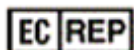


362-4 2022-08-25

**For professional use only**

BRCA mutations REAL-TIME PCR Genotyping Kit

INSTRUCTION FOR USE



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R1-H927-N3/4EU



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1. INTENDED USE

The **BRCA mutations REAL-TIME PCR Genotyping Kit** is intended for research and diagnostic applications. The **BRCA mutations REAL-TIME PCR Genotyping Kit** is in vitro Nucleic Acid Test (NAT) – human genotyping-based product. The **BRCA mutations REAL-TIME PCR Genotyping Kit** is designed to detect and discriminate eight genetic polymorphisms associated with breast and ovarian cancer (OMIM #604370; #612555): BRCA1 (185delAG, 4153delA, 5382insC, 3819delGTAA, 3875delGTCT, 300T>G (Cys61Gly), 2080delA) and BRCA2 (6174delT). The **BRCA mutations Real-Time PCR Genotyping Kit** is based on Polymerase Chain Reaction (PCR) method.

The results can be used for diagnosis of hereditary forms of breast cancer and ovarian cancer and for prediction of the corresponding hereditary forms of cancer in relatives of the first line.

There are no contradictions for use of the **BRCA mutations REAL-TIME PCR Genotyping Kit**. Bone marrow transplantation can theoretically affect the results. In this case, additional studies will be needed.

The **BRCA mutations REAL-TIME PCR Genotyping Kit** can be used in clinical and diagnostic laboratories of medical institutions and research practice.

The application of the kit does not depend on population and demographic aspects (diagnostic characteristics of the kit are determined only for women from the Russian Federation).

Potential users: personnel qualified in molecular diagnostics methods and working in the clinical and diagnostic laboratory.

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

2. METHOD

The implemented PCR method is based on amplification of a target DNA sequence.

The detection is based on melting curve analysis.

The **BRCA mutations REAL-TIME PCR Genotyping Kit** employs fluorescent probes each of one specific to one of two alleles of a gene. The PCR-mix contains two distinguishably labelled allele-specific probes bearing reporter fluorescent dyes (Fam and Hex) for each variant of polymorphism. After amplification melting of amplicon-signal probe complexes is performed. It results in changing fluorescence level and is detected by the real-time thermal cycler and is represented by the software as a graph. If the signal probe is partially complementary to the DNA-target the melting temperature will be less than in case when signal probe is absolute complementary to the DNA-target. The interpretation of results is made based on melting temperatures.

In PCR-mix for each polymorphism the system for human genomic DNA amplification is included. It allows to control quantity of human DNA in amplification tube to exclude mistakes in genotyping.

The system for human genomic DNA amplification includes DNA-probe with fluorescent tag (Cy5) and quencher molecule. While being hybridized to a target sequence, fluorescent probes are inactivated (quenched). When the amplicon is synthesized the probes denature and fluorescent tag is no more quenched and therefore provide fluorescent signal. The intensity of fluorescence is measured by Real-time PCR thermal cycler at every step and analyzed with the software provided. The application of three fluorescent dyes makes it possible to determine two alleles and estimate the amount of genomic DNA simultaneously in one tube. Table 1 shows the detection channels of PCR-mix.

Table 1. Detection channels of amplification products

PCR-mix	Detection channels				
	Fam	Hex	Rox	Cy5	Cy5.5
BRCA1: 185delAG	N	m	-	IC	-
BRCA1: 4153delA	N	m	-	IC	-
BRCA1: 5382insC	N	m	-	IC	-
BRCA1: 3819delGTAA	N	m	-	IC	-
BRCA1: 3875delGTCT	N	m	-	IC	-
BRCA1: 300 T>G (Cys61Gly)	N	m	-	IC	-
BRCA1: 2080delA	N	m	-	IC	-
BRCA2: 6174delT	N	m	-	IC	-

where N-normal allele, m-mutant allele, IC - Internal control system (system for human DNA amplification, which allows to estimate the amount of DNA in the amplification tube and eliminate genotyping errors).

