



For research use only

Bordetella Screen

REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE



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1. INTENDED USE

The **Bordetella Screen REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **Bordetella Screen REAL-TIME PCR Detection Kit** is designed to detect DNA of *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica*, *Bordetella holmesii* in human biological material (smears from nasopharyngeal and oropharyngeal mucous membranes) and bacterial cultures 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 **Bordetella Screen REAL-TIME PCR Detection Kit**.

The **Bordetella Screen REAL-TIME PCR Detection Kit** can be used in research practice.

Potential users: qualified personnel trained in 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 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.

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.

PCR mix includes internal control (IC) designed to control the quality of polymerase chain reaction.

The DNA probe used to detect the *Bordetella pertussis* amplification product includes fluorescent dye Fam. The DNA probe used to detect the *Bordetella bronchiseptica* amplification product includes fluorescent dye Rox. The DNA probe used to detect the *Bordetella parapertussis* amplification product includes fluorescent dye Cy5. The DNA probe used to detect the *Bordetella holmesii* amplification product includes fluorescent dye Cy5.5. The DNA probe used to detect the internal control amplification product includes fluorescent dye Hex.

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 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam	Hex	Rox	Cy5	Cy5.5
<i>Bordetella pertussis</i>	IC*	<i>Bordetella bronchiseptica</i>	<i>Bordetella parapertussis</i>	<i>Bordetella holmesii</i>

* - internal control

The automatic analysis available on “DNA-Technology” made instruments: DTlite, DTprime or DTprime II REAL-TIME Thermal Cyclers for **Bordetella Screen 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 **Bordetella Screen REAL-TIME PCR Detection Kit** is also approved for use with CFX96 (Bio-Rad) real-time thermal cyclers.

3. CONTENT

The **Bordetella Screen REAL-TIME PCR Detection Kit** contents is represented in Tables 2 - 4.

Table 2. The **Bordetella Screen REAL-TIME PCR Detection Kit** content, package S, strips, for R1-P039-S3/4ER

Reagent	Description	Total volume	Amount
Paraffin-sealed PCR mix	Colorless or pink transparent liquid under waxy white fraction	960 µL (20 µL in each)	tubes, 6 strips of 8
Taq polymerase solution	Colorless transparent liquid	500 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	1 tube
Positive control ¹	Colorless transparent liquid	130 µL	1 tube
Strip caps		6 strips of 8	

Table 3. The **Bordetella Screen REAL-TIME PCR Detection Kit** content, package S, tubes, for R1-P039-23/4ER

Reagent	Description	Total volume	Amount
Paraffin-sealed PCR mix	Colorless or pink transparent liquid under waxy white fraction	960 µL (20 µL in each)	48 individual tubes
Taq polymerase solution	Colorless transparent liquid	500 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	1 tube
Positive control ¹	Colorless transparent liquid	130 µL	1 tube

Table 4. The **Bordetella Screen REAL-TIME PCR Detection Kit** content, package U, for R1-P039-UA/9ER

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 **Bordetella Screen REAL-TIME PCR Detection Kit** (package S) is intended for single use and designed for 48 tests (no more than 12 runs), including analysis of test samples, negative controls and positive controls.

The **Bordetella Screen 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).

¹ - marking as C+ is allowed

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

The following equipment, reagents and consumables are required:

