DNA-TECHNOLOGY

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RUO

For research use only

SMA Screen Genotyping REAL-TIME PCR Kit

INSTRUCTION FOR USE

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1. INTENDED USE

The **SMA Screen Genotyping REAL-TIME PCR Kit** is an *in vitro* Nucleic Acid Test (NAT) – human genotyping-based product. The **SMA Screen Genotyping REAL-TIME PCR Kit** is designed to detect homozygous and heterozygous deletions of exon 7 of the SMN1 gene associated with development of spinal muscular atrophy (SMA 5q) in human biological material (whole blood) by real-time PCR.

The **SMA Screen Genotyping REAL-TIME PCR Kit** is designed for screening.

The **SMA Screen Genotyping REAL-TIME PCR Kit** can be used in research practice.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **SMA Screen Genotyping REAL-TIME PCR Kit.**

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

The method is based on amplification of TREC, KREC, exon 7 of the SMN1 gene, and a fragment of the normalizing single-copy control gene (internal control (IC)) by multiplex polymerase chain reaction (PCR). Specificity of SMN1 amplification is achieved by the presence of an unpaired nucleotide in the 3'- region of the SMN1-specific primer.

The amplification process consists of a series of repeated cycles of temperature denaturation of DNA, annealing of primers with complementary sequences, and subsequent elongation of polynucleotide chains from these primers with Taq polymerase.

To increase the sensitivity and specificity of the reaction, a “hot” start is provided by using Taq polymerase blocked by antibodies. The polymerase chain reaction only starts when the Taq polymerase and antibody complex dissociates in temperature, which eliminates nonspecific annealing of primers on the DNA target during the initial heating of the test tube.

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 amplicons formed. The fluorescence level is measured at each amplification cycle in real time.

The DNA probe used to detect the TREC DNA amplification product includes the Fam fluorescent tag.

The DNA probe used to detect the amplification product of the normalizing single-copy control gene (IC) fragment includes the Cy5 fluorescent tag.

Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam	Hex	Rox	Cy5	Cy5.5
Exon 7 of SMN1	-	-	IC*	-

* IC – endogenous internal control, a single-copy genomic locus, evaluates the genome equivalents of nucleated blood cells (leukocytes) and provides quality control of DNA preparation.

The automatic analysis is available on “DNA-Technology” made instruments: DTlite, DTprime or DTprime II REAL-TIME Thermal Cyclers **SMA Screen Genotyping REAL-TIME PCR 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.

During PCR, the indicator cycle value (Cp) for exon 7 of the SMN1 gene and the normalization gene (IC) is

determined. Substituting the Cp values into formula (1) for each test sample, the ΔCp value characterizing the content of exon 7 of SMN1 gene relative to the single-copy normalization gene is calculated:

$$\Delta Cp = Cp\ SMN1 - Cp\ IC \qquad (1),$$

where Cp is the indicator cycle value for exon 7 of SMN1 gene and for normalization gene (IC).

The presence/absence of exon 7 of SMN1 deletion is determined considering the value of ΔCp according to the recommended threshold values indicated in Table 7 (section **9. DATA ANALYSIS**).

The reagent kit allows to determine homo- and heterozygous variants of exon 7 loss ("deletion") both as true deletions (of exon 7 and exons 7-8) and as functional loss of exon 7 of SMN1 as a result of NM_000344.3:c.840C>T (SMN1> SMN2) gene conversion.

WARNING!

1. The reagent kit **does not** allow to detect the latent carriage of SMN1 exon 7 deletions (genotype 2+0), a variant of SMA q5 genetic carriage characterized by two copies of SMN1 gene on one chromosome and its lack on the second. **In this case, the analsyis using the SMA Screen Genotyping REAL-TIME PCR Kit will nor detect the carriage of SMN1 exon 7 deletion!**
2. For correct assessment of SMN1 exon 7 copies, the acceptable Cp IC value must be up to 31 (Cp IC ≤ 31), which corresponds to the genomic DNA concentration of > 1.0 ng/μL.

The analysis consists of:

- DNA extraction (sample preparation)
- DNA PCR amplification with real-time result detection using **SMA Screen Genotyping REAL-TIME PCR Kit**

3. CONTENT

The **SMA Screen Genotyping REAL-TIME PCR Kit** contents is represented in Table 2.