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **BRCA mutations REAL-TIME PCR Genotyping 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>.

3. CONTENT

The **BRCA mutations REAL-TIME PCR Genotyping Kit** contains PCR-mix, PCR-buffer, TechnoTaq MAX polymerase and mineral oil. The detailed description of content is represented in Table 2.

Table 2. The **BRCA mutations REAL-TIME PCR Genotyping Kit** content, for R1-H927-N3/4EU

Reagent	Description	Total volume	Amount
PCR-mix			
1. BRCA1: 185delAG	Colorless transparent liquid	960 µL	1 tube
2. BRCA1: 4153delA		960 µL	1 tube
3. BRCA1: 5382insC		960 µL	1 tube
4. BRCA1: 3819delGTAAA		960 µL	1 tube
5. BRCA1: 3875delGTCT		960 µL	1 tube
6. BRCA1: 300 T>G (Cys61Gly)		960 µL	1 tube
7. BRCA1: 2080delA		960 µL	1 tube
8. BRCA2: 6174delT		960 µL	1 tube
PCR-buffer	Colorless transparent liquid	4 mL (1 mL per tube)	4 tubes
TechnoTaq MAX polymerase	Colorless transparent liquid	200 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	8 mL	1 vial
Positive controls:			
“C+1” (wild-type allele homozygous)	Colorless transparent liquid	270 µL	1 tube
“C+2” (heterozygous)	Colorless transparent liquid	270 µL	1 tube

The **BRCA mutations REAL-TIME PCR Genotyping Kit** contains positive control samples: C+1 [wild-type allele homozygous] and C+2 [heterozygous]. Positive controls are mixtures of cloned parts of the target genes BRCA1 and BRCA2 detectable with an aid of the kit. Control samples are intended to control the quality of the test by the user.

The **BRCA mutations REAL-TIME PCR Genotyping Kit** is designed for 48 tests including the analysis of unknown samples and negative control samples.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

Blood sampling equipment is required. Please use only EDTA as an anticoagulant, since other substances can provide PCR inhibition.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Vortex mixer;
- Refrigerator;
- Nucleic acid extraction kit (“DNA-Technology” made **PREP-GS Genetics** REF P-023/4EU or **PREP-RAPID Genetics** REF P-021/4EU are recommended);
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 50-98 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Physiological saline solution 0.9% NaCl (Sterile);
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Vortex mixer;
- Refrigerator;
- PCR tube rack for 0.2 mL tubes;
- 0.2 mL tubes;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 50 µL, 200 µL, 1000 µL);
- 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.

All components of the **BRCA mutations REAL-TIME PCR Genotyping Kit**, except the TechnoTaq MAX polymerase, must be stored at temperatures from 2 °C to 8 °C over the storage period. The PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period. The TechnoTaq MAX polymerase must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period. The excessive temperature and light can be detrimental to product performance.

The kit can be transported by all types of roofed transport at temperatures from 2 °C to 8 °C over the transportation. It is allowed to transport TechnoTaq MAX polymerase at temperatures from 2 °C to 8 °C for no more than 5 days.

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

- components of the kit should be stored at temperatures from 2 °C to 8 °C during the storage period;
- PCR-mix for amplification should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period
- TechnoTaq MAX polymerase should be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

The kit stored in under undue regime should not be used.

An expired the **BRCA mutations REAL-TIME PCR Genotyping Kit** should not be used.

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

The conformity of the **BRCA mutations REAL-TIME PCR Genotyping Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **BRCA mutations REAL-TIME PCR Genotyping Kit**.

6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular diagnostics and the rules of work in the clinical and diagnostic laboratory are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the assay 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 assay. 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, reverse transcription, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the

degradation of nucleic acids contained in the samples or sample contamination by amplification products.

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

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

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be exclusively employed for this specific purpose. Remove PCR waste only in a closed form. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Do not open the tubes after amplification. Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

Emergency actions

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

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

Do not use the kit:

- When 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 **BRCA mutations REAL-TIME PCR Genotyping Kit** is designed to detect DNA extracted from the peripheral blood.