Equipment, reagents and consumables	Package S		Package U, dosing	
	strips	tubes	manual	automated
UV PCR cabinet	yes	yes	yes	yes
Real-time detecting thermal cycler ¹ :				
– DTprime in DTprime 5M* modification (manufactured by “DNA-Technology R&P”, LLC), hereinafter – DTprime;				
– DTprime II in DTprime II 5M* modification (manufactured by “DNA-Technology R&P”, LLC), hereinafter – DTprime II;	yes	yes	yes	yes ²
– CFX96 (Optical Reaction Module CFX96) (manufactured by Bio-Rad Laboratories, USA), hereinafter – CFX96.				
– DTlite in DTlite 5S* modification (manufactured by “DNA-Technology R&P”, LLC), (only for package S, and package U, manual dosing, tubes), hereinafter – DTlite;	yes	yes	yes ³	no
– DTprime in DTprime 5X* modification (manufactured by “DNA-Technology R&P”, LLC), hereinafter – DTprime 5X*;				
– DTprime II in DTprime II 5X* modification (manufactured by “DNA-Technology R&P”, LLC), hereinafter – DTprime II 5X*;	no	no	no	yes ⁴
Vortex mixer ⁵	yes	yes	yes	yes
Vortex rotor for 0.2 mL strips	yes	no	no	no
Refrigerator or cooling chamber	yes	yes	yes	yes
Freezing chamber	no	no	yes	yes
Tube rack for 1.5 mL tubes	yes	yes	yes	yes
Tube rack for 0.2 mL tubes	no	yes	yes ³	no
Tube rack for 0.2 mL strip tubes	yes	no	no	no
Single channel pipettes (dispensers covering 0.5-10; 2.0-20; 20-200; 200-1,000 µL volume range)	yes	yes	yes	yes
RNase and DNase free filtered pipette tips (volume 10 µL; 20 µL; 200 µL; 1,000 µL)	yes	yes	yes	yes
Pipette rack	yes	yes	yes	yes
RNase and DNase free 1.5 mL microfuge tubes with caps	no	no	yes	yes
RNase and DNase free 0.2 mL PCR tubes or 96-well microplate ⁶	no	no	yes	yes ⁷
Powder-free surgical gloves	yes	yes	yes	yes
Container for used pipette tips, tubes and other consumables	yes	yes	yes	yes
DTstream dosing instrument, version 12M1 or 15M1	yes ⁸	no	no	yes
RNase and DNase free filter pipette tips (volume 200 µL) for DTstream, or similar	yes ⁸	no	no	yes

Equipment, reagents and consumables	Package S		Package U, dosing	
	strips	tubes	manual	automated
DTpack plate sealing device	no	no	yes ⁹	yes
Centrifuge for microplates (RCF(g) at least 100)	no	no	yes ⁹	yes
Polymer thermal film for microplate sealing	no	no	yes ⁹	yes
384-well PCR microplate	no	no	no	yes
Transport medium (if necessary), the following are recommended:				
- STOR-F transport medium for biomaterial samples				
Physiological saline solution 0.9% NaCl (sterile)				
NA extraction reagent kits ¹⁰ , the following are recommended:				
<ul style="list-style-type: none"> – PREP-NA; – PREP-NA PLUS; – PREP-RAPID; – PREP-OPTIMA; – PREP-MB-RAPID II. 				
Notes:				
¹ – hereinafter – detecting thermal cycler; the required parameters are indicated below				
² – only if using 96-well PCR microplates				
³ – only if using tubes				
⁴ – only if using 384-well PCR microplates				
⁵ – DTspin laboratory shaker (DNA-Technology, Russia) is recommended				
⁶ – not used for DTlite detecting thermal cycler				
⁷ – only PCR microplates				
⁸ – in case of automated dosing				
⁹ – only if using PCR microplates				
¹⁰ – the choice of DNA extraction kit is determined by biomaterial type				

For the use of detecting thermal cyclers other than those listed in the table, please consult the reagent kit manufacturer.

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. Storage conditions

5.1.1. Package S

- All components of the **Bordetella Screen REAL-TIME PCR Detection 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 out of light over the storage period.

5.1.2. Package U

- All components of the **Bordetella Screen REAL-TIME PCR Detection 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 out of light over the storage period.

- TechnoTaq MAX polymerase (package U) must be stored in a freezer at the temperatures from minus 22 °C to minus 18 °C over the storage period.

WARNING! The excessive temperature and light can be detrimental to product performance.

5.2. Transport conditions

Transportation 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 kit components.

5.2.1. 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 thermoboxes from 2 °C to 25 °C for no longer than 5 days.

