APPROVED BY
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INSTRUCTION

Kit for mutations detection in *EGFR* gene based on real-time PCR in the probe of human genomic DNA from paraffin-fixed tissue samples (Test-EGFR-tissue) by TS 21.20.23-005-97638376-2016

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CE IVD

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1. Intended use

Intended use: kit «Test-EGFR-tissue» is intended for professional use in medical centers and clinical diagnostic laboratories of oncological profile when examining patients with non-small cells lung cancer (NSCLC). It is aimed for identification of L858R mutation and 27 deletions (del) in exon 19 of the *EGFR* gene by real-time allele-specific PCR in human genomic DNA from paraffin-fixed tissue samples for indication of targeted therapy by small molecule EGFR tyrosine kinase inhibitors.

Application area - clinical laboratory diagnostics, oncology.

Type of test sample. Material for PCR reaction is human genomic DNA from samples of paraffin-fixed tissue.

Determination principle

The testing is carried out by real-time allele-specific PCR. PCR products of the *EGFR* gene are identified in 5′-exonuclease reaction using FAM- and HEX-labeled probes. Kit contains the reagents for detection of L858R mutation and 27 deletions in exon 19 of the *EGFR* gene. PCR mixes contain all necessary reagents. Also the kit includes negative control sample (NTC) and positive control sample (PC) containing equimolar mixture of L858R mutation and deletions (del) in exon 19 in the concentration 5 %.

All reaction mixes contain HEX-labeled internal control sample (see Section 11 «Recording and interpretation of results»). It is control of DNA isolation effectiveness and possible inhibition in the probe that may cause false-negative results.

Target analyte description, information about its scientific validation

Target analyte is *EGFR* gene considered when examining patients with «non-small cells lung cancer» for identification of L858R mutation and 27 deletions (del) in exon 19 of the *EGFR* gene.

Scientific validation.

Lung cancer is one of the most extensive forms of cancer. About 60 thousand people die from lung cancer in Russia annually, that is 20% of all deaths from malignant diseases.

The identification and interpretation of the mechanism of the epidermal tumor growth factor receptor (EGFR) has become one of the promising discoveries in oncology.

During the last few years, the study of the epidermal growth factor receptor (EGFR) has become one of the most important aspects of modern oncology. It was found that the high frequency of EGFR hyperexpression is typical for non-small cell lung cancer (40-70%), ovarian cancer (35-70%), colon cancer (25-77%), etc. It is known that detection of increased expression of EGFR is a factor of unfavourable prognosis for disease. Activation of EGFR mechanisms leads to an increase in the proliferative activity of tumor cells, neoangiogenesis, delayed apoptosis, and the earlier appearance of metastases.

Most of the somatic mutations of the *EGFR* gene found in patients with non-small cell lung cancer (NSCLC) are located in the exons 18-21 encoding the tyrosine kinase domain. Mutations discovery was associated with a positive response to therapy with EGFR tyrosine kinase inhibitors.

Deletions in exon 19 are the most frequent (45% of all mutations).

Treatment order of tyrosine kinase (TK) inhibitors to NSCLC patients with deletions in exon 19 (Del19) or L858R mutation of the *EGFR* gene is an effective treatment method. It leads to a significant reduction in tumor nidus size and the disease control for a long term.

Specific pathology, conditions and risk factor – kit is intended for indication to targeted therapy for qualitative identification of mutation L858R and deletions (del) in exon 19 (detects 27 deletions but not differs) of the *EGFR* gene when examining patients with non-small cell lung cancer.

Indications and contra indications for use

Indications for use: The kit «Test-EGFR-tissue» is recommended when examining patients with « non-small cell lung cancer». It is aimed for qualitative identification of mutation L858R and deletions (del) in exon 19 of the *EGFR* gene by real-time allele-specific PCR in human genomic DNA from paraffin-fixed tissue samples for indication of targeted therapy by small molecule EGFR tyrosine kinase inhibitors.

Contra indications: none.

The kit is intended for professional use only in medical centers and clinical diagnostic laboratories of oncology profile. Potential

users are doctor of clinical diagnostic laboratory and laboratory technicians.

Total time of the analysis procedure is 2-2.5 hours.

2. Kit characteristics

The kit is produced in one version – «Test-EGFR-tissue». Each kit «Test-EGFR-tissue» contains reagents for 24 tests.

