DNA-TECHNOLOGY

611 2022-01-23

CE IVD

For professional use only

EGFR mutations REAL-TIME PCR Genotyping Kit

INSTRUCTION FOR USE

EC REP

OBELIS S.A

Registered Address:

Bd. Général Wahis, 53

1030 Brussels, Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

http://www.obelis.net

"DNA-Technology Research & Production", LLC,

142281, Russia,

Moscow Region, Protvino,

Zheleznodorozhnaya street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

http://www.dna-technology.com

Customer service department

E-mail: hotline@dna-technology.ru

REF

R1-H806-S3/4EU
R1-H806-UA/4EU
R1-H807-S3/4EU
R1-H807-UA/4EU

VER

611.2022.01.23

Digitally signed by Ceaicovschi Tudor
Date: 2023.10.18 11:30:00 EEST
Reason: MoldSign Signature
Location: Moldova

TABLE OF CONTENTS

1. INTENDED USE

The **EGFR mutations REAL-TIME PCR Genotyping Kit** is *in vitro* DNA test, which is intended for the detection of somatic mutations in the EGFR gene (deletions and insertions in the 19th exon, insertions in the 20th exon, mutations L858R, T790M, L861Q, S768I and G719X) by real-time PCR in human genomic DNA extracted from formaldehyde-fixed paraffin-embeded (FFPE) non-small-cell lung cancer samples in order to choose the patients for target therapy with tyrosinkinase inhibitors.

The **EGFR 8** variant of the kit is intended for the detection of all mentioned mutations, while the **EGFR 4** variant of the kit is a contracted version that is intended for the detection of deletions and insertions in the 19th exon and L858R, T790M mutations.

Indications for the use: the need for target tumor treatment selection for patients with non-small-cell lung cancer.

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

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

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 the allele-specific DNA amplification technology. The process of amplification includes repeating cycles of thermal DNA denaturation, annealing of primers with complementary sequences and their extension by DNA-polymerase.

To increase the sensitivity and specificity of amplification reaction, the use of a hot-start is provided. Hot-start for package S 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. Hot-start for package U is provided by the use of monoclonal antibodies for Taq-polymerase. Enzyme is activated only after 5 minutes of heating. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

The **EGFR mutations REAL-TIME PCR Genotyping Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains target-specific probes bearing reporter fluorescent dyes and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The conditions of the reactions and oligonucleotides used are set in a way for effective amplification of mutant allele. The value of threshold cycle Cp on target mutant allele significantly exceeds the background amplification on normal allele – no less than 6-9 cycles depending on the amount of DNA matrix.

Apart from allele-specific oligonucleotides for mutant alleles of EGFR gene amplification mixes contain primers and probes for the detection of 5 exon of EGFR gene (SIC) that is used for control of amplification sufficiency and semi-quantitative estimation of mutant alleles amount. For the mutant alleles detection the value of difference between Cp of mutant alleles and SIC is used: the value of dCp less than 6-9 cycles depending on SIC value indicates the presence of mutation.

For the estimation of the results validity sample intake control (SIC) value is used.

DNA probes used for the detection of mutant alleles of EGFR gene amplification products includes fluorescent dyes Fam, Hex and Rox and probes for exon 5 of EGFR gene (SIC) include Cy5 dye.

One tube contains additional probe with Rox dye label – “Marker”. It tags the strip orientation. Upon completion of run, software defines actual position of the strip (by means of “marker” position) relative to the position preset by the operator. If it mismatches, the software suggests rearrangement of the tubes by default. In accordance with the operator, order can be rearranged and saved in new file. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

EGFR 4, package S					
№ of the tube in strip with paraffin sealed PCR-mix	Dye label/detection channel				Color labeling of the PCR-mix/paraffin
	Fam	Hex	Rox	Cy5	
1	T790M	L858R	Marker	SIC	Blue/White
2	Del19ex,Ins19ex	-	Ins19ex	SIC	Colorless/White
3	T790M	L858R	Marker	SIC	Blue/White
4	Del19ex, Ins19ex	-	Ins19ex	SIC	Colorless/White
5	T790M	L858R	Marker	SIC	Blue/White
6	Del19ex, Ins19ex	-	Ins19ex	SIC	Colorless/White
7	T790M	L858R	Marker	SIC	Blue/White
8	Del19ex, Ins19ex	-	Ins19ex	SIC	Colorless/White
EGFR 4, package U					
Tube with PCR-mix	Dye label/detection channel				Color of the PCR-mix
	Fam	Hex	Rox	Cy5	
EGFR-4.1	T790M	L858R	Marker	SIC	Blue
EGFR-4.2	Del19ex, Ins19ex	-	Ins19ex	SIC	Colorless
EGFR 8, package S					
№ of the tube in strip with paraffin sealed PCR-mix	Dye label/detection channel				Color of the PCR-mix/paraffin
	Fam	Hex	Rox	Cy5	
1	T790M	L858R	Marker	SIC	Blue/White
2	Del19ex, Ins19ex	-	Ins19ex	SIC	Colorless/White
3	G719X	Ins20ex	-	SIC	Colorless/White
4	L861Q	S768I	-	SIC	Colorless/White
5	T790M	L858R	Marker	SIC	Blue/White
6	Del19ex, Ins19ex	-	Ins19ex	SIC	Colorless/White
7	G719X	Ins20ex	-	SIC	Colorless/White
8	L861Q	S768I	-	SIC	Colorless/White
EGFR 8, package U					
Tube with PCR-mix	Dye label/detection channel				Color of the PCR-mix
	Fam	Hex	Rox	Cy5	
EGFR-8.1	T790M	L858R	Marker	SIC	Blue
EGFR-8.2	Del19ex, Ins19ex	-	Ins19ex	SIC	Colorless
EGFR-8.3	G719X	Ins20ex	-	SIC	Colorless
EGFR-8.4	L861Q	S768I	-	SIC	Colorless

