DNA-TECHNOLOGY

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RUO

For research use only

BacScreen AEI 1 REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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REF

R1-P502-S3/9EU
R1-P502-23/9EU
R1-P502-UA/9EU

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1. INTENDED USE

The **BacScreen AEI 1 REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **BacScreen AEI 1 REAL-TIME PCR Detection Kit** is designed for detection of thermophilic campylobacteria *Campylobacter coli* and *Campylobacter jejuni* (without differentiation), *Shigella* bacteria and enteroinvasive *E.coli* (EIEC) (without differentiation), and *Salmonella* spp. bacteria DNA in human biological material (feces) and bacterial cultures obtained from this biomaterial by real-time PCR.

Indications for the use:

- symptoms of infectious diseases caused by acute intestinal pathogens and epidemiologically related cases;
- screening of asymptomatic carriage of *Campylobacter coli/jejuni*, *Shigella/EIEC* and *Salmonella* spp.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **BacScreen AEI 1 REAL-TIME PCR Detection Kit.**

The **BacScreen AEI 1 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: real-time polymerase chain reaction (PCR); 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 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 used to assess the quality of DNA extraction and sufficiency of DNA amount for the analysis.

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 for the detection of specific regions of the bacterial genomes contain Fam, Hex and Rox fluorescent dyes. The DNA probe used for the detection of total bacterial load (TBL) contain Cy5 fluorescent dye.

The use of several fluorescent dyes (multiplex format) reduces the number of tubes in the test by allowing simultaneous detection of all targets in the same tube.

Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam	Hex/Vic	Rox	Cy5	Cy5.5
Campylobacter coli/jejuni	*Salmonella* spp.	*Shigella/*EIEC	TBL*	-

* - control indicator Total Bacterial Load

The automatic analysis of results is available on "DNA-Technology" made instruments: DTprime *M*, *X*, DTlite *S* REAL-TIME Thermal Cyclers for **BacScreen AEI 1 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 **BacScreen AEI 1 REAL-TIME PCR Detection Kit** is also approved for use with CFX96 (Bio-Rad) and Applied Biosystems Quant Studio 5 (Life Technologies Holdings Pte. Ltd) real-time thermal cyclers.

3. CONTENT

The **BacScreen AEI 1 REAL-TIME PCR Detection Kit** content is represented in Tables 2-4.

Table 2. The **BacScreen AEI 1 REAL-TIME PCR Detection Kit** content, package S, strips, for R1-P502-S3/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless or pink transparent liquid under waxy white fraction	1.920 mL (20 µL in each)	tubes, 12 strips of 8
Taq-polymerase solution	Colorless transparent liquid	1.0 mL (500 µL in each)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL in each)	2 tubes
Positive control[1]	Colorless transparent liquid	130 µL	1 tube
Strip caps	12 strips of 8		

Table 3. The **BacScreen AEI 1 REAL-TIME PCR Detection Kit** content, package S, tubes, for R1-P502-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless or pink transparent liquid under waxy white fraction	1.920 mL (20 µL in each)	96 individual tubes
Taq-polymerase solution	Colorless transparent liquid	1.0 mL (500 µL in each)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL in each)	2 tubes
Positive control[1]	Colorless transparent liquid	130 µL	1 tube

[1] - can be marked as C+

Table 4. The **BacScreen AEI 1 REAL-TIME PCR Detection Kit** content, package U, for R1-P502-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[2]	Colorless transparent liquid	130 µL	1 tube

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

The **BacScreen AEI 1 REAL-TIME PCR Detection Kit** (package S) is designed for 96 tests (no more than 24 runs), including analysis of test samples, negative controls and positive controls.

The **BacScreen AEI 1 REAL-TIME PCR Detection Kit** (package U) is designed for 96 tests with at least 5 samples per 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;
- Sterile tubes containing transport medium: “DNA-Technology” made **STOR-F** REF P-901-1/1EU or physiological saline solution (sterile) or equivalent for transportation of the sample.

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) no less than 16,000);
- Solid-state thermostat (temperature range 50-98 °C);
- 1.5 mL tubes;
- Tube rack for 1.5 mL tubes;
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 20-1,000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL; 200 µL; 1,000 µL);
- Pipette stand;
- Magnetic homogenizer (in case of using **PREP-MB MAX** extraction kit);
- Nucleic acid extraction kit (“DNA-Technology” made, **PREP-NA** REF P-002/1EU, **PREP-NA PLUS** REF P-002/1EU, P-036-N/2EU, **PREP-GS** REF P-003/1EU, **PREP-GS PLUS** REF P-003/2EU, **PREP-**

[2] - can be marked as C+

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) (if needed);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution;
- Glycerin (for feces treatment) (if needed).