5.2.2. 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 thermoboxes from 2 °C to 25 °C for no longer than 5 days.
- It is allowed to transport TechnoTaq MAX polymerase (package U) in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermoboxes up to 25 °C for no longer than 5 days.

WARNING! Reagent kits transported with violation of temperature conditions must 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 in a refrigerator or a cooling chamber 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 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 must be stored in a freezer at temperatures from minus 22 °C to minus 18 °C over the storage period.

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

An expired **Bordetella Screen 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.

Conformity of **Bordetella Screen 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 test as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of test. 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 test. 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 cannot be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. 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 **Bordetella Screen REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from smears from nasopharyngeal and oropharyngeal mucous membranes and from bacterial cultures.

7.1. General requirements

PCR analysis refers to direct methods of laboratory research; therefore, the collection of biological material must be carried out from the site of infection localization.

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.

If biomaterial from several biotopes is required, repeat the procedure using new swab each time you collect biomaterial.

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 controversial (uncertain) results. The sign of PCR inhibition is the simultaneous absence of internal control and specific product of amplification.

PCR inhibitors include the following endogenous and exogenous interfering substances: hemoglobin, mucus (mucin) and medications (chlorhexidine bigluconate, Miramistin®, Nasol Baby, Otrivin Baby, Lasolvan®, Pinosol®, Rinofluimucil®, Tyzine® Classic, Tantum® Verde spray, Hexoral® solution, Berodual®, Pulmicort® inhalation suspension) found in the biomaterial sample due to incomplete removal during DNA extraction, as well as isopropyl alcohol and methyl acetate left in DNA sample due to incomplete removal of wash solutions during sample preparation.

Maximum interfering substance concentrations not resulting in PCR inhibition:

Biomaterial type	Interfering substance (IS)	IS concentration
<i>Endogenous substances</i>		
Smears from nasopharyngeal and/or oropharyngeal mucous membranes	Hemoglobin	0.35 mg/mL in DNA sample
	Mucus (mucin)	10 % of biomaterial sample volume
<i>Exogenous substances</i>		
Smears from nasopharyngeal and/or oropharyngeal mucous membranes	Isopropyl alcohol	10 % of DNA sample volume
Smears from nasopharyngeal and/or oropharyngeal mucous membranes	Methyl acetate	10 % of DNA sample volume
Smears from nasopharyngeal and/or oropharyngeal mucous membranes	chlorhexidine bigluconate	20 % of biomaterial sample volume
Smears from nasopharyngeal and/or oropharyngeal mucous membranes	Miramistin®	20 % of biomaterial sample volume
Smears from nasopharyngeal and/or oropharyngeal mucous membranes	Nasol Baby	20 % of biomaterial sample volume
Smears from nasopharyngeal and/or oropharyngeal mucous membranes	Otrivin Baby	20 % of biomaterial sample volume
Smears from nasopharyngeal and/or oropharyngeal mucous membranes	Lasolvan®	20 % of biomaterial sample volume
Smears from nasopharyngeal and/or oropharyngeal mucous membranes	Pinosol®	20 % of biomaterial sample volume
Smears from nasopharyngeal and/or oropharyngeal mucous membranes	Rinofluimucil®	20 % of biomaterial sample volume
Smears from nasopharyngeal and/or oropharyngeal mucous membranes	Tyzine® Classic	20 % of biomaterial sample volume
Smears from nasopharyngeal and/or oropharyngeal mucous membranes	Tantum® Verde spray	20 % of biomaterial sample volume
Smears from nasopharyngeal and/or oropharyngeal mucous membranes	Hexoral® solution	20 % of biomaterial sample volume
Smears from nasopharyngeal and/or oropharyngeal mucous membranes	Berodual®	20 % of biomaterial sample volume
Smears from nasopharyngeal and/or oropharyngeal mucous membranes	Pulmicort® inhalation suspension	20 % of biomaterial sample volume

To reduce the amount of PCR inhibitors, please observe the rules of biomaterial collection. If you suspect a significant amount of PCR inhibitors in the sample, we recommend that you choose NA extraction method that allows to remove them as fully as possible and not express NA extraction methods.