Sampling, sample processing procedures and storage are carried out in accordance with the instructions to the DNA extraction kit from biological material.

Peripheral blood sampling is carried out in vacuum plastic tube. It may be 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL. After taking the material, it is necessary to mix the blood with anticoagulant inverting the tube 2 – 3 times.



It is not allowed to use heparin as an anticoagulant.

Transportation and storage of the samples

Samples may be stored at temperatures from 2 °C to 8 °C for no more than 24 h. When it is impossible to deliver the material in the laboratory during the day, a one-time freezing of the material is allowed. The frozen material is allowed to be stored at temperatures from minus 18 °C to minus 22 °C for 6 months.



The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-GS Genetics** and **PREP-RAPID Genetics** extraction kits user manuals.

8. PROCEDURE

DNA extracting from biological material

DNA extraction is carried out according to the extraction kit instructions. **PREP-GS Genetics** and **PREP-RAPID Genetics** extraction kits are recommended. The **PREP-GS Genetics DNA Extraction Kit** is intended for long-term storage of the extracted DNA (up to 6 months). The DNA extracted with aid of **PREP-RAPID Genetics DNA Extraction Kit** should be stored no more than one month. The extracted DNA can be used for about 50 reactions to determine genetic polymorphisms.



Independently of DNA extraction kit used, a negative control sample should go through all stages of DNA extraction. Physiological saline solution can be used as a negative control in volumes as indicated.



The quantity of DNA to be analyzed must be greater than or equal to 1.0 ng per reaction (the Cp parameter for IC must not be more than 32). The violation of this requirement will affect the validity of analysis and void the manufacturer guarantee.

Assay procedure



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

- 8.1** Mark the required number of 0.2 mL PCR-tubes for each of the polymorphisms to be tested (one tube for each sample to be tested and three extra - one for negative control “C-” and two for positive controls “C+”).

Example: to test 5 samples in one PCR run, mark 40 tubes for samples, 8 tubes for “C-” and 16 tubes for “C+”. The resulting number of tubes is 64. See the Table 3 below.

Table 3. Tube marking

Sample	PCR-mix / tube №							
	BRCA1							BRCA2
	185 delAG	4153 delA	5382 insC	3819 delGTAAA	3875 delGTCT	300 T>G	2080 delA	6174 delT
1	1	2	3	4	5	6	7	8
2	9	10	11	12	13	14	15	16
3	17	18	19	20	21	22	23	24
4	25	26	27	28	29	30	31	32
5	33	34	35	36	37	38	39	40
C-	41	42	43	44	45	46	47	48
C+1	49	50	51	52	53	54	55	56
C+2	57	58	59	60	61	62	63	64

8.2 Vortex the tubes with PCR-mixes for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

8.3 Add 20 µL of corresponding PCR-mix into the marked tubes (use a new pipette tip for each type of PCR-mix).

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



TechnoTaq MAX polymerase must be stored at temperatures from minus 18 °C to minus 22 °C. Room temperature exposure is permitted only for a short time. Remove from freezer just prior to use and place on ice.

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

- 10×(N+1) µL of PCR-buffer,
 - 0.5×(N+1) µL of TechnoTaq MAX polymerase,
- N — number of the marked tubes including “C-” and “C+”.

Example: for simultaneous testing of 5 samples, “C-” and two “C+” in one PCR run. Mix 650 µL of PCR-buffer and 32.5 µL of TechnoTaq MAX polymerase (calculate final volume for 65 (64+1) tubes).

8.6 Vortex the tube for 3-5 seconds, then spin for 1-3 seconds to collect the drops.



The mixture of PCR-buffer and TechnoTaq MAX polymerase must be prepared just prior to use.

8.7 Add 10 µL of PCR-buffer and TechnoTaq MAX polymerase mixture into each PCR-tube.

8.8 Add one drop (~20 µL) of mineral oil into each tube. Close the tubes.



Follow the steps listed in pp. 8. 9 – 8.14 within two hours after addition of PCR-buffer and TechnoTaq MAX polymerase mix to PCR-mix.