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator with freezing chamber;
- Vortex mixer;
- Vortex rotor for 0.2 ml strips (in case of using package S, strips);
- PCR tube rack for 0.2 mL tubes (in case of using package S, tubes, and for manual dosing in case of using package U with tubes);
- PCR tube rack for 0.2 mL strips (in case of using package S, strips);
- Tube rack for 1.5 mL tubes;
- Single channel pipettes (dispensers covering 2.0-1,000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL; 200 µL; 1,000 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 (only for manual dosing in case of using package U);
- 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 *M1 dosing device (only for automated dosing in case of using package U);
- Device for plate sealing DTpack (“DNA-Technology”, LLC) (in case of using package U, manual dosing into microplates or automated dosing);
- Centrifuge for microplates (RCF(g) at least 100) (in case of using package U, manual dosing into microplates or automated dosing);
- 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);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

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. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

5.1. Transport conditions

The **BacScreen AEI 1 REAL-TIME PCR Detection Kit** must be transported in thermoboxes with ice packs by all types of roofed transport at temperatures inside the thermoboxes corresponding to storage conditions of the kit components.

5.1.1. Package S

- 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.

5.1.2. Package U

- 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! The kits transported with violation of temperature conditions must not be used.

5.2. Storage conditions

5.2.1. Package S

- All components of **BacScreen AEI 1 REAL-TIME PCR Detection Kit Detection 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 stored out of light over the storage period.

5.2.2. **Package U**

- All components of **BacScreen AEI 1 REAL-TIME PCR Detection Kit Detection Kit**, except TechnoTaq MAX polymerase, must be stored at the temperature from 2 °C to 8 °C over the storage period. PCR-mix must be stored out of light over the storage period.
- TechnoTaq MAX polymerase must be stored at the temperature from minus 22 °C to minus 18 °C over the storage period.

Kits stored under undue regime must not be used.

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

- Components of the kit, except TechnoTaq MAX polymerase (package U), must be stored at temperatures from 2 °C to 8 °C over the storage period;
- PCR-mix and paraffin sealed PCR-mix must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C and out of light over the storage period;
- TechnoTaq MAX polymerase (package U) must be stored in a freezing chamber at temperatures from minus 22 °C to minus 18 °C over the storage period.

An expired **BacScreen AEI 1 REAL-TIME PCR Detection Kit** must not be used.

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

The conformity of the **BacScreen AEI 1 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 coming in contact with the biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °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 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. Work surfaces, as well as rooms where PCR is performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work. Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

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

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

7. SAMPLES

The **BacScreen AEI 1 REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from human biological material (feces) and bacterial cultures obtained from this biomaterial.

7.1. General recommendations

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 and specific product amplification.

PCR inhibitors include the following substances: hemoglobin, bilirubin, cholesterol, triglycerides, mucus (mucin) and medications in the DNA sample, as well as the presence of isopropyl alcohol and methyl acetate in the DNA sample as a result of incomplete removal of wash solutions during sample preparation.

The maximum concentrations of interfering substances at which PCR inhibition was not observed are shown in the table below.

Type of biomaterial	Interfering substance	Interfering substance concentration
Endogenous substances		
Feces	bilirubin	684 µmoL/L
	cholesterol	13 mmoL/L
	hemoglobin	0.35 mg/mL
	triglycerides	37 mmoL/L
	mucus (mucin)	20%
Exogenous substances		

Feces	isopropyl alcohol	10%
	methyl acetate	10%
	Ibuprofen suppositories	5.0%
	Espumisan emulsion	5.0%
	Viferon suppositories	10%

Impurities contained in the biomaterial sample, such as mucus, blood, topical medications, including those contained in rectal suppositories, are removed during NA extraction using sample preparation kits /sets. To reduce the amount of PCR inhibitors, it is necessary to follow the rules of biological material collection.

7.3. Sample collection

WARNING! Before DNA extraction, preparation of biological material samples is needed.

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 cap and marked.

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 culture media is made with single-use microbiological loop or spatula.

Prepare the necessary amount of single-use 1.5-2.0 mL plastic tubes with pre-added 500 µL of sterile physiological saline solution. Place a sole colony of cells or 100 µL of liquid media in a tube. Tightly close and mark the tube.

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.