7.3. Sample collection

WARNING! Sample preparation may be required before DNA extraction!

7.3.1 Smears from nasopharyngeal and/or oropharyngeal mucous membranes

Material is taken using special authorized medical devices according to the procedure established depending on the source of biological material.

Biomaterial is taken from mucous membranes of nasopharynx or of the back of oropharynx.

For differential diagnostics we recommend that both types of smears are taken from each examinee. In this case, combine and use both samples taken by different probes into one tube as a single sample.

Method limitations: Meal later than 2 hours before the test; use of medical supplies irrigating the nasopharynx or oropharynx and oropharyngeal resorption preparations less than 6 hours before the test. The collection of biological material is carried out before other types of research or procedures (including rinsing) are applied, in good light

Biomaterial is taken in accordance with the NA extraction kit instruction for use.

In case of using PREP-RAPID for NA extraction:

WARNING! Material is taken into tubes with **PREP-RAPID** reagent using a dry probe! It is necessary to exclude contact of the solution with skin, eyes and mucous membranes.

After taking the material transfer the probe into the tube with **PREP-RAPID** reagent and rinse it thoroughly in the liquid for 10-15 seconds, avoiding splashing.

Remove the probe from the solution and press the probe against the inner wall of the tube above the solution level with a rotating motion to squeeze out excess liquid. Remove the probe completely from the tube and discard.

Close the tube tightly and mark it.

7.3.2 Bacterial cultures

The material is taken in accordance with the instruction to the NA extraction reagent kit.

Biomaterial is taken in accordance with the NA extraction kit instruction for use.

In case of using PREP-NA PLUS for NA extraction:

Collect bacterial culture from liquid and dense media into plastic 1.5-2.0 mL tubes with 0.5-1.0 mL of physiological solution.

Place a single cell colony or 100 µL of liquid medium into each tube using a microbiological loop or a spatula.

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 store biomaterial according to the conditions indicated in Table 5 (if it does not contradict the requirements stated in the instructions for use of the NA extraction reagent kits or the transport media used for transport and storage of samples):

Table 5. Biomaterial transport and storage conditions prior to DNA extraction

Biomaterial	Transport and storage temperature	Time period before DNA extraction
Smears from nasopharyngeal and/or oropharyngeal mucous membranes	From 2 °C to 8 °C	Up to 24 hours
	From minus 22 °C to minus 18 °C	Up to 1 month
Bacterial cultures (culture suspension in physiological solution)	From 2 °C to 8 °C	Up to 24 hours
	From minus 22 °C to minus 18 °C	Up to 7 days
	Minus 70 °C	Prolonged period

WARNING! Only one freezing-thawing of the material is allowed.

7.5. Sample preparation to DNA extraction

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

For bacterial cultures and PREP-NA PLUS extraction kit:

- 7.5.1. Centrifuge the tube at RCF(g) 12,000 – 16,000 for 10 minutes.
- 7.5.2. Remove supernatant leaving approximately 100 µL (precipitate + liquid fraction) in the tube.
- 7.5.3. Close the tubes tightly.

Sample is ready for DNA extraction.

8. PROCEDURE

DNA extraction from biological material

We recommend the authorized DNA extraction kits for the corresponding biomaterial types: **PREP-NA, PREP-NA PLUS, PREP-RAPID, PREP-OPTIMA, PREP-MB RAPID II** (see Table 6).

Table 6. Kits recommended for DNA extraction (manufactured by “DNA-Technology”):

Reagent kit	Biomaterial	Minimal eluate volume, µL
PREP-NA	Smears from nasopharyngeal and/or oropharyngeal mucous membranes	50
	Smears from nasopharyngeal and/or oropharyngeal mucous membranes (according to shortened method in Annex A)	
PREP-NA PLUS	Smears from nasopharyngeal and/or oropharyngeal mucous membranes	100
	Bacterial cultures	300
	Smears from nasopharyngeal and/or oropharyngeal mucous membranes (according to shortened method in Annex A)	100
PREP-RAPID	Smears from nasopharyngeal and/or oropharyngeal mucous membranes	500
PREP-OPTIMA	Smears from nasopharyngeal and/or oropharyngeal mucous membranes, bacterial cultures	500
PREP-MB RAPID II	Smears from nasopharyngeal and/or oropharyngeal mucous membranes	100

DNA is extracted in accordance with the instruction to the NA extraction reagent kit or with Annex A in case of using **PREP-NA/PREP-NA PLUS** kits for shortened DNA extraction method.