Discrimination between a deletion and insertion in 19th exon is made according to the presence of signal on Rox channel: in case of presence of signal on Fam channel (Del19ex, Ins19ex) and absence of signal in Rox channel there is a deletion in the 19th exon while in case of presence of signal on Fam channel (Del19ex, Ins19ex) and presence of signal in Rox channel (Ins19ex) there is a insertion in the 19th exon.

Del19ex, Ins19ex	Ins19ex	Conclusion
mutation	mutation	Insertion in 19th exon
mutation	norm	Deletion in 19th exon
norm	norm	norm

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

3. CONTENT

The **EGFR mutations REAL-TIME PCR Genotyping Kit** is manufactured in two variants: **EGFR 4** and **EGFR 8**.

The kit in **EGFR 8** variant allows to detect deletions and insertions in the 19th exon, insertions in the 20th exon, mutations L858R, T790M, L861Q, S768I and G719X.

The kit in **EGFR 4** variant allows to detect deletions and insertions in the 19th exon and L858R, T790M mutations.

The detailed description of content is represented in Tables 2-5.

Table 2. The **EGFR mutations REAL-TIME PCR Genotyping Kit** content, **EGFR 4,** package S (standard) for R1-H806-S3/4EU

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

Table 3. The **EGFR mutations REAL-TIME PCR Genotyping Kit** content, **EGFR 4,** package U (universal) for R1-H806-UA/4EU

Reagent	Description	Total volume	Amount
PCR-mix EGFR - 4.1	Colorless transparent liquid	300 µL	1 tube
PCR-mix EGFR - 4.2	Colorless transparent liquid	300 µL	1 tube
PCR-buffer	Colorless transparent liquid	600 µL	1 tube
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	30 µL	1 tube
Positive control	Colorless transparent liquid	130 µL	1 tube

Table 4. The **EGFR mutations REAL-TIME PCR Genotyping Kit** content, **EGFR 8,** package S (standard) for R1-H807-S3/4EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless or blue transparent liquid under waxy white fraction	3840 µL (20 µL in each tube)	24 8-tube strips
Taq-polymerase solution	Colorless transparent liquid	2000 µL (500 µL in each tube)	4 tubes
Mineral oil	Colorless transparent viscous oily liquid	4.0 mL (1.0 mL in each tube)	4 tubes
Positive control	Colorless transparent liquid	130 µL	1 tube
Strip's caps	24 8-caps		

Table 5. The **EGFR mutations REAL-TIME PCR Genotyping Kit** content, **EGFR 8,** package U (universal) for R1-H807-UA/4EU

Reagent	Description	Total volume	Amount
PCR-mix EGFR – 8.1	Colorless transparent liquid	300 µL	1 tube
PCR-mix EGFR – 8.2	Colorless transparent liquid	300 µL	1 tube
PCR-mix EGFR – 8.3	Colorless transparent liquid	300 µL	1 tube
PCR-mix EGFR – 8.4	Colorless transparent liquid	300 µL	1 tube
PCR-buffer	Colorless transparent liquid	1200 µL (600 µL in each tube)	2 tubes
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	60 µL	1 tube
Positive control	Colorless transparent liquid	130 µL	1 tube

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

The **EGFR mutations REAL-TIME PCR Genotyping Kit** is intended for single use and designed for 48 tests.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile 1.5 mL tubes to collect clinical material.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF 16000 x g);
- Solid-state thermostat (temperature range 40-95 °C);

- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- Single channel pipettes (dispensers covering 2.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- RNase and DNase free pipette tips for aspirator with trap flask;
- Nucleic acid extraction kit intended for DNA extraction from formaldehyde-fixed paraffin-embedded (FFPE) samples ("DNA-Technology" made **PREP-PK** (REF P-028-N/2EU, REF P-030-N/2EU) for primary processing and **PREP-NA PLUS** (REF P-002/2EU) for subsequent DNA extraction) kits are recommended**;**
- Physiological saline solution 0.9% NaCl (Sterile) for the preparation of negative control sample (if needed);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

For preprocessing of FFPE samples additionally required:

- D-limonene (depending on the way of paraffin removal);
- 96% distilled ethyl alcohol (depending on the way of paraffin removal).

