in composition to solid household waste). Unused reagents in accordance with SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, soils, residential premises, operation of industrial and public premises, organization and conduct of sanitary and anti-epidemic (preventive) measures" they are collected in reusable containers or disposable bags of any color (except yellow and red).

The remaining tubes and materials after the work are disposed of in accordance with MU 287-113 (Guidelines for disinfection, pre-sterilization cleaning and sterilization of medical devices).

Liquid components (reagents) are destroyed by draining into the sewer with preliminary reagent dilution with tap water 1:100 and removal of the remaining packaging as industrial or household waste.

NA-Extra-SW reagent kit consumer packaging is subject to mechanical destruction with the removal of residues as industrial or household waste.

Personnel destroying a reagent kit must comply with the safety rules of a particular destruction method.

14. Warranty, contacts

The manufacturer guarantees the reagent kit quality and safety during the shelf life in compliant with the kit transportation and storage requirements, as well as rules of operation.

In case of complaints about the kit quality, undesirable events or incidents, submit information to:

Limited Liability Company TestGene (TestGene LLC),
9, 44th Inzhenerny Proezd, office 13, Ulyanovsk, 432072
Phone number: +7 (499) 705 03 75
www.testgene.com

Technical Support Service:
Phone number: +7 927 981 58 81
E-mail: help@testgen.ru

Annex A

GOST ISO 14971-2021	Medical devices. Application of risk management to medical devices.
GOST R 51088-2013	In vitro diagnostic medical devices. Reagents, kits, the test-systems, control materials, culture media. Requirements to devices and to supporting documentation.
GOST R ISO 23640-2015	In vitro medical devices. Evaluation of stability of in vitro diagnostic reagents.
GOST R ISO 18113-1-2015	In vitro diagnostic medical devices. Information supplied by the manufacturer (labelling). Part 1. Terms, definitions and general requirements.
GOST R ISO 18113-2-2015	In vitro diagnostic medical devices. Information supplied by the manufacturer (labelling). Part 2. In vitro diagnostic reagents for professional use.
GOST R ISO 15223-1-2020	Medical devices. Symbols to be used with medical device labels, labelling and information to be supplied. Part 1. General requirements.
GOST ISO 13485-2017	Medical devices. Quality management systems. Requirements for regulatory purposes.

Annex B

Labeling symbols

	Contains sufficient for < n > tests
	Consult instructions for use
	In vitro diagnostic medical device
	Temperature limitation
	Batch code or Lot number
	Use by…
	Date of manufacture
	Fragile, handle with care
	This icon shows the correct position of the load in space. This side up. Do not turn over or tip on its side a transport packaging with this symbol. Store and transport it vertically only.