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.

8.1 Preparing PCR for package S

WARNING!

1. The reagents and tubes should be kept away from direct sun light.
2. 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!
3. For package S, strips automated dosing is available using DTstream dosing instrument. Please refer to DTstream operation manual.

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 12 runs assuming a variable number of test samples, 1 negative control and 1 positive control per run.

Example: To test 4 samples, mark 4 tubes for samples, one negative control tube "C-" and one positive control tube "C+". Total number of tubes is 6.

8.1.2 Shake the tubes with Taq polymerase solution on vortex for 3-5 seconds and centrifuge at RCF(g) 1,000-3,000 on vortex 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 for 3-5 seconds and centrifuge at RCF(g) 1,000-3,000 on vortex 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** extraction kits, shake the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and centrifuge at RCF(g) 1,000-3,000 for 1-3 seconds.

3. 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 Centrifuge the tubes/strips at RCF(g) 1,000-3,000 for 3-5 seconds in a vortex mixer.

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

8.1.11 Launch the operating software for DT instrument². Add corresponding test³, specify the number and IDs 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 7.

8.1.12 For CFX96 detecting thermal cycler: perform PCR considering reaction mixture volume of 35 µL according to amplification programs shown in Table 8.

² - Please, apply to Operation Manual for DTprime, DTprime II and DTlite Real-Time PCR instruments PART II.

³ - Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website <https://www.dna-technology.com/assaylibrary>.

Table 7. The PCR program for DTlite, DTprime and DTprime II thermal cyclers (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 measurement

¹ - Holding at 10 °C is allowed

Table 8. 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, Cy5, Cy5.5 channels at 64 °C

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 microplates⁴, sealed hermetically with thermal film. It is not recommended to use strips due to postamplification contamination hazard.
2. The reagents and tubes should be kept away from direct sun light.

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 Shake the tube with PCR mix on vortex for 3-5 seconds, then centrifuge at RCF(g) 1,000-3,000 for 1-3 seconds.

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 centrifuge at RCF(g) 1,000-3,000 for 1-3 seconds.

⁴ - 96-well plates are not used with DTlite detecting thermal cycler

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) μ L of PCR buffer,

0.3 x (N+1) μ L 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 μ L of PCR buffer and 2.1 μ L of TechnoTaq MAX polymerase.

8.2.6 Shake the tube with the mixture of PCR buffer and TechnoTaq MAX polymerase on vortex for 3-5 seconds, then centrifuge at RCF(g) 1,000-3,000 for 1-3 seconds.

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

8.2.7 Add 6.0 μ L 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 centrifuge at RCF(g) 1,000-3,000 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** extraction kits, shake the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and centrifuge at RCF(g) 1,000-3,000 for 1-3 seconds.
3. 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.

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

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

8.2.11 Add 6.0 μ L 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:**

Centrifuge the tubes at RCF(g) 1,000-3,000 for 3-5 seconds.

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

8.2.15 Launch the operating software for DT instrument⁵. Add corresponding test⁶, specify the number and IDs of the samples, positive and negative controls. Specify position of the samples in thermal unit (see 8.2.14) and run PCR. See Table 9.

8.2.16 For CFX96 thermal cyclers perform PCR considering the volume of reaction mixture of 18 μ L. See Table 8.

Table 9. The PCR program for DTlite, DTprime and DTprime II thermal cycler (package U)

Step	Temperature, °C	Min	Sec	Number of cycles	Optical measurement	Type of the step
1	80	0	5	15		Cycle
	94	0	5			
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

8.3 Preparing PCR for package U, using DTStream⁷

WARNING!