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Vortex mixer;
- Vortex rotor for strips;
- Refrigerator;
- Freezing chamber (using detection kit in the packaged U REF R1-H806-UA/4EU, REF R1-H807-UA/4EU);
- Tube rack for 1.5 mL tubes;
- PCR tube rack for 0.2 mL tubes;
- PCR tube rack for strips of eight 0.2 mL tubes;
- 0.2 mL PCR tubes or strips (using detection kit in the packaged U REF R1-H806-UA/4EU, REF R1-H807-UA/4EU);
- Single channel pipettes (dispensers covering 2.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- DTstream M1 dosage instrument (only for automated dosing using detection kit in the packaged U REF R1-H806-UA/4EU, REF R1-H807-UA/4EU);
- Device for tray sealing DTpack ("DNA-Technology", LLC) (only for automated dosing using detection kit in the packaged U REF R1-H806-UA/4EU, REF R1-H807-UA/4EU);
- Centrifuge for microtrays (only for automated dosing using detection kit in the packaged U REF R1-H806-UA/4EU, REF R1-H807-UA/4EU);

- Polymer thermal seal for microtray sealing (only for automated dosing using detection kit in the packaged U REF R1-H806-UA/4EU, REF R1-H807-UA/4EU);
- PCR mictotray (only for automated dosing using detection kit in the packaged U REF R1-H806-UA/4EU, REF R1-H807-UA/4EU);
- 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 http://dna-technology.com/software.

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

5. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 months from the date of production.

All components of the **EGFR mutations REAL-TIME PCR Genotyping Kit**, except TechnoTaq MAX polymerase, must be stored at temperatures from 2 °C to 8 °C during the storage period. PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period. TechnoTaq MAX polymerase must be stored in freezing chamber at temperature 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 has to be transported in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions of the kit components.

Transportation of the kit, except the TechnoTaq MAX polymerase, is allowed in termobox with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but no more than 5 days and should be stored at temperatures from 2 °C to 8 °C immediately on receipt.

It is allowed to transport the TechnoTaq MAX polymerase in termobox with ice packs by all types of roofed transport at temperatures up to 25 °C but no more than 5 days and should be stored at temperatures from minus 18 °C to minus 22 °C immediately on receipt.

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

- components of the kit, except TechnoTaq MAX polymerase, 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 **EGFR 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 **EGFR 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 **EGFR 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, 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 **EGFR mutations REAL-TIME PCR Genotyping Kit** is designed to detect DNA extracted from the FFPE samples depending on professional prescription.

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

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 are the presence of hemoglobin in a DNA sample as a result of incomplete removal during DNA extraction from biomaterial sample containing blood impurities, as well as the presence of isopropyl alcohol and methyl acetate in a DNA sample as a result of incomplete removal of washing solutions during sample preparation.

The maximum concentrations of interfering substances, that have no effect on the amplification of the laboratory control sample and internal control are: hemoglobin – 0.35 mg/mL of the DNA sample, isopropyl alcohol – 100 µL/mL of the DNA sample, methyl acetate – 100 µL/mL of the DNA sample.

Impurities contained in the biomaterial sample are almost completely removed during the DNA extraction. To reduce the count of PCR inhibitors, it is necessary to follow the principles of taking biological material. Suspecting a large count of PCR inhibitors in the sample, it is recommended to choose DNA extraction methods that allow to remove PCR inhibitors from the sample as much as possible. It is not recommended to use express methods of DNA extraction.

The quality of analysis can be influenced by large amount of healthy and necrotic tissue in the sample. Samples should contain no less than 50% of tumor tissue and no more than 20 % of necrotic tissue (both parameters are estimated by a pathologist during sample preparation).

General requirements

To interpret results successfully and robustly, a high quality of sample and appropriate conditions of storage, transport, and handling are required.

Features of FFPE tumor tissues sampling

Sample taking is made only by a specialist pathologist from a tumor site. The sample should contain no less than 50% of tumor tissue. 10-20% of necrotized tissue is allowed (both parameters are estimated by a specialist pathologist while preparing a tissue section stained with haematoxylin-eosin).

Fixation on formaldehyde must be made within 2 hours after obtaining a biomaterial. Time of fixation in formaldehyde depends on sample volume but should not exceed 48-72 hours. While calculating the time needed for fixation note that formaldehyde treatment of 1 mm of tissue is carried out during 1 hour.

