DNA-TECHNOLOGY

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For research use only

Shigella/EIEC and Salmonella spp.
REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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R1-P503-UA/9EU

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1. INTENDED USE

The **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit** is designed to detect DNA of Shigella and enteroinvasive E.coli (EIEC) (without differentiation) and Salmonella genus pathovar bacteria in human biological material (feces) and bacterial cultures obtained from this biomaterial, by real-time PCR.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit.**

The **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit** can be used in research practice.

Potential users: qualified personnel trained in molecular research methods and rules of work in the laboratory.

It is necessary to apply the kit only as directed in this instruction for use.

2. METHOD

Method: polymerase chain reaction (PCR) with real time results detection; qualitative multiplex analysis.

The implemented PCR method is based on amplification of a target DNA sequence. The amplification process consists of a series of repeated cycles of temperature denaturation of DNA, annealing of primers with complementary sequences, and subsequent completion of the polynucleotide chains from these primers with Taq polymerase.

To increase the sensitivity and specificity of the amplification reaction, the use of a “hot” start is provided. For package S, “hot” start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin. The polymerase chain reaction starts only when paraffin is melted. This eliminates the nonspecific anchoring of primers with the DNA target at lower temperatures. “Hot” start for package U is provided by using polymerase which activity is blocked by antibodies. The activation of the enzyme occurs only after preheating the reaction mixture at 94 °C. This eliminates the nonspecific anchoring of primers with the DNA target at lower temperatures.

The PCR-mix includes reagents for total bacterial load (TBL) detection. TBL value is necessary as a sample intake control (SIC) as well as for analysis of quality of DNA extraction from biomaterial samples and allows to determine if the amount of obtained DNA is sufficient for the test.

DNA probes each containing a fluorescent label and a fluorescence quencher are introduced into the PCR-mix. When a specific product is formed, the DNA probe is destroyed and the quencher stops affecting the fluorescent label, which leads to an increase in the fluorescence level. The number of destroyed probes (and hence the fluorescence level) increases in proportion to the number of specific amplification products formed. The fluorescence level is measured at each amplification cycle in real time.

The DNA probes used to detect the amplification products of genome fragments of the bacteria being detected include the Hex, Rox, and Cy5 fluorescent tags. The DNA probes used to detect total bacterial load (TBL) amplification product include the fluorescent dye Fam. Table 1 shows the detection channels of amplification products.

The use of several fluorescent dyes allows to reduce the number of tubes since it makes simultaneous detection of the results of different amplification reactions taking place in the same tube.

Table 1. Detection channels of amplification products

Fam	Hex	Rox	Cy5	Cy5.5
TBL*	*Salmonella spp.*	*Shigella/EIEC(ipaH)*	*Shigella/EIEC(invE)*	-

* - control indicator

The automatic analysis available on "DNA-Technology" made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit** (see the catalogue at https://www.dna-technology.com to see available supply options). The current version of the software is available for download at https://www.dna-technology.com/software.

The **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit** is also approved for use with CFX96 (Bio-Rad) real-time thermal cyclers.

3. CONTENT

The **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit** contents is represented in Tables 2 - 4.

Table 2. The **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit** content, package S, strips, for R1-H503-S3/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless or pink transparent liquid under waxy white fraction	1920 µL (20 µL in each)	tubes, 12 strips of 8
Taq-polymerase solution	Colorless transparent liquid	1000 µL (500 µL in each tube)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL in each tube)	2 tubes
Positive control[1]	Colorless transparent liquid	130 µL	1 tube
Strip caps	12 strips of 8		

Table 3. The **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit** content, package S, tubes, for R1-H503-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless or pink transparent liquid under waxy white fraction	1920 µL (20 µL in each)	96 individual tubes
Taq-polymerase solution	Colorless transparent liquid	1000 µL (500 µL in each tube)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL in each tube)	2 tubes
Positive control[1]	Colorless transparent liquid	130 µL	1 tube

[1] - marking as C+ is allowed

Table 4. The **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit** content, package U, for R1-H503-UA/9EU

Reagent	Description	Total volume	Amount
PCR-mix	Colorless or pink transparent liquid	600 µL	1 tube
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	30 µL	1 tube
PCR-buffer	Colorless transparent liquid	600 µL	1 tube
Positive control[1]	Colorless transparent liquid	130 µL	1 tube

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

The **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit** (package S) is intended for 96 samples (no less than 24 runs), including the unknown samples, negative and positive controls.

The **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit** (package U) is intended for 96 samples and requires no less than 5 samples in a single run (3 test samples, negative control and positive control).