Table 2. The **SMA Screen Genotyping REAL-TIME PCR Kit** content, package N, for R1-H821-N3/9EU

Reagent	Description	Total volume	Amount
PCR-mix	Colorless or pink transparent liquid	3 mL (1.5 mL in each)	2 tubes
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	50 μL (25 μL in each)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL in each)	2 tubes
Positive control	Colorless transparent liquid	130 μL	1 tube
Tubes, strips of 8	12 pcs		
Strip caps	12 strips of 8		

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

If dosing manually, the kit is designed for 96 tests (no more than 4 runs), including analysis of test samples, negative controls and positive controls.

If dosing automatically, the kit is designed for 96 tests (one run of 96 samples or two runs of 48 samples), including analysis of test samples, negative controls and positive controls.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

– 2.0 - 4.0 mL Vacuette blood collection tubes with anticoagulant, for example, salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant.

Please use only salt of EDTA or sodium citrate as an anticoagulant, since other substances can provide PCR inhibition.

4.2. DNA extraction and PCR

Equipment, reagents and consumables	Dosing	
	manual	automatic
UV PCR cabinet	yes	yes
Real-time detecting thermal cycler[1]	yes	yes
Vortex mixer	yes	yes
Refrigerator with freezer	yes	yes
Tube rack for 0.2 mL tubes[2]	yes	no
Tube rack for 0.2 mL stripped tubes[3]	yes	no
Tube rack for 1.5 mL tubes	yes	yes
Tube rack for 2.0 mL skirted tubes	yes	no
Single channel pipettes (dispensers covering 2.0-20, 20-200, 200-1,000 µL volume range)	yes	yes
RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL)	yes	yes
Pipette rack	yes	yes
RNase and DNase free 1.5 mL microfuge tubes or 2.0 mL microfuge tubes with caps	yes	no
RNase and DNase free 0.2 mL PCR tubes with caps or 96-well PCR microplate[4]	yes	yes
Powder-free surgical gloves	yes	yes
Container for used pipette tips, tubes and other consumables	yes	yes
DTstream *M4 dosing instrument (manufactured by DNA-Technology Research&Production, LLC)	no	yes
DTstream *M1 dosing instrument (manufactured by DNA-Technology Research&Production, LLC)	no	yes
RNase and DNase free filtered 200 µL pipette tips for DTstream dosing instrument or similar tips recommended for an equivalent dosing instrument	no	yes
DTpack sealing device (manufactured by DNA-Technology Research&Production, LLC)[5]	yes	yes
Centrifuge with RCF(g) at least 100 with microplate adapter[5]	yes	yes
Polymer thermal film for 96-well microplates[5]	yes	yes
PREP-MB MAX DNA Extraction Kit (REF P-103-N/4EU, P-103-A/8EU)		
Notes: [1] - DTprime detecting thermal cycler (version DTprime *M*) manufactured by DNA-Technology Research&Production, LLC; - DTprime II detecting thermal cycler (version DTprime *M*) manufactured by DNA-Technology		

Equipment, reagents and consumables	Dosing	
	manual	automatic
Research&Production, LLC; - DTlite detecting thermal cycler (version DTlite *S*) manufactured by DNA-Technology Research&Production, LLC (only for manual dosing with strips). [2] – if using tubes. [3] – if using strips. [4] – optional; PCR microplates are not used for the DTlite *S* thermal cycler. [5] – if using PCR microplates.		

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.

All components of the **SMA Screen Genotyping REAL-TIME PCR 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.

The TechnoTaq MAX polymerase must be stored in a freezer at the temperature from minus 22 °C to minus 18 °C over the storage period.

The **SMA Screen Genotyping REAL-TIME PCR Kit** must be transported in thermoboxes with ice packs by all types of roofed transport at temperatures inside the thermoboxes orresponding to storage conditions of the kit components.

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 the TechnoTaq MAX polymerase 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.

Reagent kits transported with violation of temperature conditions must not be used.

Shelf-life of the kit following the first opening of the primary container:

- 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.

The kit stored under undue regime should not be used.

An expired **SMA Screen Genotyping REAL-TIME PCR Kit** shall not be used.

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

Conformity of **SMA Screen Genotyping REAL-TIME PCR Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

6. WARNINGS AND PRECAUTIONS

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

Handle and dispose all biological samples, reagents and materials used to carry out the analysis as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the analysis. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

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

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

All the liquid solutions are designed for single use and 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 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 **SMA Screen Genotyping REAL-TIME PCR Kit** is designed to detect DNA extracted from whole peripheral blood.