1. For amplification use 96-well or 384-well⁸ PCR microplates hermetically sealed with thermal film.
2. The reagents and tubes should be kept away from direct sun light.

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 centrifuge at RCF(g) 1,000-3,000 for 1-3 seconds.

8.3.2 Vortex the tube with PCR buffer and TechnoTaq MAX polymerase for 3-5 seconds, then centrifuge at RCF(g) 1,000-3,000 for 1-3 seconds.

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 centrifuge at RCF(g) 1,000-3,000 for 1-3 seconds.

8.3.5 Vortex the tubes with positive control for 3-5 seconds and centrifuge at RCF(g) 1,000-3,000 for 1-3 seconds.

⁵ - Please, apply to Operation Manual for DTprime, DTprime II and DTlite Real-Time PCR instruments PART II.

⁶ - Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website

<https://www.dna-technology.com/assaylibrary>.

⁷ - only for DTprime, DTprime II and CFX96 thermal cyclers

⁸ - only for DTprime 5X* and DTprime II 5X* thermal cyclers

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** extraction kits, shake the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and centrifuge at RCF(g) 1,000-3,000 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 instrument⁹. Add corresponding test¹⁰, specify the number and IDs of the samples, positive and negative controls. Specify position of the samples in thermal unit (see 8.3.10) and run PCR. See Table 9.

8.3.12 For CFX96 thermal cyclers perform PCR considering the volume of reaction mixture of 18 µL. See Table 8.

9. CONTROLS

The **Bordetella Screen 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.

The PCR mix from the kit includes the internal control (IC). IC is intended to assess the quality of PCR performance.

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 from an extraction kit can be used as a negative control 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.

⁹ - Please, apply to Operation Manual for DTprime, DTprime II 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 detection thermal cyclers, use regression type analysis (Cq Determination Mode: Regression). In the “Baseline Setting” tab select “Baseline Subtraction Curve Fit”.
- 10.3** Result interpretation is carried out according to Table 10. The results are valid if the conditions for the interpretation of results obtained for control samples are met.

WARNING! In case of a mixed infection simultaneous DNA detection for two types of microorganisms on the corresponding detection channels is possible.

- 10.4** Unreliable result may be due to the presence of inhibitors in the NA preparation obtained from biological material; incorrect execution of the analysis protocol, noncompliance with the amplification temperature regime, etc. In this case it is necessary to repeat PCR with the available DNA preparation, or to re-extract DNA and perform PCR for this sample, or to re-collect biological material from the patient (performed sequentially).
- 10.5** If a positive result is obtained for negative control, the results of the whole batch should be deemed invalid. In this case special measures are required for detection and elimination of possible contamination.
- 10.6** If a negative result is obtained for positive control, the results of the whole batch should be deemed invalid. In this case it is required to repeat PCR for the whole batch of samples.

Table 10. PCR results interpretation

Specified	Not considered	Specified	Specified	Specified	Positive result Run results are valid
* - The obtained result indicates that the target microorganism DNA level is at the limit of detection, which may be due to low microorganism load in the test sample, cross-contamination with high-copy samples or PCR inhibition. The result may not be confirmed by a repeated test. Please repeat biomaterial collection and/or DNA extraction and PCR. If the result repeats, the conclusion should be "...DNA is detected".					

11. SPECIFICATIONS

a. Analytical specificity

For biomaterial samples containing DNA of *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica* or *Bordetella holmesii*, thermal cycler software must register positive amplification result for specific products (*Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica* or *Bordetella holmesii* genome fragments) on specified detection channels.

For biomaterial samples not containing DNA of *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica* or *Bordetella holmesii*, thermal cycler software must register negative amplification result for specific products (*Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica* or *Bordetella holmesii* genome fragments) on specified detection channels or $C_p \geq 39$ and positive amplification result for IC on Hex detection channel.