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile containers to collect clinical material.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) at least 16000);
- Thermostat (temperature range 50-98 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Pipette stand;
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- Nucleic acid extraction kit ("DNA-Technology" made, **PREP-NA** REF P-002/1EU, **PREP-NA PLUS** REF P-002/2EU, **PREP-GS** REF P-003/1EU, **PREP-GS PLUS** REF P-003/2EU, **PREP-MB MAX** REF P-103-N/4EU, P-103-A/8EU, **PREP-OPTIMA** REF P-016-1/2EU, REF P-016-N/2EU are recommended);
- Physiological saline solution 0.9% NaCl (sterile);
- Container for used pipette tips, tubes and other consumables;

- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Freezing chamber (in case of using package U);
- Vortex mixer;
- Vortex rotor for 0.2 mL strips (in case of using package S, strips);
- PCR tube rack for 0.2 mL tubes or strips;
- 1.5 mL tubes;
- Tube rack for 1.5 mL tubes;
- Single channel pipettes (dispensers covering 2.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- Pipette stand;
- RNase and DNase free 1.5 mL microfuge tubes with caps;
- RNase and DNase free 0.2 mL PCR tubes or 96-well microplate;
- DTstream *M1 dosing device (“DNA-Technology”, LLC) (only for automated dosing in case of using package U);
- RNase and DNase free filtered pipette tips (volume 200 µL) for DTstream dosing device (only for automated dosing in case of using package U);
- Device for plate sealing DTpack (“DNA-Technology”, LLC) (only for automated dosing in case of using package U);
- Centrifuge for microplates (RCF(g) at least 100) (only for automated dosing in case of using package U);
- Polymer thermal film for microplate sealing (in case of using package U);
- 384-well microplate (only for automated dosing in case of using package U);
- Powder-free surgical gloves;
- Disinfectant solution;
- Container for used pipette tips, tubes and other consumables.

Post-amplification – amplification detection area:

Real-time PCR thermal cycler.

Software:

The most recent version of the DT thermal cyclers software can be downloaded from https://www.dna-technology.com/software.

The OS supported: all versions of Windows starting from 7.

5. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 months from the date of production.

5.1. Transport and storage conditions. Package S

- All components of the reagent kit must be stored in a refrigerator or a cooling chamber at the temperature from 2 °C to 8 °C over the storage period.
- Paraffin sealed PCR-mix must be kept away from light.
- Transport of the reagent kit is carried out in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container corresponding to the storage conditions of the components included in the kit.
- It is allowed to transport the kit in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container from 2 °C to 25 °C for no longer than 5 days.

5.2. Transport and storage conditions. Package U

- All components of the reagent kit, except for TechnoTaq MAX polymerase, must be stored in a refrigerator or a cooling chamber at the temperature from 2 °C to 8 °C over the storage period.
- PCR-mix must be kept away from light.
- TechnoTaq MAX polymerase must be stored in a freezer at the temperature from minus 22 °C to minus 18 °C over the storage period.
- Transport of the reagent kit is carried out in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container corresponding to the storage conditions of the components included in the kit.
- It is allowed to transport the kit, except for TechnoTaq MAX polymerase, in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container from 2 °C to 25 °C for no longer than 5 days.
- It is allowed to transport TechnoTaq MAX polymerase in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container up to 25 °C for no longer than 5 days.

WARNING! Reagent kits transported with violation of temperature conditions shall not be used.

5.3. Shelf-life of the kit following the first opening of the primary container

5.3.1. Package S

- All components of the kit must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C over the storage period.
- Paraffin sealed PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light over the storage period.

5.3.2. Package U

- All components of the kit, except for TechnoTaq MAX polymerase, must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C over the storage period;
- PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light over the storage period;
- TechnoTaq MAX polymerase must be stored in a freezer at temperatures from minus 22 °C to minus 18 °C during the storage period.

WARNING! The kits stored under undue regime should not be used.

An expired **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit** should not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

The conformity of the **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular research are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the analysis 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 analysis. Wear powder-free surgical gloves. Wear 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 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, PCR-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.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial DNA synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and can not 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. Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be exclusively employed for this specific purpose. Remove PCR waste only in a closed form. 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.

Do not open the tubes after amplification. 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.

Emergency actions

Inhalation: Inhalation of the PCR-mix contained within this kit is unlikely, however care should be taken.

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, 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:

- When transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When 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.

7. SAMPLES

The **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from human biological material (feces) and bacterial cultures obtained from this biomaterial.

WARNING! It is not recommended to carry out the analysis earlier than two days after the end of enterosorbents intake.

7.1. General requirements

The quality of sampling, sample storage, transport and pretreatment are of great importance for obtaining correct results.

Incorrect sampling may lead to unreliable results and, therefore, to the necessity for repeated sampling.

Use RNase and DNase free filtered tips during biomaterial preparation and NA extraction.

To prevent contamination, only open the cap of the tube you are working with and close it before proceeding to the next tube.

7.2. Interfering substances

The presence of PCR inhibitors in a sample may cause doubtful (uncertain/unreliable) results. The sign of PCR inhibition is the simultaneous absence of TBL amplification and specific product amplification.

PCR inhibitors include the following substances: hemoglobin, bilirubin, cholesterol, triglycerides, mucus (mucin) and pharmaceuticals left in DNA sample as a result of their incomplete removal during DNA extraction from biomaterial sample, as well as isopropyl alcohol and methyl acetate that can be present in DNA sample as a result of wash solutions' incomplete removal during sample preparation.

The maximum concentrations of interfering substances are indicated in the table below.