7.1. General requirements

To obtain correct results, the quality of biomaterial sampling, storage, transport and pretreatment are of major importance.

Incorrect biomaterial sampling may lead to unreliable results and, thus, to the necessity of repeated sampling.

7.2. Interfering substances

The presence of PCR inhibitors in a sample of biological material can cause unreliable results. Signs of PCR inhibition are the absence of internal control amplification, distortion of a curve shape (curve does not have the S-shape, does not reach the plateau phase, is of flat nature) or late Cp values.

Anticoagulants used for blood sampling, as well as interfering substances of endogenous nature (hemoglobin, bilirubin, cholesterol and triglycerides) are referred to PCR inhibitors. The maximum allowable concentrations of potential interfering substances in a sample of original biomaterial are given in Table 3.

Potential inhibitors in DNA preparations include wash solution No. 2 of the **PREP-MB MAX** reagent kit.

NOTE. The maximum amount of the **PREP-MB MAX** wash solution No. 2 that does not inhibit PCR is up to 9 % of the DNA sample volume (considering DNA elution is 200 µL).

Provided that the instruction for use to the DNA extraction kit is followed strictly, PCR inhibition with wash solutions is not observed.

Table 3. Maximum allowable concentrations of potential interfering substances in the original biomaterial sample.

Biomaterial type	Interfering substance	Studied concentration in sample
Exogenous		
Whole blood	EDTA	3.60 mg/mL
Endogenous		
Whole blood, dried blood spots	Hemoglobin	250 g/L
	Bilirubin	500 µmol/L
	Cholesterol	12 mmol/L
	Triglycerides	500 mg/L

7.3. Sample collection

Biomaterial collection is performed in accordance with the instruction for use to the **PREP-MB MAX DNA Extraction Kit**.

Method limitations: Intravenous injections of heparin, infusions of parenteral nutrition are not allowed if done less than 6 hours before the test.

7.4. Transport and storage of samples

Transport and storage conditions for biomaterial samples are indicated in the instruction for use to the **PREP-MB MAX DNA Extraction Kit**.

In case the material cannot be delivered to the laboratory within 24 hours, a one-time freezing is allowed.

8. PROCEDURE

8.1. DNA extraction from biological material

PREP-MB MAX DNA Extraction Kit is validated for DNA extraction.

DNA extraction is carried out according to the **PREP-MB MAX** kit instruction for use.

WARNING!

1. DNA elution must be performed in **200 µL** of elution solution.
2. Simultaneously with DNA extraction from biological material, perform all the sample preparation stages on negative control. Use negative control included in the **PREP-MB MAX DNA Extraction Kit** in the volume indicated in the instruction for use to the **PREP-MB MAX DNA Extraction Kit**.
3. If using DNA extraction kits not validated to be used together with **SMA Screen Genotyping REAL-TIME PCR Kit**, the manufacturer cannot guarantee the correct analysis result interpretation.

The obtained DNA preparation can be stored at temperatures from 2 °C to 8 °C for no longer than 7 days or at temperatures from minus 22 °C to minus 18 °C for no longer than 6 months.

WARNING! If you intend to store DNA preparation for longer than 7 days, transfer supernatant to a new tube.

8.2. PCR analysis. Automated dosing using DTstream

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

1. For amplification, use 0.2 mL single-use stripped PCR tubes[1] or 96-well PCR microplates[2] sealed

[1] - included in the reagent kit

[2] - 96-well PCR microplates are not for use with DTlite detecting thermal cycler

with thermal film.

2. In the course of the analysis, keep tubes with PCR-mix away from direct sunlight!
3. Make sure the strips and strip caps belong to the same reagent kit. Do not use strip caps from other reagent kits!

8.1.1. Mark the necessary amount of stripped tubes or a 96-well microplate for test samples, negative control “C-“ and positive control “C+”.

WARNING! The reagents are calculated for:

- 1 run with 94 test samples, negative control “C-“ and positive control “C+”;
- 2 runs with 46 test samples, negative control “C” and positive control “C+” in each run.