Feces

Native samples of feces can be transported and stored[3]:

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

Suspension of feces with glycerin can be transported and stored[3]:

- at minus 20 °C for 1 week;
- at minus 70 °C for a prolonged period.

Bacterial cultures

Bacterial cultures can be transported and stored[3]:

- at temperature from 2 °C to 8 °C for no more than 1 day;

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

- at temperature from minus 20 °C to minus 18 °C for no more than 1 week;
- at minus 70 °C for a prolonged period.

7.5. Sample preparation

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:

Feces

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

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

7.5.3. 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.

7.5.4. 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.

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

7.5.6. 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.

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

Samples are ready for DNA extraction.

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.

Samples are ready for DNA extraction.

8. PROCEDURE

DNA extraction from biological material

It is recommended to use DNA extraction reagent kits registered as medical devices and compatible with the relevant biomaterial for further DNA analysis by PCR, e.g. **PREP-NA**, **PREP-NA PLUS**, **PREP-GS**, **PREP-GS PLUS, PREP-OPTIMA**, **PREP-MB MAX** (see Table 5).

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

Table 5. Reagent kits validated for DNA extraction for further analysis using **BacScreen AEI 1**

Reagent kit	Biomaterial	Minimum elution volume, µL
PREP-NA	Feces, bacterial cultures	50
PREP-NA PLUS	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	300

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 in volumes as indicated in the instructions for use of extraction kits or negative control that is included in the corresponding extraction kit.

8.1. Preparing PCR for package S

WARNING!

1. The reagents and tubes should be kept away from direct sun light.
2. Strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips from 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 intended 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 on 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 (~20 µL) of mineral oil into each strip 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), 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.

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. Close the tubes/strips.

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

8.1.10 Set the strips into the thermoblock of the real-time thermal cycler.

8.1.11 Launch the operating software for DT instrument[4]. Add corresponding test[5], specify the number and ID's of the samples, positive and negative controls. Specify the position of the tubes/strips in the thermal unit (see 8.1.10) and run PCR. See Table 6.

8.1.12 For CFX96 and Applied Biosystems QuantStudio 5 detecting thermal cyclers: perform PCR considering reaction mixture volume of 35 µL according to amplification programs shown in Tables 7, 8.

Table 6. 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*		…	Holding		Holding
√ - optical measurement * - holding at 10 °C is allowed						

Table 7. The PCR program for CFX96 thermal cyclers (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			

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

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

Table 8. The PCR program for Applied Biosystems QuantStudio 5 thermal cyclers (packages S, U)

Stage	Step	Temperature, °C	Time min: sec	Number of cycles (repeats)
Holding	1	80	01:00	1
	2	94	01:30	1
PCR	1	94	00:20	50
	2	64 **√**	00:20	
√- data collection for fluorophores (Fam, Vic (Hex), Rox, Cy5) is on				

8.2. Preparing PCR for package U, manual dosing

WARNING!

1. For amplification use 0.2 mL single-use amplification tubes or 96-well PCR microplates[6], sealed hermetically with thermal film. It is not recommended to use strips due to increased risk of postamplification contamination.
2. The reagents and tubes should be kept away from direct sunlight.

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 analysis (3 test samples, negative control and positive control).

Example: to test 4 samples, mark 4 tubes/reserve 4 microplate 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 Shake the tube with PCR-mix for 3-5 seconds on vortex, 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 Shake the tubes with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds on vortex, 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 number of marked tubes/number of microplate wells considering "C-", "C+".

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

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

WARNING! Mix 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 marked tube/necessary well with PCR-mix. Cover the tubes loosely.

[6] - 96-well plates are not used with DTlite detecting thermal cycler

WARNING! Follow the steps 8.2.8 – 8.2.14 within two hours after adding PCR-buffer and TechnoTaq MAX polymerase mixture to the tubes/wells with 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 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 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 PCR microplate 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:
Close the tubes. Spin the tubes for 3-5 seconds on vortex mixer.

8.2.14 Set all the tubes/PCR microplate into the real-time thermal cycler.

8.2.15 Launch the operating software for DT instrument[7]. Add corresponding test[8], specify the number and ID's of the samples, positive and negative controls. Specify position of the tubes in thermal unit (see 8.2.14) and run PCR. See Table 9.

8.2.16 For CFX96 and Applied Biosystems QuantStudio 5 thermal cyclers perform PCR considering the volume of reaction mixture of 18 µL. See Tables 7, 8.