Biological material	Interfering substance	Interfering substance concentration
Endogenous substances		
Feces	Bilirubin	684 µmol/L
	Cholesterol	13 mmol/L
	Hematoglobulin	0.35 mg/mL
	Triglycerides	37 mmol/L
	Mucin	20%
Exogenous substances		
Feces	Isopropyl alcohol	10%
	Methyl acetate	10%
	Ibuprofen suppositories	5%
	Espumisan emulsion	5%
	Viferon suppositories	10%

Impurities contained in the biomaterial sample, such as mucus, blood, local medicines, including those that are contained in rectal suppositories should be removed during the DNA extraction using sample preparation kits. To reduce the amount of PCR inhibitors, it is necessary to follow the principles of taking biological material.

7.3. Sample collection

Feces

Method limitations: it is recommended to conduct the analysis not earlier than two days after finishing the intake of enterosorbents.

Sampling is performed in accordance with the instructions to the used NA extraction reagent kits.

If using PREP-NA, PREP-NA PLUS, PREP-GS, PREP-GS PLUS reagent kits for DNA extraction: Samples of feces with mass (volume) 1.0-3.0 g (1.0-3.0 mL) are transferred to a sterile dry flask by a single-use filtered pipette tip or single-use spoon in the amount of approximately 1.0 g.

After sample collection the flask is tightly closed with a lid and marked.

WARNING! Before DNA extraction pre-processing of biological material samples is needed.

Bacterial cultures

Sampling is performed in accordance with the instructions to the used NA extraction reagent kits.

If using PREP-NA, PREP-NA PLUS, PREP-GS, PREP-GS PLUS reagent kits for DNA extraction: Sample taking from liquid and solid media is made with single-use microbiological loop or spatula. Place a sole colony of cells or 100 µL of liquid media in single-use 1.5-2.0 mL tube with 500 µL of sterile physiological saline solution.

Close the tubes tightly and mark them.

7.4. Transport and storage of the samples

Transport and storage conditions of biomaterial samples are stated in the instructions for use of the NA extraction reagent kits or the transport media used for transport and storage of samples.

It is allowed to transport and store[2]:

[2] - if it does not contradict the requirements for the used NA extraction reagent kits

Feces

Feces samples can be transported and stored:

- at room temperature from 18 °C to 25 °C for no longer than 6 hours;
- at temperature from 2 °C to 8 °C for no longer than 3 days.

Feces suspension with glycerin:

- at minus 20 °C for one week;
- at minus 70 °C for prolonged period.

Bacterial cultures

Bacterial cultures samples can be transported and stored:

- at room temperature from 2 °C to 8 °C for no longer than 24 hours;
- at temperature from minus 20 °C to minus 18 °C for no longer than one week.
- at minus 70 °C for prolonged period.

7.5. Sample preparation (preparation of the suspension)

Sample preparation (if necessary) is performed in accordance with the instructions for use for the NA extraction reagent kits.

If using PREP-NA, PREP-NA PLUS, PREP-GS, PREP-GS PLUS, PREP-MB MAX reagent kits for DNA extraction:

7.5.1. Feces

7.5.1.1 Prepare the necessary amount of 1.5 mL single-use plastic tubes with 1.0 mL of sterile physiological saline solution.

7.5.1.2 Place approximately 0.1-0.2 g (mL) of feces into each tube.

7.5.1.3 Resuspend the contents of the tubes thoroughly on vortex for 5-10 seconds.

Note:

If it is impossible to analyze the biomaterial on the same day and/or long-term storage is necessary, glycerol at a final concentration of 10-15% is added to the fecal suspension in sterile isotonic sodium chloride solution. Samples prepared in this way are frozen only after thorough homogenization and exposure to glycerol for 30-40 minutes.

7.5.1.4 Centrifuge the tubes with feces suspension at RCF(g) 13,000 at room temperature (from 18 °C to 25 °C) for 30 seconds to precipitate debris to the bottom of the tube.

7.5.1.5 Mark one 1.5 mL single-use tube for each test sample and negative control “C-“.

7.5.1.6 Add middle fraction from tubes with feces suspension into the corresponding marked tubes. To do this, collect bacterial fraction (upper white and yellow part of the precipitate) using a new tip for each tube: 100 µL (for PREP-NA, PREP-NA PLUS or PREP-MB MAX extraction kits) or 50 µL (for PREP-GS or PREP-GS PLUS extraction kits). If there is no white-yellow boundary layer between the precipitate and supernatant, take 100 or 50 µL from the precipitate-supernatant boundary, partial capture of the precipitate is allowed.

7.5.1.7 Add 100 or 50 µL of negative control into the tube “C-“ according to the instruction of the used extraction kit.

Samples are ready for DNA extraction.

7.5.2. Bacterial cultures

Resuspend the contents of the tube thoroughly for 5-10 seconds on a vortex mixer, spin down the drops for 1-3 seconds. 100 µL (for PREP-NA, PREP-NA PLUS extraction kits) or 50 µL (for PREP-GS or PREP-GS PLUS extraction kits) are used for DNA extraction.

8. Samples are ready for DNA extraction.

PROCEDURE

DNA extracting from biological material

DNA extraction is carried out according to the instruction for use for the kit of reagents.