Table 4 – Amount of reagents required for the analysis

Component	Reagents required per run	
	96 test samples (1 run[1])	48 test samples (2 runs)
PCR-mix	2 tubes	1 tube
TechnoTaq MAX polymerase	2 tubes	1 tube
Mineral oil	2 tubes	1 tube
Positive control	1 tube[1]	1 tube[2]
[1] - for detecting thermal cyclers DTprime and DTprime II [2] - used twice; after the first run close the tube with the cap from the kit and store at 2 °C – 8 °C until the next run		

8.1.2. Shake the tube/tubes (see Table 4) with PCR-mix on vortex for 3-5 seconds and spin on vortex for 1-3 seconds.

8.1.3. Spin the tube/tubes (see Table 4) with TechnoTaq MAX polymerase on vortex for 1-3 seconds.

8.1.4. Spin the tube/tubes with mineral oil on vortex for 1-3 seconds.

WARNING! TechnoTaq MAX polymerase should be taken out of the freezer immediately before use.

8.1.5. Shake the tube with positive control on vortex for 3-5 seconds and spin on vortex for 1-3 seconds.

WARNING! Before dosing spin the tubes with DNA preparations and negative control on vortex for 1-3 seconds. **Do not shake the tubes!** After spinning, place the tubes into the magnetic rack. If after DNA extraction the supernatant containing DNA was transferred into new tubes, shake the tubes with supernatant and negative control on vortex for 3-5 seconds, then spin on vortex for 1-3 seconds.

8.1.6. Place the tubes with PCR-mix, mineral oil, TechnoTaq MAX polymerase, DNA preparations, negative control and positive control as well as amplification strips or a PCR microplate, onto the DTstream worktable, them proceed to dosing according to the dosing instrument user manual.

8.1.7. In case of using a 96-well PCR microplate:

8.1.6.1. Carefully, without shaking, place the 96-well microplate into the DTpack sealing device plate rack.

8.1.6.2. Seal the 96-well microplate with polymer thermal film according to the DTpack user manual.

8.1.6.3. Spin the 96-well microplate at RCF(g) 100 for 30 seconds.

8.1.8. In case of using strips:

8.1.7.1. Close the strips tightly.

8.1.7.2. Spin all the strips in vortex mixer for 3—5 seconds.

8.1.9. Set the strips/96-well microplate into the thermal cycler.

8.1.10. Run operating software for thermal cycler. Add corresponding test[3]. For further runs create the corresponding analysis protocol: specify the number and IDs of samples, including positive and negative controls, specify the positions of samples in the heat block and run PCR. For the PCR program see Table 5.

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

Step	Temperature, °C	Min	Sec	Number of cycles	Optical measurements	Type of the step
1	80	0	30	1		Cycle
	94	5	00			
2	94	0	30	5		Cycle
	60	0	15		√	
3	94	0	10	40		Cycle
	60	0	15		√	
4	94	0	5	1		Cycle
5	25[1]	…	…	Holding		Holding

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

8.3. PCR analysis. Manual dosing

WARNING!

1. For amplification, use 0.2 mL single-use stripped PCR tubes[4], or 0.2 mL single-use PCR tubes and 96-well PCR microplates[5] sealed with thermal film.
2. In the course of the analysis, keep tubes with PCR-mix away from direct sunlight!
3. Make sure the strips and strip caps belong to the same reagent kit. Do not use strip caps from other reagent kits!

8.3.1 Mark the necessary amount of tubes/stripped tubes or a 96-well microplate for test samples, negative control “C-“ and positive control “C+”.

WARNING! The reagents are calculated for no more than 4 runs considering variable number of test samples, 1 negative control and 1 positive control in each run.

E x a m p l e . To test 6 samples, mark 6 tubes/reserve 6 microplate wells for test samples, 1 tube/well for “C-“ and 1 tube/well for “C+”. The total number of tubes/wells is 8.

8.3.2 Shake the tube/tubes with PCR-mix on vortex for 3-5 seconds and spin on vortex for 1-3 seconds

8.3.3 Spin the tube/tubes with TechnoTaq MAX polymerase and mineral oil on vortex for 1-3 seconds.

WARNING! TechnoTaq MAX polymerase should be taken out of the freezer immediately before use.

8.3.4 To prepare PCR-mix with TechnoTaq MAX, mix in separate tubes:

- 30×(N+1) µL of PCR-mix,
- 0.5×(N+1) µL of TechnoTaq MAX polymerase,

where N is the number of marked tubes/wells, considering “C-“ and “C+”.