Features of sampling

For sections preparation single-use microtome knife is used. Cut 1-3 FPE tissue sections 5.0 µm thick from preliminarily cut paraffin-treated tissue block (average square of a section is up to 0.5-1.5 sm^2). FFPE tissue sections are put in dry plastic 1.5 mL tubes. Do not exceed the recommended amount of tissue as excess paraffin-treated tissue can decrease total DNA yield.

Transportation and storage of the samples

Samples may be stored at temperatures from 18 °C to 25 °C.

Samples may be transported at temperatures from 2 °C to 25 °C.

ATTENTION! The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements are cited in **PREP-PK** and **PREP-NA PLUS** kits user manuals.

8. PROCEDURE

DNA extracting from biological material

DNA extraction is carried out according to the extraction kit instructions.

PREP-PK Extraction Kit is recommended for preprocessing of samples and **PREP-NA PLUS Extraction Kit** is recommended for subsequent DNA extraction. It is allowed to use kits of reagents registered as a medical device and recommended by manufacturers for the extraction of DNA from FFPE samples.

ATTENTION! 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.

Assay procedure

8.1 Preparing PCR for package S

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

ATTENTION! Strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips of the other kits!

8.1.1 Mark the required number of tubes with paraffin sealed PCR-mix for each test sample, positive control (C+) and negative control (C-) (2 tubes for **EGFR 4** or 4 tubes for **EGFR 8** variant of the test).

Example: to test 4 samples,

in the variant **EGFR 4** it is required to mark 8 tubes for test samples, two tubes for “C+” and two tubes for “C-“. The resulting number of tubes is 12 or 1.5 strips;

in the variant EGFR8 it is required to mark 16 tubes for analyzed samples, 4 tubes for “C+” and 4 tubes for “C-“. The resulting number of tubes is 24 or 3 strips.

8.1.2 Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

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

8.1.4 Add one drop (~20 µL) of mineral oil into each tube of the strip. Close strips.

8.1.5 Vortex the tubes with samples, "C+" and "C-" and for 3-5 seconds and spin down drops for 1-3 seconds.

ATTENTION! Close the strip before proceeding to the next DNA sample (or control sample) to prevent contamination. Use filter tips. Close the strips tightly.

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

8.1.7 Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into corresponding tubes of the strip. Add 5.0 µL of positive control sample (C+) into corresponding tubes of the strip. Avoid paraffin layer break.

8.1.8 Spin strips for 3-5 seconds.

8.1.9 Set the strips into the Real-time Thermal Cycler.

8.1.10 Launch the RealTime_PCR application in "Device operation" mode. Upload the .ini file "EGFR_4s_en.ini" or "EGFR_8s_en.ini" depending on the kit variant, supplied with the kit before first run. Please refer to DTlite or DTprime thermal cycler's user manual for details on working with .ini files. In subsequent runs add corresponding test to the protocol, specify the number and ID's of the samples, specify the position of the strips in the thermal unit (p. 8.1.9) and run PCR. See Table 6.

Selecting a test, Table 6 should be displayed in the "Start run" window.

Table 6. 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	00	30	1		Cycle
	94	01	30			
2	94	00	30	20		Cycle
	71.1 Temperature increment Δt – 0.3	00	20		√	
3	94	00	10	25		Cycle
	65	00	20		√	
4	80	00	01	1		Cycle
5	10			Holding		Holding

8.2 Preparing PCR for package U (manual dosing)

ATTENTION! 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 0.2 mL tubes in strips for each test sample, positive control (C+) and negative control (C-)(2 tubes for **EGFR 4** variant of the test, EGFR-4.1 and EGFR-4.2 mixes or 4 tubes for **EGFR 8** variant of the test, EGFR-8.1, EGFR-8.2, EGFR-8.3 and EGFR-8.4 mixes).

Example: to test 4 samples,
in the variant **EGFR 4** it is required to mark 8 tubes for test samples, two tubes for "C+" and two tubes for "C-". The resulting number of tubes is 12 or 1.5 strips;
in the variant **EGFR 8** it is required to mark 16 tubes for analyzed samples, 4 tubes for "C+" and 4 tubes for "C-". The resulting number of tubes is 24 or 3 strips.