Kits recommended for DNA extraction (manufactured by "DNA-Technology"):

Reagent kit	Biomaterial	Minimal eluate volume, µL
PREP-NA (including short method according to Annex A)	Feces, bacterial cultures	50
PREP-NA PLUS (including short method according to Annex A)	Feces, bacterial cultures	300
PREP-GS	Feces, bacterial cultures	100
PREP-GS PLUS	Feces, bacterial cultures	300
PREP-OPTIMA	Feces, bacterial cultures	400
PREP-MB MAX	Feces	50

WARNING! Simultaneously with DNA extraction from biological material a negative control must be prepared and carried through all the stages of sample preparation. Physiological saline solution can be used as a negative control sample in volumes as indicated in the instructions for use of extraction kits or negative control sample that is include in the corresponding extraction kit.

Analysis procedure

8.1 Preparing PCR for package S:

WARNING! The reagents and tubes should be kept away from direct sun light.

WARNING! When using package S, strips, strictly observe the completeness of the strips and caps. Do not use the caps for the strips of the other kits!

8.1.1 Mark one tube/stripped tube with the paraffin sealed PCR-mix for each test sample, negative control (C-), positive control (C+).

WARNING! The volume of reagents is calculated for no more than 24 runs assuming a variable number of test samples, 1 negative control and 1 positive control per run.

Example: To test 2 samples, mark 2 tubes for samples, one negative control tube "C-" and one positive control tube "C+". Total number of tubes is 4.

8.1.2 Shake the tubes with Taq-polymerase solution on vortex mixer for 3-5 seconds and spin in a vortex mixer for 1-3 seconds.

8.1.3 Add 10 µL of Taq-polymerase solution to each tube. Avoid paraffin layer break.

8.1.4 Add one drop of mineral oil (~20 µL) to each tube. Cover the tubes/strips loosely with caps.

8.1.5 Shake the tube with positive control on vortex mixer and spin down the drops for 1-3 seconds.

WARNING!

1. Before introducing DNA preparation and negative control into tubes with PCR-mix, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using **PREP-NA**, **PREP-NA PLUS**, **PREP-GS**, **PREP-GS PLUS** extraction kits (only if supernatant containing the extracted DNA was transferred to new tubes after extraction), mix the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.
3. In case of using **PREP-MB MAX** DNA extraction kit, it is necessary to carefully, without shaking, spin the tubes with DNA preparation and negative control on vortex for 1-3 seconds and place the

tubes into magnetic rack. If, after extraction, the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation and negative control "C-" on vortex for 3-5 seconds and spin down the drops for 1-3 seconds.

4. To prevent contamination, only open the caps of the tubes into which the sample is to be added and close them before adding the next sample. If strips are used, close the strip caps after adding the sample before proceeding with the next sample. Close the tubes/strips tightly. Use filter tips.

8.1.6 Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the tubes "C-", and "C+". Avoid paraffin layer break.

8.1.7 Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into "C-" tube. Avoid paraffin layer break.

8.1.8 Add 5.0 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

8.1.9 Spin the tubes/strips for 3-5 seconds in a vortex mixer.

8.1.10 Set the tubes/strips into the real-time thermal cycler.

8.1.11 For DT instruments: Launch the operating software for DT instrument[3]. Add corresponding test[4], specify the number and ID's of the samples, positive and negative controls. Specify position of the tubes/strips in thermal unit (see 8.1.10) and run PCR. See Table 5.

8.1.12 For CFX96 thermal cyclers: perform PCR considering reaction mixture volume of 35 µL according to amplification programs shown in Table 6.

Table 5. The PCR program for DTlite and DTprime thermal cyclers for package S

Step	Temperature, °C	Min	Sec	Number of cycles	Optical measurement		Type of the step
1	80	0	30	1			Cycle
	94	1	30				
2	94	0	30	5			Cycle
	64	0	15		√		
3	94	0	10	45			Cycle
	64	0	15		√		
4	94	0	5	1			Cycle
5	25[1]	…	…	Holding			Holding
√- optical measurements [1] - holding at 10 °C is allowed							

Table 6. The PCR program for CFX96 (Bio-Rad) thermal cyclers for packages S, U

Step	Temperature, °C	Time, min:sec	Number of cycles (repeats)
1	80	01:00	1
2	94	01:30	1
3	94	00:15	50
4	64 **√**	00:20	
√ - optical measurements (Plate Read), set the fluorescence measurement on the Fam, Hex, Rox and Cy5 channels at 64 °C			

[3] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.

[4] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

8.2 Preparing PCR for package U, manual dosing

WARNING! The reagents and tubes should be kept away from direct sun light.

WARNING! For amplification use 0.2 mL single-use amplification tubes or 96-well PCR microplates[5], sealed hermetically with thermal film. It is not recommended to use strips due to postamplification contamination hazard.

8.2.1 Mark the required number of 0.2 mL tubes or a 96-well microplate for each test sample, negative control (C-) and positive control (C+).

Note: It is recommended to test at least 5 samples per test (3 test samples, negative control and positive control).

Example: to test 4 samples, mark 4 tubes/reserve 4 wells for samples, 1 tube/well for "C-" and 1 tube/well for "C+". The resulting number of tubes/wells is 6.