E x a m p l e . To test 6 samples with “C-“ and “C+” in 8 marked tubes/wells, prepare a mix of TechnoTaq MAX polymerase and PCR-mix for 9 (8+1) tubes, i.e. 270 µL of PCR-mix and 4.5 µL of TechnoTaq MAX polymerase.

[3] - Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.
[4] - included in the reagent kit
[5] - 96-well PCR microplates are not for use with DTlite detecting thermal cycler

8.3.5 Shake the tube with the prepared PCR-mix and TechnoTaq MAX polymerase mixture on vortex for 3-5 seconds and spin on vortex for 1-3 seconds.

WARNING! Prepare the PCR-mix with TechnoTaq MAX polymerase immediately prior to use.

8.3.6 Add 30 µL of PCR-mix with TechnoTaq MAX polymerase into each marked tube/well of the microplate (including "C-", "C+").

WARNING! After adding PCR-mix with TechnoTaq MAX polymerase into the tubes/wells immediately proceed to 8.3.7 – 8.3.15.

8.3.7 Add 20 µL of mineral oil into each tube/microplate well with PCR-mix. Cover the strips loosely with caps.

8.3.8 Shake the tube with positive control on vortex for 3-5 seconds and spin on vortex for 1-3 seconds.

WARNING!

1. Before adding DNA preparation and negative control into the tubes/wells with PCR-mix, 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 on vortex for 3-5 seconds and spin down the drops for 1-3 seconds.
2. 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.

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

8.3.10 Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into "C-" tube/microplate well.

8.3.11 Add 5.0 µL of positive control sample (C+) into the corresponding tube/microplate well.

8.3.12 In case of using a 96-well PCR microplate:

8.3.12.1. Carefully, without shaking, place the 96-well microplate into the DTpack sealing device plate rack.

8.3.12.2. Seal the 96-well microplate with polymer thermal film according to the DTpack user manual.

8.3.12.3. Spin the 96-well microplate at RCF(g) 100 for 30 seconds.

8.3.13 In case of using tubes/strips:
Spin all the tubes/strips on vortex for 3-5 seconds.

8.3.14 Set the tubes/strips/ or a 96-well PCR microplate into the thermal cycler.

8.3.15 Run operating software for thermal cycler. Add corresponding test[6]. For further runs create the corresponding analysis protocol: specify the number and IDs of samples, including positive and negative controls, specify the positions of samples in the heat block and run PCR. For the PCR program see Table 5.

9. DATA ANALYSIS

9.1. Registration of the amplification results is carried out automatically during amplification by the software provided with detecting thermal cycler.

9.2. Interpretation of PCR results for each sample is carried out considering the Cp values of SMN1 DNA (Fam detection channel) and normalization gene (NG) (Cy5 detection channel) in accordance with Table 6. Interpretation of the screening results is performed according to Table 7. The run results are valid if the conditions for interpretation of the results obtained for controls are met

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

(Table 6).

WARNING! The reagent kit does not allow to detect forms of hidden carriage of SMN1 exon 7 deletions (genotype 2+0) – a variant of SMA q5 genetic carrier characterized by two copies of the SMN1 gene on one chromosome and its absence on the second chromosome. In this case, the study using the reagent kit will not reveal carriage of SMN1 exon 7 deletion!

Table 6. PCR results interpretation for controls

Detection channel		**|ΔCp|** (|Cp SMN1 (Fam) – Cp ВК (Cy5)|)	**Result interpretation**
Fam (SMN1), Cp	**Cy5 (**ВК), Cp		
Negative control			
Not specified or **≥ 38**	Not specified or **≥ 38**	Not considered	**Negative result** Run results are valid
Positive control			
< 38	**< 38**	**≤ 1,0**	**Positive result** Run results are valid

9.3. If the results for the negative control differ from the values specified in Table 6, the results of the whole run are considered unreliable. In this case it is necessary to carry out special measures to identify and eliminate possible contamination.

9.4. If the results for the positive control differ from the values specified in Table 6, a repeated amplification for the whole batch of samples is required.