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

Table 9. 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*			Holding		Holding
√ - optical measurements * - holding at 10 °C is allowed						

8.3. Preparing PCR for package U, using DTstream (only for DTprime 5X*)

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 Shake the tube with PCR-mix for 3-5 seconds on vortex, then spin for 1-3 seconds to collect the drops.

8.3.2 Shake the tubes with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds on vortex, then spin for 1-3 seconds to collect the drops.

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

8.3.3 Prepare the mixture of PCR-buffer and TechnoTaq MAX.

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

8.3.5 Shake the tubes with positive control for 3-5 seconds on vortex, then spin 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 microplate into the real-time thermal cycler.

8.3.11 Launch the operating software for DT instrument[9]. Add corresponding test[10], specify the number and ID's of the samples, positive and negative controls. Specify position of the samples in thermal unit (see 8.3.10) and run PCR. The volume of reaction mixture is 18 µL. See Table 9.

9. CONTROLS

The **BacScreen AEI 1 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 biomaterial sampling quality control and DNA extraction quality control.

To reveal possible contamination a negative control is required.

WARNING! A negative control 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 PCR stage for all samples in the batch. It may be caused by operation error or violation of storage and handling of the reagent kit.

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

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

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 (Bio-Rad) 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 10, 11. The results are valid if the conditions for the interpretation of results obtained for control samples are met.

Table 10. PCR results interpretation

Detection channel				**Result interpretation**
Fam, Cp/Cq/Ct	**Hex/Vic**, Cp/Cq/Ct	**Rox**, Cp/Cq/Ct	**Cy5**, Cp/Cq/Ct	
Test samples				
< 37	> 39 or not specified	Not specified	Not considered	***Campylobacter coli/jejuni*** **DNA is detected**
Not specified	**< 37**	Not specified	Not considered	***Salmonella*** **spp. DNA is detected**
Not specified	> 39 or not specified	**< 37**	Not considered	***Shigella/*****EIEC DNA is detected**
Not specified	> 39 or not specified	Not specified	**≤ 35**	DNA of the target microorganisms is not detected
≥ 37 or not specified	> 39 or not specified	≥ 37 or not specified	> 35 or not specified	Unreliable result[11]
Negative control				
Not specified	> 39 or not specified	Not specified	> 35 or not specified	**Negative result** Run results are valid
Positive control				
Specified	**Specified**	**Specified**	**Specified**	**Positive result** Run results are valid

Table 11. Other possible PCR results

Detection channel				**Result interpretation ***
Fam, Cp/Cq/Ct	**Hex/Vic**, Cp/Cq/Ct	**Rox**, Cp/Cq/Ct	**Cy5**, Cp/Cq/Ct	
Test samples				
≥ 37	> 39 or not specified	Not specified	**≤ 35**	**Low content of** ***Campylobacter coli/jejuni*** **DNA**

[11] - A repeated PCR with the ready DNA preparation, or a repeated DNA extraction and PCR for this sample, or a repeated sample collection are required (preformed sequentially).

Not specified	**≥ 37, but ≤ 39**	Not specified	**≤ 35**	**Low content of *Salmonella* spp. DNA**
Not specified	> 39 or not specified	**≥ 37**	**≤ 35**	**Low content of *Shigella/*EIEC DNA**
* - if Cp/Cq/Ct value ≤ 35 on Cy5, while Cp/Cq/Ct value ≥ 37 but ≤ 39 on Hex/Vic, and/or Cp/Cq/Ct value ≥ 37 on Fam, and/or Rox channels, the obtained result indicates low specific DNA content that can be due to lower load in clinical sample, cross-contamination from 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. The value of total bacterial load (TBL, Cy5 detection channel) must be considered in the results analysis:
The value of TBL Cp/Cq/Ct > 35 in the absence of specific positive results in the tube should be interpreted as an insufficient amount of biomaterial or a possible PCR inhibition. The amplification result in "invalid".

10.5. For the clinical samples containing the sought analytes' DNA software registers positive result on the corresponding detection channel (Fam, Hex/Vic or Rox). The amplification result is "detected" ("+").

10.6. For the clinical samples not containing the sought analytes' DNA software registers negative result on the corresponding detection channel (Fam, Hex/Vic or Rox). The amplification result is "not detected" ("-").

10.7. If the results obtained for negative control differ from those indicated in Table 10, the results of the whole run are considered invalid. In this case, special measures to detect and eliminate a possible contamination are required.

10.8. If the results obtained for positive control differ from those indicated in Table 10, a repeated amplification for the whole batch of samples is required.

11. SPECIFICATIONS

a. Analytical specificity

For clinical samples containing DNA of the detected analytes, the software must register positive amplification result for specific products on the corresponding detection channels[12].