8.2.2 Vortex the tube with PCR-mix for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

8.2.3 Add 6.0 µL of PCR-mix to each tube/well (including "C-" and "C+").

8.2.4 Vortex the tube with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

WARNING! Take TechnoTaq MAX polymerase out from the freezer immediately prior to use.

8.2.5 Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase. Add into the one tube:

6.0 x (N+1) µL of PCR-buffer,
0.3 x (N+1) µL of TechnoTaq MAX polymerase,
where N is the quantity of samples to be tested taking to account "C-", "C+".

Example: to test 4 samples, "C-" and "C+" in one PCR run, mark 6 tubes/reserve 6 wells (4 tubes/wells for test samples, 1 tube/well for "C-" and 1 tube/well for "C+"). Prepare the mixture of PCR-buffer and Taq-polymerase for 7 (6+1) tubes/wells. Mix 42 µL of PCR-buffer and 2.1 µL of TechnoTaq MAX polymerase.

8.2.6 Vortex the tube with the mixture of PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin on vortex for 1-3 seconds to collect the drops.

WARNING! Mixture of PCR-buffer and TechnoTaq MAX polymerase must be prepared immediately prior to use.

8.2.7 Add 6.0 µL of PCR-buffer and TechnoTaq MAX polymerase mixture into each tube/well with PCR-mix. Cover the tubes loosely.

WARNING! Follow the steps listed in pp. 8.2.8 – 8.2.14 within two hours after adding PCR-buffer and TechnoTaq MAX polymerase mixture to PCR-mix.

8.2.8 Vortex the tube with positive control "C+" for 3-5 seconds and spin down the drops for 1-3 seconds.

WARNING!

1. Before introducing DNA preparation and negative control into tubes/wells with PCR-mix, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using **PREP-NA**, **PREP-NA PLUS**, **PREP-GS**, **PREP-GS PLUS** extraction kits (only if supernatant containing the extracted DNA was transferred to new tubes after extraction), shake the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.
3. In case of using **PREP-MB MAX** DNA extraction kit, it is necessary to carefully, without shaking, spin the tubes with DNA preparation and negative control on vortex for 1-3 seconds and place the

[5] - 96-well plates are not used with DTlite detecting thermal cycler

tubes into magnetic rack. If, after extraction, the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation and negative control "C-" on vortex for 3-5 seconds and spin down the drops for 1-3 seconds.

4. To prevent contamination, only open the caps of the tubes into which the sample is to be added and close them before adding the next sample. Close the tubes/strips tightly. Use filter tips.

8.2.9 Add 6.0 µL of DNA sample into corresponding tubes/wells. Do not add DNA into the "C-", "C+" tubes/wells.

8.2.10 Add 6.0 µL of negative control (C-) which passed whole DNA extraction procedure into the corresponding tube/well.

8.2.11 Add 6.0 µL of positive control sample (C+) into the corresponding tube/well.

8.2.12 In case of using 96-well microplates:

8.2.12.1. Place the plate carefully, without shaking into the DTpack sealing device.

8.2.12.2. Seal the PCR microplate with polymer thermal film according to the DTpack operation manual.

8.2.12.3. Centrifuge the plate at RCF(g) 100 for 30 seconds.

8.2.13 In case of using tubes:

Spin the tubes for 3-5 seconds on vortex mixer.

8.2.14 Set the tubes into the Real-time Thermal Cycler.

8.2.15 Launch the operating software for DT instrument[6]. Add corresponding test[7], specify the number and IDs of the samples, positive and negative controls. Specify position of the tubes in thermal unit (see 8.2.14) and run PCR. See Table 7.

8.2.16 For CFX96 thermal cyclers perform PCR considering the volume of reaction mixture of 18 µL. See Table 6.

Table 7. The PCR program for DTlite and DTprime thermal cyclers for package U

Step	Temperature, °C	Min	Sec	Number of cycles	Optical measurement	Type of the step
1	80	0	05	15		Cycle
	94	0	05			
2	94	5	00	1		Cycle
3	94	0	30	5		Cycle
	64	0	15		√	
4	94	0	10	45		Cycle
	64	0	15		√	
5	94	0	5	1		Cycle
6	25[1]			Holding		Holding

√ - optical measurements
[1] - holding at 10 °C is allowed

[6] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[7] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

8.3 Preparing PCR for package U, using DTStream (only for DTprime *X*)

WARNING! The reagents and tubes should be kept away from direct sun light.

WARNING! For amplification use 384-well microplates hermetically sealed with thermal film.

Note – It is recommended to test at least 5 samples in 1 run (3 test samples, negative control and positive control).

8.3.1 Vortex the tube with PCR-mix for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

8.3.2 Vortex the tube with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

WARNING! TechnoTaq MAX polymerase should be got out from the freezer immediately prior to use.

8.3.3 Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase according to the software for DTstream.

8.3.4 Vortex the tube with the mixture PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

8.3.5 Vortex the tubes with positive control for 3-5 seconds and spin in vortex for 1-3 seconds to collect the drops.

WARNING!