There were no nonspecific positive amplification results in the presence of RNA of Influenza virus A(H3N2), Influenza virus A(H1N1pdm09), Influenza B virus, Human Coronavirus 229E, Human Coronavirus HKU-1, Human Coronavirus NL-63, Human Coronavirus OC-43, Human Metapneumovirus, Human Parainfluenza virus type 2, Human Parainfluenza virus type 3, Human Parainfluenza virus type 4, Human Parainfluenza virus type 1, Human Rhinovirus, Respiratory syncytial virus, SARS-CoV-2, DNA of Human Adenovirus, Human Bocavirus, *Chlamydophila pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Staphylococcus aureus* (methicillin-resistant), *Streptococcus pneumoniae*, *Escherichia coli*, as well as human DNA in concentration 20 ng/ μ L.

b. Analytical sensitivity (limit of detection)

Limit of detection is 5 copies of each microorganism's DNA per amplification tube.

Limit of detection was established via analysis of laboratory control's serial dilutions.

Limit of detection corresponds to the following DNA concentration values when using the specified NA extraction kits and the end elution (dilution) volumes of the extracted DNA:

Biomaterial	DNA extraction kit	Preparation volume, μ L	Limit of detection, copies per sample
Nasopharyngeal, oropharyngeal smears in transport medium	PREP-NA	50	50
	PREP-NA PLUS	100	100
	PREP-RAPID	500	500
	PREP-OPTIMA ¹¹	500	500
	PREP-MB-RAPID II	100	100
Bacterial cultures in transport medium ¹²	PREP-OPTIMA	500	500
	PREP-NA PLUS	300	300

c. Reproducibility and repeatability

Reproducibility is 100%.

Repeatability is 100%.

Note. The claimed specifications are guaranteed when DNA extraction is performed with **PREP-RAPID**, **PREP-NA**, **PREP-NA PLUS**, **PREP-OPTIMA** and **PREP-MB RAPID II** extraction kits.

¹¹ - STOR-F transport medium was used

¹² - Physiological saline solution was used

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 encounter 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 **Bordetella Screen REAL-TIME PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

<https://www.dna-technology.com>

Manufacturer:

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142281, Russia, Moscow Region,
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Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

<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
VER	Version		Non-sterile
	Caution		

REF

R1-P039-S3/4ER

R1-P039-23/4ER

R1-P039-UA/9ER

VER

1250.2025.08.08

Annex A

Shortened method of DNA extraction from biomaterial (nasopharyngeal, oropharyngeal smears) 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 a 1.5 mL plastic tube for negative control (C-).
2. Enter 300 µL of lysis solution into each marked tube with 100 µL of biomaterial and into the "C-" tube. Do not touch the edges of the tube.
3. Add 100 µL of negative control into the "C-" tube.
4. Close the tubes tightly and shake on vortex for 3-5 seconds.
5. Heat the tubes on thermostat at 65 °C for 5 minutes.
6. Spin the tubes on vortex for 3-5 seconds.
7. Add 400 µL of precipitation buffer into each tube without touching the edges 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 fully, using separate tip for each tube. Do not touch the precipitate.
10. Add 500 µL of wash solution No. 1 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 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 fully, using separate tip for each tube. Do not touch the precipitate.
13. Add 300 µL of wash solution No. 2 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 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 fully, using separate tip for each tube. Do not touch the precipitate. It is allowed to leave up to 20-30 µL of liquid above the precipitate.
16. Open the tubes and dry the precipitate at 65 °C for 5 minutes.
17. Add the corresponding amount of dilution buffer to the precipitate according to the extraction kit instruction. Shake the tubes on vortex for 3-5 seconds and spin down the drops for 3-5 seconds.
18. Heat the tubes on thermostat at 65 °C for 5 minutes. Shake the tubes on vortex for 3-5 seconds.
19. Centrifuge the tubes at RCF(g) 12,000-16,000 for 30 seconds at room temperature (from 18 °C to 25 °C) to spin down the condensate.

DNA preparation is ready to be introduced to PCR mix.

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 DNA 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 to PCR mix.