For clinical samples not containing DNA of the detected analytes, the software must register negative result of amplification for specific products on the corresponding detection channels[13] and positive amplification result for total bacterial load (TBL)[14] on Cy5 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 biological 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, Campylobacter coli, 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 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

Analytical sensitivity, or LOD (limit of detection) of microorganisms detected by the **BacScreen AEI 1 REAL-TIME PCR Detection Kit** is determined by the analysis of serial dilutions of the laboratory controls (LC) and is presented in the table below.

LOD depends on the used kit/set of reagents for DNA extraction and on the final volume of extracted DNA elution:

[12] result interpretation is shown in Tables 10, 11

[13] for *Salmonella* spp. the Cp/Cq/Ct value > 39 on Hex detection channel is allowed

[14] the Cp/Cq/Ct value ≤ 35 on Cy5 detection channel is considered a positive amplification result for TBL

Detected analyte	Copies per amplification tube	Copies 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 (elution volume 300 µL)
Campylobacter coli/jejuni	10	$2.0x10^3$	$1.0x10^3$	$4.0x10^3$	$1.2x10^4$	$1.8x10^4$	$6.0x10^3$
Salmonella spp.	10	$2.0x10^3$	$1.0x10^3$	$4.0x10^3$	$1.2x10^4$	$1.8x10^4$	$6.0x10^3$
Shigella/EIEC	10	$2.0x10^3$	$1.0x10^3$	$4.0x10^3$	$1.2x10^4$	$1.8x10^4$	$6.0x10^3$

c. Diagnostic characteristics

Type of biomaterial	Analyte	Diagnostic sensitivity	Diagnostic specificity
Feces	*Campylobacter coli/jejuni*	100% (95% CI: 92.89% – 100%)	100% (95% CI: 92.89% – 100%)
	Shigella/EIEC	100% (95% CI: 92.89% – 100%)	100% (95% CI: 92.89% – 100%)
	Salmonella spp.	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
Bacterial cultures	*Campylobacter coli/jejuni*	100% (95% CI: 92.89% – 100%)	100% (95% CI: 92.89% – 100%)
	Shigella/EIEC	100% (95% CI: 92.89% – 100%)	100% (95% CI: 92.89% – 100%)
	Salmonella spp.	100% (95% CI: 86.28% – 100%)	100% (95% CI: 86.28% – 100%)
Total		100% (95% CI: 98.54–100%)	100% (95% CI: 98.54–100%)

d. Reproducibility and repeatability

Reproducibility is 100%.

Repeatability is 100%.

12. TROUBLESHOOTING

Table 12. 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
TBL	Invalid	Low amount of DNA / PCR inhibition	Repeat whole test Resample

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

Phone: +7(495)640.16.93

E-mail: hotline@dna-technology.ru

https://www.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 Department with quality issues of **BacScreen AEI 1 REAL-TIME PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

Technical support:

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https://www.dna-technology.com

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
	Do not reuse		Non-sterile

REF R1-P502-S3/9EU
R1-P502-23/9EU
R1-P502-UA/9EU

VER 994.2025.03.31

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 it is necessary to:
   - preheat the thermostat to 65 °C;
   - take out of the refrigerator the NA extraction reagent kit and check the absence of precipitate in the lysis solution. In case of precipitation it is necessary to heat the vial with lysis solution on the thermostat preheated to 65 °C, until complete dissolution of the precipitate. Then mix the solution by turning the vial upside down 5-10 times, avoid 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) within approximately 12 hours.
2. Tube caps may open during heating! Use tubes with self-lock caps (e.g. Eppendorf Safe-Lock Tubes) or programmable thermostats with clamp cover (e.g. solid-state programmable thermostat TT-1-DNA-Tech manufactured by "DNA-Technology R&P", LLC).

Procedure

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. Heat the tubes on thermostat 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 for 10 minutes at room temperature (from 18 °C to 25 °C).
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 for 1 minute at room temperature (from 18 °C to 25 °C).
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 for 1 minute at room temperature (from 18 °C to 25 °C).
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. Heat the tubes on thermostat 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 for 30 seconds at room temperature (from 18 °C to 25 °C) 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 DNA preparation for PCR, defreeze the tubes with DNA preparation and negative control at room temperature (from 18 °C to 25 °C) or at temperature from 2 °C to 8 °C, shake on vortex for 3-5 seconds and spin down the drops for 1-3 seconds.

DNA preparation is ready to be introduced into reaction mixture.