1. Before dosing of DNA preparation and negative control, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using **PREP-NA**, **PREP-NA PLUS**, **PREP-GS**, **PREP-GS PLUS** extraction kits (only if supernatant containing the extracted DNA was transferred to new tubes after extraction), shake the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.
3. In case of using **PREP-MB MAX** DNA extraction kit, it is necessary to carefully, without shaking, spin the tubes with DNA preparation and negative control on vortex for 1-3 seconds and place the tubes into magnetic rack. If, after extraction, the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation and negative control "C-" on vortex for 3-5 seconds and spin down the drops for 1-3 seconds.

8.3.6 Set the tubes with PCR-mix, the mixture of PCR-buffer and TechnoTaq MAX polymerase, DNA samples, positive and negative controls and PCR microplate on the DTstream working table and conduct dosage of the components according to DTstream user manual.

8.3.7 After the end of dosing program on DTstream put the PCR microplate without shaking on the working table of DTpack sealing device.

8.3.8 Run the process of sealing of PCR microplate according to the user manual of DTpack sealing device.

8.3.9 Centrifuge the microplate at RCF(g) 100 for 30 seconds.

8.3.10 Set the PCR microtray into the Real-time Thermal Cycler.

8.3.11 Launch the operating software for DT instrument[8]. Add corresponding test[9], specify the number and ID's of the samples, positive and negative controls. Specify position of the tubes in thermal unit (see 8.3.10) and run PCR. The volume of reaction mixture is 18 µL. See Table 7.

[8] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[9] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

9. CONTROLS

The **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit** contains positive control C+.

Positive control is a cloned part of the bacterial genome. It is produced with genetic engineering techniques and characterized by automatic sequencing.

TBL value is used for sample intake quality control and DNA extraction quality control.

To reveal possible contamination a negative control is required.

WARNING! A negative control sample should go through all stages of DNA extraction. Physiological saline solution or negative control sample from an extraction kit can be used as a negative control sample in volumes indicated in supplied instructions.

If positive control (C+) does **not** express growing fluorescence of the specific product or positive result, it is required to repeat the whole test. It may be caused operation error or violation of storage and handling.

If negative control (C-) expresses growing fluorescence of the specific product or positive result, all tests of the current batch are considered false. Decontamination is required.

10. DATA ANALYSIS

10.1 Registration of the results is carried out automatically during amplification by the software provided with detecting thermocycler.

10.2 When using CFX96 detection thermal cyclers, use regression type analysis (Cq Determination Mode: Regression). In the "Baseline Subtraction" tab select "Baseline Subtraction Curve Fit".

10.3 Result interpretation is carried out according to Tables 8, 9. The results are valid if the conditions for the interpretation of results obtained for control samples are met.

Table 8. PCR results interpretation

Detection channel			Result interpretation
Fam, Cp/Cq	**Hex**, Cp/Cq	**Rox**, **Cy5**, Cp/Cq	
Analyzed samples			
Is not considered	< 37	Is not specified (on both detection channels)	*Salmonella spp.* DNA is detected, *Shigella/EIEC* DNA is not detected
Is not considered	> 39 or is not specified	< 37 (on one or both detection channels)	*Shigella/EIEC* DNA is detected, *Salmonella spp.* DNA is not detected
Is not considered	< 37	< 37 (on one or both detection channels)	*Salmonella spp.* and *Shigella/EIEC* DNA is detected
≤ 35	> 39 or is not specified	Is not specified (on both detection channels)	*Salmonella spp.* and *Shigella/EIEC* DNA is not detected
> 35 or not specified	> 39 or is not specified	Is not specified (on both detection channels)	Invalid result[10]
Negative control			
> 35 or not specified	> 39 or is not specified	Is not specified (on both detection channels)	Negative result. The results are valid
Positive control			
Is specified	Is specified	Is specified (on both detection channels)	Positive result. The results are valid

[10] - repeating of PCR with the existing DNA preparation, or repeating of DNA extraction and PCR, or repeating of biomaterial sampling is required (performed sequentially).

Table 9. Other possible PCR results

Detection channels			Result interpretation*
Fam, Cp/Cq	**Hex**, Cp/Cq	**Rox, Cy5**, Cp/Cq	
≤ 35	≥ 37, but < 39	Is not specified (on both detection channels)	Low content of *Salmonella spp.* DNA*, Shigella/EIEC* DNA is not detected
≤ 35	> 39 or is not specified	≥ 37 (on one or both detection channels)	Low content of *Shigella/EIEC* DNA*, Salmonella spp.* DNA is not detected
≤ 35	≥ 37, but < 39	≥ 37 (on one or both detection channels)	Low concentration of *Salmonella spp.* and *Shigella/EIEC* DNA
* if at the Cp/Cq value on Cy5 detection channel ≤ 35, on Hex detection channel the Cp/Cq value ≥ 37, but ≤ 39 and/or on Fam and/or Hex and/or Rox detection channels the Cp/Cq value ≥ 37 at Cp/Cq value >35 on Cy5 detection channel, the obtained result indicates low specific DNA content that can be due to lower load in clinical sample, cross-contamination, high-copy samples or PCR inhibition. A one-time repeating of biomaterial sampling and/or repeated DNA extraction and PCR is required. In case of result repetition, the final result should be "… DNA is detected".			