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

8.2.3 Add to each marked tube 6.0 µL of corresponding PCR-mix (with new pipette tip for each mix) according to the scheme:

EGFR 4								
Tube	1	2	3	4	5	6	7	8
Added PCR-mix	EGFR - 4.1	EGFR - 4.2	EGFR - 4.1	EGFR - 4.2	EGFR - 4.1	EGFR - 4.2	EGFR - 4.1	EGFR - 4.2
Sample	Sample 1		Sample 2		Sample 3		Sample 4	
EGFR 8								
Tube	1	2	3	4	5	6	7	8
Added PCR-mix	EGFR - 8.1	EGFR- 8.2	EGFR- 8.3	EGFR - 8.4	EGFR - 8.1	EGFR - 8.2	EGFR - 8.3	EGFR - 8.4
Sample	Sample 1				Sample 2			

8.2.4 Vortex the TechnoTaq MAX polymerase and PCR-buffer for 3-5 seconds, then spin for 1-3 seconds.

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

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

- 6.0 x (N+1) µL of PCR-buffer,
- 0.3 x (N+1) µL of TechnoTaq MAX polymerase,

N is a quantity of marked tubes taking to account "C-", "C+".

Example: for simultaneous testing of 5 samples, "C-" and "C+" in one PCR run,

in the variant **EGFR 4** the number of marked tubes is 12. Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase for 13 (12+1) tubes. Mix 78 µL of PCR-buffer and 3.9 µL of TechnoTaq MAX polymerase.

in the variant **EGFR 8** the number of marked tubes is 24. Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase for 25 (24+1) tubes. Mix 150 µL of PCR-buffer and 7.5 µL of TechnoTaq MAX polymerase.

8.2.6 Vortex the tube with the mixture of PCR-buffer and TechnoTaq MAX polymerase (3-5 seconds) and spin briefly in vortex mixer (1-3 seconds).

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

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

ATTENTION! Follow the steps listed in pp. 8.2.8 – 8.2.13 within two hours after addition of PCR-buffer and TechnoTaq MAX polymerase mixture to PCR-mix.

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

ATTENTION! Open the cap of the tube, add DNA sample (or control sample), then close the tube before proceeding to the next tube to prevent contamination. In case of using tubes in strips, close the strip before proceeding to the next DNA sample (or control sample) to prevent contamination. Close the tubes/strips tightly. Use filter tips.

8.2.9 Add 6.0 µL of DNA sample into corresponding tubes. Do not add DNA into the “C+”, “C-“ tubes.

8.2.10 Add 6.0 µL of negative control (C-) which passed whole DNA extraction procedure into corresponding tubes. Add 6.0 µL of positive control sample (C+) into corresponding tubes.

8.2.11 Spin tubes/strips for 3-5 seconds.

8.2.12 Set the tubes/strips into the Real-time Thermal Cycler.

8.2.13 Launch the RealTime_PCR application in “Device operation” mode. Upload the .ini file “EGFR_4u_en.ini” or “EGFR_8u_en.ini” depending on the kit variant, supplied with the kit before first run. Please refer to DTlite or DTprime thermal cycler’s user manual for details on working with .ini files. In subsequent runs add corresponding test to the protocol, specify the number and ID’s of the samples, specify the position of the strips in the thermal unit (p. 8.2.12) and run PCR with reaction volume 18 µL. See Table 7.

Selecting a test, Table 7 should be displayed in the “Start run” window.

Table 7. 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	00	30	1		Cycle
	94	5	00			
2	94	00	30	20		Cycle
	71.1 Temperature increment Δt – 0.3	00	20		√	
3	94	00	10	25		Cycle
	65	00	20		√	
4	80	00	01	1		Cycle
5	10			Holding		Holding

8.3 Preparing PCR using DTstream (only for package U)

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

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

8.3.2 Vortex the TechnoTaq MAX polymerase and PCR-buffer for 3-5 seconds, then spin for 1-3 seconds.

ATTENTION! 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. Follow the DTstream instructions.

8.3.4 Vortex the tube with the mixture of PCR-buffer and TechnoTaq MAX polymerase (3-5 seconds) and spin briefly (1-3 seconds).

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

8.3.6 Set tubes with PCR-mix, mixture of PCR-buffer and TechnoTaq MAX, analyzed samples, positive and negative controls and PCR microtray to the DTstream working table and dispense the components according to the instruction manual.

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

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

8.3.9 Centrifuge the microtray on 100 x g during 30 seconds.

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

8.3.11 Launch the RealTime_PCR application in “Device operation” mode. Upload the .ini file “EGFR_4u_en.ini” or “EGFR_8u_en.ini” depending on the kit variant, supplied with the kit before first run. Please refer to DTlite or DTprime thermal cycler’s user manual for details on working with .ini files. In subsequent runs add corresponding test to the protocol, specify the number and ID’s of the samples and run PCR with reaction volume 18 µL. See Table 7.

9. CONTROLS

The **EGFR mutations REAL-TIME PCR Genotyping Kit** contains positive control sample. It is produced with genetic engineering techniques and characterized by automatic DNA sequencing. The PCR-mix contains primers and probes for the detection of sample intake control (SIC), that is used for control of amplification sufficiency and semi-quantitative estimation of mutant alleles amount. For the mutant alleles detection the value of difference between Cp of mutant alleles and SIC is used: the value of dCp less than 6-9 cycles depending on SIC value indicates the presence of mutation.