Kit contents

Kit «Test-EGFR-tissue» includes:

Table 1 – Contents of the kit «Test-EGFR-tissue»

No.	Reagent	Marking on the tube cap	Description	Tube quantity, volume, µl
1	PCR Mix del	**del**	Pink-colored transparent liquid	1 tube (120 µl)
2	PCR Mix L858R	**L858R**	Pink-colored transparent liquid	1 tube (120 µl)
3	PC	**К+**	Transparent colorless liquid	1 tube (120 µl)
4	NTC	**К-**	Transparent colorless liquid	1 tube (120 µl)
5	Taq-polymerase	**Taq**	Transparent colorless liquid	1 tube (580 µl)

Positive control (PC) is ready-to-use and is the mix of genomic DNA from Jurkat human cell culture with concentration of 400 GE/ µl (genome-equivalent per µl) of *EGFR* gene and artificial-synthesized insertion with 300 bps size in plasmid vector pAL-TA with concentration 20 GE/µl containing equimolar mixture of L858R mutation and 27 deletions (del) in exon 19. It contains 5% of mutant and 95% of normal DNA copies.

Deionized water is used as NTC.

All reaction mixes contain HEX-labeled internal control (see Section 11 «Recording and interpretation of the results»). It is control of

DNA isolation effectiveness and possible inhibition in the probe that may cause false-negative results.

3. Operating principle

Qualitative identification of L858R mutation and deletions (del) in exon 19 (detects 27 deletions but not differs) of the *EGFR* gene by real-time allele-specific PCR in human genomic DNA from paraffin-fixed tissue samples for indication of targeted therapy includes three stages:

1) PCR preparation;
2) DNA PCR-amplification and real-time hybridization-fluorescent detection;
3) Interpretation of results.

Genomic DNA samples from paraffin-fixed tissue are used for amplification reactions of *EGFR* gene sites in the reaction buffer, using primers specific for these DNA sites and Taq-polymerase enzyme. The reaction mixture for amplification includes allele-specific fluorescent-labeled oligonucleotide probes that are hybridized with the complementary site of the amplified DNA-target and destroyed by Taq-polymerase resulting increased fluorescence intensity. This enables to observe specific amplification product accumulation by measuring the fluorescent signal intensity. The fluorescent signal is detected directly during PCR using a cycler with a system for fluorescent signal detection in «real-time» mode.

DNA amplification product of normal variant of *EGFR* gene is detected by the channel conforming **HEX** fluorophore. DNA amplification product of mutant variant and deletions in exon 19 (detects 27 deletions but not differs) of *EGFR* gene is detected by the channel conforming **FAM** fluorophore.

4. Analytical and diagnostic characteristics

4.1 Analytical characteristics

Table 2 – Analytical characteristics of the kit «Test-EGFR-tissue»

Analytical specificity	Specific to L858R mutation and 27 deletions (del) in exon 19 of the *EGFR* gene
Analytical sensitivity	10 copies of *EGFR* gene to 1 µl of DNA solution

The list of detected mutations with mutation ID is presented in the Table 3.

Table 3 - List of deletions and COSMIC identities*

Deletions detected by «Test-EGFR-tissue»	COSMIC ID*
2235_2249del15	6223
2235_2252>AAT (complex)	13551
2236_2253del18	12728
2237_2251del15	12678
2237_2254del18	12367
2237_2255>T (complex)	12384
2236_2250del15	6225
2238_2255del18	6220
2238_2248>GC (complex)	12422
2238_2252>GCA (complex)	12419
2239_2247del9	6218
2239_2253del15	6254
2239_2256del18	6255
2239_2248TTAAGAGAAG>C	12382
2239_2258>CA (complex)	12387
2240_2251del12	6210
2240_2257del18	12370
2240_2254del15	12369
2239_2251>C (complex)	12383

Deletions detected by «Test-EGFR-tissue»	COSMIC ID*
2236_2252>AT (complex)	26680
2236_2251>T (complex)	26513
2238_2252del15	23571
2237_2252>T (complex)	12386
2235_2255>GGT (complex)	85797
c.2235_2246del12	28517
2235_2251>AG (complex)	13549
2236_2253>CAA (complex)	22999

* COSMIC IDs are taken from the Catalog of Somatic Mutations in Cancer: (www.sanger.ac.uk/genetics/CGP/cosmic).

4.2 Diagnostic characteristics:

Diagnostic specificity – 94,3 % with 90% confidence interval.

Diagnostic sensitivity – 90,9 % with 90% confidence interval.

Specificity of the testing is determined by oligonucleotide primers matched to homologous genes regions and by specific fluorescent oligonucleotide probes for hybridization with complementary regions of amplicons (specific amplification products). It excludes cross-reactions.

Limitations

Detection of mutations depends on sample integrity and amount of amplifiable DNA present in the sample. Purity of isolated DNA expressed in ratio of optical dense (A260/280nm) should be no less than 1,4. DNA amount sufficient for testing must be 1-100 ng/μl.