10.4 When analyzing the results, it is necessary to consider total bacterial load value (TBL, Fam channel):

- TBL Cp/Cq value ≥ 35 or absent without specific positive results in the tube should be interpreted as an insufficient amount of biomaterial or possible PCR inhibition. Amplification result is "invalid".

10.5 When amplification is finished, in test samples containing DNA of detected analytes the software registers positive result on corresponding channel (Hex, Rox or Cy5). Result interpretation is "detected" ("+"). Positive amplification result for both or any one Shigella/EIEC (ipaH, invE) marker indicated the presence of Shigella/EIEC DNA in the sample.

10.6 When amplification is performed, in test samples not containing DNA of detected analytes the software registers negative result on corresponding channel (Hex, Rox or Cy5) in the tube. Result interpretation is "not detected" ("-").

10.7 If results obtained for negative control do not match the results indicated in Table 8, the results of the whole run are considered unreliable. In this case special actions for indication and elimination of possible contamination are required.

10.8 If values obtained for positive control do not match the values indicated in Table 8, a repeated amplification run for the whole batch of samples is required.

11. SPECIFICATIONS

a. Analytical specificity

For biomaterial samples containing DNA of the detected analytes, the software must register exponential growth of fluorescence level in the corresponding tube in the corresponding detection channels.

For biomaterial samples not containing DNA of the detected analytes, the software does not register exponential growth of fluorescence level in the corresponding tube in the corresponding detection channels and does for total bacterial load (TBL) on Fam detection channel.

Specific positive amplification results were observed in the presence of DNA of the declared pathogens of acute intestinal infections in the samples. Reference strains were obtained from Leibniz-Institut DSMZ — Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, FSBI FSRC of Epidemiology and Microbiology named after N.F. Gamaleya and the collection of L.A. Vorokhobov State Clinical Hospital No. 67.

Confirmation of the kit's analytical specificity was performed on DNA samples from the cultures of claimed microorganisms as well as from cultures of microorganisms that are closely related or are present in clinical sample in significant concentration ($1.0x10^{10}$ CFU/mL): *Akkermansia muciniphila, Alistipes finegoldii, Allisonella histaminiformans, Anaerococcus spp., Astrovirus, Bacteroides ovatus, Bifidobacterium bifidum, Bilophila wadsworthia, Blautia coccoides, Butyricimonas virosa, Campylobacter jejuni, Candida albicans, Clostridioides difficile, Clostridium hathewayi, Clostridium symbiosum, Collinsella aerofaciens, Coprobacter fastidiosus, Coprococcus comes, Desulfovibrio piger, Dialister spp., Dorea spp., Eggerthella lenta, Enterococcus faecalis, Enterococcus faecium, Enterovirus, Escherichia coli K12, Eubacterium limosum, Faecalibacterium prausnitzii, Fusobacterium nucleatum, Lactobacillus salivarius, Lactococcus lactis, Megasphaera cerevisiae, Methanobrevibacter smithii, Methanosphaera stadtmanae, Neisseria flava, Norovirus, Parabacteroides merdae, Peptostreptococcus anaerobius, Prevotella copri, Proteus vulgaris, Pseudomonas aeruginosa, Rotavirus, Ruminococcus spp., Saccharomyces cerevisiae, Salmonella spp., Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Slackia piriformis, Staphylococcus aureus, Streptococcus oralis, enteroinvasive Escherichia coli (EIEC)*, and/or human DNA in concentration up to $1,0×10^8$ copies per mL of sample.

The absence of cross non-specific reactions for each specific system in the kit towards analytes detected by other specific systems in the reagent kit is shown, as well as the absence of non-specific positive amplification results at presence of other microorganisms and/or human DNA in concentration up to $1.0×10^8$ copies per mL of sample in DNA samples.

b. Analytical sensitivity (limit of detection)

LOD (limit of detection) is determined by the analysis of serial dilutions of the laboratory control (LC) and is presented in the table below.
LOD corresponds to the following DNA concentration values obtained using the specified DNA extraction reagent kits/sets and the end elution (dilution) volume of the extracted DNA:

Detected indicator	Copies per amplification tube	Copies per mL of DNA preparation	GE per mL of DNA preparation	Copies per mL of biomaterial (fecal suspension)				
				PREP-NA	**PREP-GS**	**PREP-GS PLUS**	**PREP-OPTIMA**	**PREP-MB MAX, PREP-NA PLUS**
Salmonella spp.	15	$3x10^3$	$1x10^3$	$1.5x10^3$	$5x10^3$	$1.5x10^4$	$2.5x10^4$	$1x10^4$
EIEC/ Shigella (ipaH)	15	$3x10^3$	$1x10^3$	$1.5x10^3$	$5x10^3$	$1.5x10^4$	$2.5x10^4$	$1x10^4$
EIEC/ Shigella (invE)	15	$3x10^3$	$1x10^3$	$1.5x10^3$	$5x10^3$	$1.5x10^4$	$2.5x10^4$	$1x10^4$

c. Analytical sensitivity (limit of detection)

Type of biomaterial	Analyte	Diagnostic sensitivity	Diagnostic specificity
Feces	*Salmonella* spp.	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	Shigella/EIEC	100% (95% CI: 92.89% – 100%)	100% (95% CI: 92.89% – 100%)
Bacterial cultures	*Salmonella* spp.	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
	Shigella/EIEC	100% (95% CI: 92.89% – 100%)	100% (95% CI: 92.89% – 100%)
Total		100% (95% CI: 97.57% - 100%)	100% (95% CI: 97.57% - 100%)

d. Reproducibility and repeatability

Reproducibility is 100%.