ATTENTION! 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 test result is considered valid when:

- For positive control sample exponential growth of fluorescence on corresponding detection channels Fam, Hex, Rox и Cy5 is present (the value of the indicator cycle is determined). The proportion of mutant alleles must be 10-100%;
- For negative control sample exponential growth of fluorescence on corresponding detection channels Fam, Hex, Rox и Cy5 is absent (the value of the indicator cycle is absent). Cp for SIC on Cy5 channel must be absent or exceed 38^{th} cycle.

The test result is considered invalid in case of absence of exponential growth of fluorescence on corresponding detection channels and absence of the value of the indicator cycle or discrepancy of the proportion of mutant alleles to the determined values.

For positive control sample: in case of absence of exponential growth of fluorescence on corresponding detection channels and absence of the value of the indicator cycle or discrepancy of the proportion of mutant alleles to the determined values the results are considered invalid. In this case repeat of amplification of all samples is required.

For negative control sample: on presence of amplification on Fam, Hex, Rox detection channels or Cp for SIC on Cy5 channel less than 38^{th} cycle all results of the current PCR run are considered invalid. In this case decontamination procedures are required.

10. DATA ANALYSIS

Registration and interpretation of the PCR results are held in automatic mode. Analysis will be performed by Real-Time PCR application.

The graph shows the dependence of fluorescence on the cycle number for each tube.

The table shows the sample ID, the name of the assay, and the Cp indicative cycle.

On data analysis presence or absence of mutant allele is determined. In case of mutant allele presence its proportion in the sample is estimated.

It is recommended to have a training on conducting analysis and result interpretation. Contacts of technical support service are present in the current manual (“Quality control” paragraph).

11. SPECIFICATIONS

a. The analytical specificity of the **EGFR mutations REAL-TIME PCR Genotyping Kit**

Tables 8-9 contain the list of mutations detected by the EGFR reagent kit.

Table 8. The list of mutations detected by the kit

Mutation	**Exon**	**Nucleotide substitutions, insertions and deletions NM_005228.5**	**Cosmic ID or ClinVar RCV Id**
p.T790M	20	c.2369C>T	COSM6240
p.L858R	21	c.2573T>G	COSM6224
		c.2573_2574delinsGT	COSM12429
Deletions in the 19th exon del2235-2258 (without differentiation)			
p.L747_T751delinsS	19	c.2240_2251del	COSM6210
p.L747_E749del		c.2239_2247del	COSM6218
p.E746_S752delinsD		c.2238_2255del	COSM6220
p.E746_A750del		c.2235_2249del	COSM6223
p.E746_A750del		c.2236_2250del	COSM6225
p.L747_S752del		c.2239_2256del	COSM6255
p.E746_S752delinsA		c.2237_2254del	COSM12367
p.L747_T751del		c.2240_2254del	COSM12369
p.L747_P753delinsS		c.2240_2257del	COSM12370
p.L747_A750delinsP		c.2239_2248delinsC	COSM12382
p.L747_T751delinsP		c.2239_2251delinsC	COSM12383
p.E746_S752delinsV		c.2237_2255delinsT	COSM12384
p.E746_S752delinsI		c.2235_2255delinsAAT	COSM12385
p.E746_T751delinsV		c.2237_2252delinsT	COSM12386
p.L747_P753delinsQ		c.2239_2258delinsCA	COSM12387
p.L747_S752delinsQ		c.2239_2256delinsCAA	COSM12403
p.E746_T751delinsVA		c.2237_2253delinsTTGCT	COSM12416
p.L747_T751delinsQ		c.2238_2252delinsGCA	COSM12419
p.L747_A750delinsP		c.2238_2248delinsGC	COSM12422
p.E746_T751delinsA		c.2237_2251del	COSM12678
p.E746_T751del		c.2236_2253del	COSM12728
p.E746_A750delinsIP		c.2235_2248delinsAATTC	COSM13550
p.E746_T751delinsI		c.2235_2252delinsAAT	COSM13551
p.E746_T751delinsIP		c.2235_2251delinsAATTC	COSM13552
p.E746_A750delinsAP		c.2237_2248delinsCAC c.2237_2248delinsCCC	COSM144207 RCV000150618
p.E746_T751delinsVP		c.2237_2251delinsTTC	COSM18421
p.E746_S752delinsV		c.2237_2256delinsTC	COSM18426
p.E746_P753delinsVS		c.2237_2257delinsTCT	COSM18427
p.L747_T751delinsP		c.2238_2251delinsGC	COSM22944
p.K745_E749del		c.2233_2247del	COSM26038
p.E746_T751delinsI		c.2236_2252delinsAT	COSM26680
p.L747_A750delinsS		c.2240_2248del	COSM4170221
p.L747_E749del		c.2235_2243del	RCV000154252
p.L747_P753delinsT		c.2239_2257delinsA	RCV000154456
p.L747_P753delinsQS		c.2239_2257delinsCAAT	RCV000154485
p.E746_E749delinsA		c.2237_2246delinsC	RCV000155025