Table 7. PCR result interpretation

Detection channel		**ΔCp** **(**Cp SMN1 (Fam) – Cp IC (Cy5))	**Result interpretation**
Fam (SMN1), Cp	**Cy5 (**IC), Cp		
Test samples			
Specified	**≤ 31**	**> 6.0**	**Homozygous deletion is detected** (deldel)
Not specified	**≤ 31**	Not considered	
Specified	**≤ 31**	**0.8≤** ΔCp **≤2.0**	**Heterozygous deletion is detected** (Ndel)
Specified	**≤ 31**	**<0.8** (including negative values)	**Deletion is not detected** (NN)
Specified	≤ 31	2.0<ΔCp ≤6.0	Doubtful result (?)
Not considered	Not specified or >31	Not considered	Unreliable result

Table 8 – Exon 7 of SMN1 gene analysis result interpretation

PCR results interpretation	**PCR screening result**	**Comments**
Homozygous deletion is detected (deldel)	**Deletion of exon 7 of SMN1 gene in homozygous state is detected. WARNING! High risk of SMA q5.** Consult with a geneticist. Advanced diagnostics of SMN1 and SMN2 genes by quantitative MLPA analysis and/ or sequencing is required.	We recommend to confirm the result with a repeated analysis starting with sample preparation.

PCR results interpretation	PCR screening result	Comments
Heterozygous deletion is detected (Ndel)	**Deletion of exon 7 of SMN1 gene in heterozygous state is detected.** Consultation with a geneticist is recommended. Confirmation of the result by quantitative MLPA analysis and advanced genetic diagnosis of SMN1 and SMN2 genes by sequencing is recommended.	We recommend to confirm the result with a repeated analysis starting with sample preparation.
Deletion is not detected (NN)	Deletion of exon 7 of SMN1 gene is not detected.	-
Doubtful result	Alternative SMN1 and SMN2 genes analysis method is recommended (quantitative MLPA analysis and/or sequencing)	Repeated analysis starting with sample preparation is required. If doubtful result reoccurs, alternative analysis methods are recommended.
Unreliable result	Repeat the exon 7 of SMN1 gene analysis.	A repeated PCR with an existing DNA preparation, or a repeated DNA extraction and PCR for that sample, or a repeated collection of biological material from the patient (performed sequentially) are required. An unreliable result may be due to the presence of inhibitors in the DNA preparation obtained from biological material; incorrect execution of the test protocol; failure to comply with the amplification temperature regime, etc.

10. SPECIFICATIONS

a. Analytical specificity

In the course of research, the specificity of the reagent kit was verified by bioinformatic methods. The absence of non-specific cross-reactions was demonstrated for each of the oligonucleotide systems (SMN1 and IC) included in the reagent kit.

Analytical specificity of the reagent kit was confirmed using genetically engineered constructs (recombinant plasmids) corresponding to the DNA sequences of the detected indicators (exon 7 of SMN1 and IC), as well as a homologous fragment of SMN2. The specificity of SMN1 oligonucleotide systems was additionally confirmed using control reference DNA samples characterized by the number of SMN1 and SMN2 gene copies by MLPA.

b. Analytical sensitivity (limit of detection)

The detection limit for homozygous and heterozygous deletions of exon 7 of the SMN1 gene is 1.0 ng/µL (IC Cp ≤ 31).

The analytical detection limit (the thermal cycler registers a positive result) is 5 copies of DNA of each analyte (exon 7 of SMN1 and IC) per amplification tube.

c. Diagnostic characteristics

Diagnostic characteristics for exon 7 of SMN1 deletion in homozygous state

Number of samples	247
Diagnostic sensitivity (CI 95%)	100.00% (63.06%-100.00%)
Diagnostic specificity (CI 95%)	100.00% (98.47%-100.00%)

Diagnostic characteristics for exon 7 of SMN1 deletion in heterozygous state

Number of samples	247
Diagnostic sensitivity (CI 95%)	100.00% (86.28%-100.00%)
Diagnostic specificity (CI 95%)	100.00% (98.35%-100.00%)

d. Results precision

Precision amounts to 100%.

Repeatability amounts to 100%.

11. TROUBLESHOOTING

Table 9. Troubleshooting

	Result	Possible cause	Solution
C+	Does not meet the acceptable Cp values (see Tables 6, 7)	Operation error Violation of storage and handling requirements	Repeat the analysis Dispose of the current batch
C-	Does not meet the acceptable Cp values (see Table 6, 7)	Contamination	Dispose of the current batch Perform decontamination procedures
IC	Invalid	Low amount of DNA\ PCR inhibition	Repeat the analysis 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://dna-technology.com/support

12. 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 **SMA Screen Genotyping REAL-TIME PCR Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

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13. 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

REF R1-H821-N3/9EU VER 1048.2025.03.10