8.9 Vortex the tubes with samples and “C-” and “C+” for 3-5 seconds and spin down drops for 1-3 seconds.



In case of using **PREP-GS Genetics DNA Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g) 16000 for one minute to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.



Open the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA sample to prevent contamination. Close the tubes tightly. Use filter tips.

- 8.10** Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the “C-” and “C+” tubes.
- 8.11** Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure and positive controls (C+1, C+2) into corresponding tubes.
- 8.12** Spin tubes briefly for 3-5 seconds.
- 8.13** Set the tubes into the Real-time Thermal Cycler.
- 8.14** Launch the operating software for DT instrument¹. Add corresponding test², specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes in the thermal unit (8.13) and run PCR. See Table 4.



The type of positive and negative controls tubes must be specified as “Sample”.

Table 4. The PCR program for DTlite and DTprime Thermal Cyclers

Step	Temperature, °C	Temperature Increment, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80		2	00	1		Cycle
	94		5	00			
2	94		0	30	5		Cycle
	64		0	15		v	
	67		0	5			
3	94		0	5	45		Cycle
	64		0	15		v	
4	25		0	30	1		Cycle
5	25	1	0	15	50	v	Melting, Δt=1°C; T _{fin} =75°C
6	10		Holding		Holding

¹ Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.

² Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website <https://www.dna-technology.com/assaylibrary>.

9. CONTROLS

The **BRCA mutations Real-Time PCR Genotyping Kit** contains positive control samples: C+1 [wild-type allele homozygous] and C+2 [heterozygous]. Positive control is a cloned part of the genome. It is produced with genetic engineering techniques and characterized by automatic DNA sequencing. The PCR-mix from the kit includes the Internal control (IC). IC is an artificial plasmid intended to assess the quality of PCR performance.

To reveal possible contamination a negative control is required.



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

The results for "C+1" should be wild-type allele homozygous.

The results for "C+2" should be heterozygous.

The test result is considered valid when genotype is defined.

The test result is considered invalid when the Cp of IC (Cy5) is less than 32 or absent.

If the signal for "C-" is present, whole tests of current batch considered false. Decontamination is required.

10. DATA ANALYSIS

Registration and interpretation of the PCR results held in automatic mode. The graph will show the fluorescence dependence of the melting temperature for each tube in the thermoblock. The table will show the sample ID, the name of the polymorphism being detected, and the genotyping result of each sample. It is possible to create and print a report based on the analysis results. Please refer to DTlite or DTprime thermal cycler's user manual for details on working with software.

The software registers the result of human genomic DNA amplification (IC) for all samples. For samples containing a sufficient quantity of DNA for correct analysis, the software defines the genotype, which is displayed in the table in the "Polymorphism" column. The samples containing an insufficient quantity of DNA (less than 1.0 ng per reaction or Cp>32) will be analyzed as "invalid" (uncertain result).

Results Statistics Melting Curve IC				
N°	Identifier	Test	Polimorphism	
A1	Sample_1	BRCA1:185delAG	N	N
B1	Sample_1	BRCA1:4153delA	N	N
C1	Sample_1	BRCA1:5382insC	N	N
D1	Sample_1	BRCA1:3819delGTAAA	N	N
E1	Sample_1	BRCA1:3875delGTCT	N	N
F1	Sample_1	BRCA1:300 T>G (Cys61G	N	N
G1	Sample_1	BRCA1:2080delA	N	N
H1	Sample_1	BRCA2:6174delT	N	N
A2	Sample_2	BRCA1:185delAG	N	N
B2	Sample_2	BRCA1:4153delA	N	m
C2	Sample_2	BRCA1:5382insC	N	N
D2	Sample_2	BRCA1:3819delGTAAA	N	N
E2	Sample_2	BRCA1:3875delGTCT	N	N
F2	Sample_2	BRCA1:300 T>G (Cys61G	N	N
G2	Sample_2	BRCA1:2080delA	N	N
H2	Sample_2	BRCA2:6174delT	N	N



Because of high medical and social significance of BRCA1 or BRCA2 mutation carrier status, it is recommended to retest heterozygous samples, starting from the DNA extraction step.