Mutation	Exon	Nucleotide substitutions, insertions and deletions NM_005228.5	Cosmic ID or ClinVar RCV Id
Insertions in the 19th exon (without differentiation**)**			
p.I744_K745insKIPVAI	19	c.2214_2231dup	COSM53176
		c.2215_2232dup	
p.K745_E746insIPVAIK		c.2217_2234dup	No data
p.K745_E746insVPVAIK		c.2219_2236	No data
p.K745_E746insTPVAIK		c. 2234_2235ins AACTCCCGTCGCTATCAA	COSM53172
p.G719X (without differentiation**)**			
p.G719A	18	c.2156G>C	COSM6239
p.G719S		c.2155G>A	COSM6252
p.G719C		c.2155G>T	COSM6253
p.S768I	20	c.2303G>T	COSM6241
p.L861Q	21	c.2582T>A	COSM6213
Insertions in the 20th exon (without differentiation**)**			
p.A767_V769dup	20	c.2300_2308(dup)	COSM12376
p.H773dup		c.2317_2319dup	COSM12377
p.D770_N771insG		c.2310_2311insGGT	COSM12378

Table 9. The list of detected mutations depending of the kit variant

Detected mutations in the EGFR gene	Variant of the kit EGFR 8	Variant of the kit EGFR 4
T790M	ν	ν
L858R (without differentiation)	ν	ν
Insertions in the 19th exon (without differentiation)	ν	ν
Deletions in the 19th exon (without differentiation)	ν	ν
G719X (without differentiation)	ν	-
Insertions in the 20th exon (without differentiation)	ν	-
L861Q	ν	-

In human biomaterial samples containing DNA of mutant alleles of EGFR gene the software determines indicator cycles (Cp) after the end of amplification.

For mutations T790M, L858R, G719X, S768I, L861Q and insertions in 20th exon background amplification of the matrix of normal allele is possible. Thus on determination of mutation the difference between Cp of mutant allele and SIC is considered: Cp less than 6-9 cycles indicates the presence of mutation (Table 10).

Due to drawbacks of the PCR method there is a “grey zone” for Cp: mutation status is not determined, result is considered uncertain (invalid).

If dCp exceeds the values of “grey zone” (more than 6.5-9.5 cycles depending on SIC value) result is considered as norm.

Table 10. Threshold values dCp mutant allele/SIC for the detection of mutant allele

Mutation	Threshold values dCp for the detection of mutant allele		
	Cp of SIC≤28 (package S) Cp of SIC ≤27 (package U)	28<Cp of SIC ≤31.5 (package S) 27<Cp of SIC ≤30.5 (package U)	31.5<Cp of SIC ≤34 (package S) 30.5<Cp of SIC ≤33 (package U)
T790M	dCp< 9	dCp< 7	dCp< 6
L858R	dCp< 9	dCp< 7	dCp< 6
G719X	dCp< 9	dCp< 7	dCp< 6
S768I	dCp< 9	dCp< 7	dCp< 6
L861Q	dCp< 9	dCp< 7	dCp< 6
Ins20ex	dCp< 9	dCp< 7	dCp< 6

b. Influence of closely related genes

It was shown that presence of DNA of closely related genes as tyrosine kinases of epidermal growth factor receptors, particularly ERBB2/HER2, ERBB3/HER3, ERBB4/HER4 do not influence on the results of analysis using the kit.

Oligonucleotides using in the kit are developed in the way to exclude amplification on matrix of closely related genes. During the analysis of blood samples from 50 healthy controls there were no false positive results obtained.

c. Influence of lung microbiota

Addition of microorganisms cultures that can be present in lung microbiota, in concentration 10^6 CFU/mL to samples containing normal and mutant alleles on the stage of DNA extraction have not influenced the results of analysis using the kit.

d. Limit of detection

Limit of detection (LOD) is the minimal amount of mutant allele in the allele pool (wild type and mutation) that is detected with reproducibility 95% with result interpretation as “mutation”.

LOD is limited by DNA amount and is 0.3% (T790M, L858R, G719X, S768I, L861Q, insertions in 20^{th} exon) and 0.1% (deletions and insertions in 19^{th} exon) in a sample with Cp of SIC ≤27-28 on Cy5 detection channel, 1.0% if 27-28< Cp of SIC ≤30-31.5, 5% if 30-31.5< Cp of SIC 33-34 (Table 11).