Repeatability is 100%.

12. TROUBLESHOOTING

Table 11. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	+	Contamination	Dispose current batch Perform decontamination procedures
IC	Invalid	low amount of DNA\ PCR inhibition	Repeat whole test Resample

If you face to any undescribed issues contact our customer service department:

Phone: +7(495) 640.16.93

E-mail: hotline@dna-technology.ru

https://dna-technology.com/support

13. QUALITY CONTROL

The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our customer service with quality issues of **Shigella/EIEC and Salmonella spp. REAL-TIME PCR Detection Kit**.

Technical support:

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14. KEY TO SYMBOLS

RUO	For research use only		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
	Caution	VER	Version
	Non-sterile		

REF R1-P503-S3/9EU
R1-P503-23/9EU
R1-P503-UA/9EU

VER 840-2.2025.03.31

Annex A

Shortened method of DNA extraction from feces and bacterial cultures) using PREP-NA, PREP-NA PLUS reagent kits

WARNING!

1. Before starting work:
   - preheat the thermostat to 65 °C;
   - take out the NA extraction reagent kit from the refrigerator and check that there is no precipitate in the lysis solution. In case of precipitation heat the vial with lysis solution on thermostat preheated to 65 °C to dissolve the precipitate completely. Then stir the solution by turning the vial upside down 5-10 times, avoiding foaming. Before use, cool the solution to room temperature (18 °C to 25 °C). The precipitate can also be dissolved at room temperature (18 °C to 25 °C) for approximately 12 hours.
2. Tube caps may open during heating! Use tubes with locking caps (e.g. Eppendorf Safe-Lock Tubes) or programmable thermostats with a clamp lid (e.g. solid-state programmable small-size thermostat TT-1-DNA-Technology, manufactured by "DNA-Technology R&P", LLC.).

1. Mark one 1.5 mL single-use plastic tube for each test sample and negative control (C-).
2. Add 300 µL of lysis solution into each tube without touching the edge of the tube
3. Centrifuge the tubes with feces suspension at RCF(g) 13,000 at room temperature (from 18 °C to 25 °C) for 30 seconds to precipitate debris to the bottom of the tube. Add middle fraction from tubes with feces suspension into the corresponding marked tubes. To do this, collect 100 µL of bacterial fraction (upper white and yellow part of the precipitate) using a new tip for each tube. If there is no white-yellow boundary layer between the precipitate and supernatant, take 100 µL from the precipitate-supernatant boundary, partial capture of the precipitate is allowed.
   If the DNA extraction material is bacterial suspension, add 100 µL of samples into the corresponding marked tubes.
4. Add 100 µL of negative control into the C- tube. Close the tubes tightly and shake on vortex for 3-5 seconds.
5. Thermostat the tubes at 65 °C for 5 min.
6. Spin the tubes on vortex for 3-5 seconds.
7. Add 400 µL of precipitation reagent without touching the edge of the tube, close the tubes and shake on vortex for 3-5 seconds.
8. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 10 minutes.
9. Remove supernatant without touching the precipitate. Use separate tip for each tube.
10. Add 500 µL of wash solution No. 1 into each tube without touching the edge of the tube. Close the tubes and turn them upside down carefully 3-5 times.
11. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 1 minute.
12. Remove supernatant without touching the precipitate. Use separate tip for each tube.
13. Add 300 µL of wash solution No. 2 into each tube without touching the edge of the tube. Close the tubes and turn them upside down carefully 3-5 times.
14. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 1 minute.
15. Remove supernatant without touching the precipitate. Use separate tip for each tube. It is allowed to leave liquid covering the precipitate, but no more than 20-30 µL.
16. Open the tubes and dry the precipitate at 65 °C for 5 minutes.
17. Add **50 µL (PREP-NA)** or **300 µL (PREP-NA PLUS)** of solution buffer to precipitate, close the tubes.
18. Shake the tubes on vortex for 3-5 seconds and spin down the drops for 3-5 seconds.
19. Thermostat the tubes at 65 °C for 5 min. Shake the tubes on vortex for 3-5 seconds.
20. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 30 seconds to precipitate the condensation.

DNA preparation is ready for PCR.
DNA preparation can be stored at temperature from minus 22 °C to minus 18 °C for no longer than one month or at from minus 72 °C to minus 68 °C for no longer than one year.
Before using the DNA preparation for PCR thaw the DNA preparation and negative control at room temperature (from 18 °C to 25 °C) or at temperature from 2 °C to 8 °C, then shake the tubes with SDNA preparation and negative control on vortex for 3-5 seconds and spin on vortex for 1-3 seconds.
WARNING! It is only allowed to thaw DNA preparation once!
DNA preparation is ready to be introduced into reaction mixture.