The software defines corresponding genotype in positive control samples:

C+1 [wild-type allele homozygous] -

N	N
---	---

C+2 [heterozygous] -

N	m
---	---

For samples with insufficient quantity of DNA for analysis (less than 1.0 ng per reaction or Cp>32.0 on the IC detection channel), the software defines the uncertain result:

invalid	invalid
---------	---------

In the case of uncertain result, PCR method with the same DNA sample, or DNA extraction and PCR, or blood taking (carry out sequentially) is required to repeat.

For negative control samples, the software defines an uncertain result:

invalid	invalid
---------	---------

In case of positive result in a negative control, the results of the whole tests of current batch are considered to be uncertain. It is necessary to carry out special measures to eliminate contamination.



DNA-Technology Genotyping assays provide genetic information for some, but not all polymorphic loci known to be associated with certain medical conditions. This information estimates a probability of disease development but does not provide a definitive diagnosis, since other genes may contribute to the odds of disease onset. Moreover, the professional medical consultation regarding complex diseases cannot solely rely on genetic testing. The medical recommendations should also consider behavioral, physical, nutritional and familial information of a patient. On the basis of DNA-Technology Genotyping assays, a specialist can conclude whether a person of a certain genotype has lower or higher chance of disease development in relation to average risk. The definitive diagnosis is a derivative of a physicians experience and the depth of clinical information.

At the assay development stage we review the most up-to-date scientific literature on genetic associations repeatedly confirmed by independent research. We restrict our genotyping assays to a relatively small set of genetic markers because we believe they provide the most helpful and unbiased information about possible genetic susceptibility to common diseases.

11. SPECIFICATIONS

a. The analytical **specificity** of the **BRCA mutations REAL-TIME PCR Genotyping Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

Interferants in concentrations: bilirubin - 684 µmol/L, hemoglobin - 2 g/L, cholesterol - 13 mmol/L, triglycerides - 37 mmol /L do not affect the specificity of the kit.

b. In a determination of analytical **sensitivity**, the **BRCA mutations REAL-TIME PCR Genotyping Kit** demonstrated the ability to reproducibly detect 1 or more genome equivalents per PCR reaction.

The lower limit of detection is not less than 1.0 ng of human DNA per amplification tube, which corresponds to Cp≤32.0 on the IC detection channel (Cy5). When the amount of DNA is smaller (Cp>32.0 on the IC detection channel), the manufacturer does not guarantee the correct result of the kit.

After the amplification reaction for samples with insufficient quantity of DNA (less than 1.0 ng per amplification tube), the result is defined as uncertain.

c. Diagnostic characteristics

Number of samples (n) - 635;

Diagnostic sensitivity (95% CI) - 100.0% (93.9-100.0%);

Diagnostic specificity (95% CI) – 100.0% (99,6-100%).



The claimed specifications are guaranteed when DNA extraction is performed with **PREP-GS Genetics** **REF** P-023/4EU or **PREP-RAPID Genetics** **REF** P-021/4EU extraction kits.

12. TROUBLESHOOTING

Table 5. Troubleshooting

	Genotype	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	-	PCR inhibition Insufficient amount of DNA	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

“DNA-Technology Research&Production”, LLC declares that the above mentioned products meet the provision of the Council Directive 98/79/EC for *in vitro* Diagnostic Medical Devices. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016.

Contact our official representative in EU by quality issues of **BRCA mutations REAL-TIME PCR Genotyping Kit**.

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14. KEY TO SYMBOLS

	<i>In vitro</i> diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for <n> tests		Catalogue number
	Use-by date		Manufacturer
	Batch code		Keep away from sunlight
	Version		Do not reuse
	Non-sterile		Positive control
	Authorized representative in the European Community		Caution

REF

R1-H927-N3/4EU

VER

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