Table 11. Limit of detection

Minimal amount of DNA (copies of allele pool in the amplification tube)	10^4	10^3	200
Corresponding value of Cp for SIC			
Package S	Cp≤28	28<Cp≤31.5	31.5<Cp≤34
Package U	Cp≤27	27<Cp≤30.5	30.5<Cp≤33
Detection threshold of the mutant allele proportion in a sample	0.1% 0.3% *	1%	5%

*0.1% for deletions and insertions in 19 exon, 0.3% for T790M, L858R, G719X, S768I, L861Q, insertions in 20^{th} exon

In case if Cp of SIC is more than 34 for package S and more than 33 for package U the amount of biomaterial is considered insufficient for correct result, and the result is registered as invalid.

e. Reproducibility

Reproducibility of analysis has been determined for EGFR 8 variant of the test, package S and U in the tests performed in one laboratory on different devices by different operators in different days by testing positive and negative DNA samples. The results are presented in Table 12.

Table 12. Reproducibility of analysis

Type of sample	**Reproducibility within the batch**		**Intralaboratory reproducibility between S and U packages**	
	Number	Match of results	Number	Match of results
Deletions in 19th exon	8	8 from 8	14	14 from 14
L858R	4	4 from 4	6	6 from 6
Insertions in 19th exon	3	3 from 3	4	4 from 4
Insertions in 20th exon	1	1	1	1
T790M all	1	1	4	4
L861Q	1	1	1	1
G719X	0	0	0	0
S768I	0	0	0	0
Norm	29	29 from 29	28	28 from 28
Sum	46	46 из 46 (100%)	54	54 from 54 (100%)

f. Diagnostic characteristics

Analit	**Diagnostic characteristics**		**Number of samples (n)**
	Diagnostic sensitivity (%)	**Diagnostic specificity (%)**	
Deletions in 19th exon	100% (93.5 – 100)	99.2% (97.0 – 99.2)	179
Insertions in 19th exon	100% (65.7 – 100)	100% (97.1 – 100)	90
Insertions in 20th exon	100% (34.8 – 100)	99.2% (97.6 – 99.2)	124
L858R	100% (92.1 – 100)	99.3% (97.2 – 99.3)	179
T790M all	83.3% (43.9 – 88.3)	100% (98.4 – 100)	158
L861Q	100% (22.7 – 100)	100% (98.7 – 100)	124
S768I	100% (22.7 – 100)	100% (98.7 – 100)	124
G719X	100% (47.3 – 100)	100% (98.2 – 100)	124
Sum	99.1% (95.6 – 100)	99.7% (99.3 – 99.8)	-

ATTENTION!The claimed specifications are guaranteed when DNA extraction is performed with **PREP-PK**(REF P-028-N/2EU, REF P-030-N/2EU) and **PREP-NA PLUS** (REF P-002/2EU) kits.

12. TROUBLESHOOTING

Table 13. Troubleshooting

	Result		Possible cause of invalid result	Solution
	Valid	**Invalid**		
C+	Cp on Fam, Hex, Rox and Cy5 detection channels are determined. All mutations are detected, the proportion of mutant alleles is 10-100%	Cp on Fam, Hex, Rox and Cy5 detection channels are not determined and the proportion of mutant alleles does not correspond to the indicated limit	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	Cp on Fam, Hex, Rox detection channels are not determined, Cp on Cy5 more than 38	Cp on Fam, Hex, Rox detection channels are determined, Cp on Cy5 less than 38	Contamination	Dispose current batch Perform decontamination procedures
SIC	Cp of SIC ≤ 33 for package U or ≤ 34 for package S	Cp of SIC is more than 34 for package S and more than 33 for package U	Insufficient amount of DNA Operation error PCR inhibition	Low DNA concentration is probable. Repeat sample preparation and DNA extraction
	Cp SIC differs in the test tubes with (dCp) ≤ 1.7	Cp SIC differs in the test tubes with (dCp) > 1.7		Repeat PCR or DNA extraction (performed sequentially)

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

www.dna-technology.com

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:

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

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

Technical support:

E-mail: hotline@dna-technology.ru

http://www.dna-technology.com

Manufacturer: "DNA-Technology Research & Production", LLC,

142281, Russia, Moscow Region,

Protvino, Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

http://www.dna-technology.com

Seller: "DNA-Technology" LLC,

117587, Russia, Moscow,

int. ter. Municipal District Chertanovo Severnoye,

Varshavskoye shosse, 125 Zh, building 5, floor 1, office 12;

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

http://www.dna-technology.com

Authorized representative in EU:

OBELIS S.A

Registered Address:

Bd. Général Wahis, 53

1030 Brussels, Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

http://www.obelis.net

14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		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	CONTROL +	Positive control
EC REP	Authorized representative in the European Community		Caution

REF R1-H806-S3/4EU
R1-H806-UA/4EU
R1-H807-S3/4EU
R1-H807-UA/4EU

VER 611.2022.01.23