Tumor tissue is not homogenous, so test results may not coincide with results received from other tissue sections of the similar tumor. Besides, tumor samples may include normal tissue (non-tumor tissue). Kit «Test-EGFR-tissue» is not able to detect mutations of *EGFR* gene if genomic DNA is isolated from non-tumor tissue.

During PCR procedure samples may be contaminated. Use caution to avoid contamination of DNA samples and reaction mixtures by PC tube or PCR products.

Kit «Test-EGFR-tissue» may not be used for diagnosis of any disease. It is intended only for identification of L858R mutation and deletions (del) in exon 19 (detects 27 deletions but not differs) of the *EGFR* gene.

5. List of risks

Border risk zone includes:

- loss of functional properties of the reagents included in the kit, due to transportation, storage or operation under inappropriate conditions,
- utilization of the kit with violation of safety and deactivation measures;
- crossover contamination of the samples;
- contamination of materials by inhibitors;
- contamination of reaction mixes with DNA samples by PC tube or PCR products;
- failure to meet the requirements for sample preparation, testing procedure and utilization because of unqualified personnel.

No risks have been identified in the unacceptable zone.

The total residual risk of using the medical product «Kit for mutations detection in *EGFR* gene based on real-time PCR in the probe of human genomic DNA from paraffin-fixed tissue samples (Test-EGFR-tissue) by TS 21.20.23-005-97638376-2016» produced by TestGene LLC, is acceptable, and the benefit of its using exceeds the risk.

6. Precautions for handling

Class depending on the potential risk of use is 2b in accordance with the nomenclature classification of medical devices approved by order of the Ministry of Health of the Russian Federation dd. 06.06.2012 N 4n.

All components and reagents contained in composition of «Test-EGFR-tissue» are of 4 hazard class (low-hazard substances) in accordance with GOST 12.1.007-76 «Occupational safety standards system. Noxious substances. Classification and general safety

requirements». Precautions against any special, unusual environmental risks are not provided during use or implementation of the product.

Reagents included in «Test-EGFR-tissue» kit have low vapor pressure and eliminate the possibility of inhalation toxicity.

Reagents included in «Test-EGFR-tissue» kit are not toxic as they are prepared by mixing of separate nontoxic components.

The work shall be carried out in a laboratory performing bimolecular (PCR) testing of clinical material in compliance with the sanitary and epidemiologic rules SanPiN 2.1.7.2790-10 «Sanitary and Epidemiologic Requirements to the address with Medical Waste Products». The following requirements should always be fulfilled during the work:

- Dispose of unused reagents in accordance with p. 4.28 of SanPiN 2.1.7.2790-10 «Sanitary and Epidemiologic Requirements to Handling of Medical Waste».

ATTENTION! When discarding waste products after amplification (tubes containing PCR products), it is not allowed to open the tubes and spray their contents because this may result in contamination of the laboratory area, equipment and reagents with PCR products.

- Use the kit strictly for its intended use as per this instruction.
- Admit only specially trained staff to the work with the kit.
- Do not use the kit after the expiry date.
- Avoid contact with skin, eyes and mucous membranes. In case of contact, wash immediately the affected area with water and seek medical attention.

The necessary precautions regarding the influence of magnetic fields, external electric actions, electrostatic discharges, pressure or pressure drops, overload, sources of explosion or ignition are not provided.

As a part of the kit, there are no substances of human or animal origin that have a potential infectious nature. Therefore precautions against any special, unusual risks are not provided during using or implementation of the product.

7. Required equipment and materials

Equipment:

1. PCR-box (e.g., BAV-PCR-Laminar-S, Laminar Systems, Russia).
2. Vortex (e.g., TETA-2, Biocom, Russia).
3. Kit of electronic or automatic variable volume dispensers (e.g., Eppendorf, Germany).
4. Refrigerator with a temperature from 2 °C to 8 °C with a freezing chamber (max minus 16 °C).
5. Cycler of rotary type, e.g., Rotor-Gene 3000 or 6000 (Corbett Research, Australia), or cycler of plate type, e.g. Real-Time CFX96 Touch (e.g., BioRad, USA), DT-Prime (e.g., DNA-Technology, Russia) or equivalent ones.

Materials and reagents not included in the kit:

ATTENTION! When working with DNA it is necessary to use only sterile disposable plastic consumables with a special marking «DNase-free».

1. Disposable tips with an aerosol barrier of up to 200 µl, up to 100 µl, up to 20 and 10 µl (e.g., Axygen, USA).
2. Racks for tips (e.g., Axygen, USA) and 0.5 (0.2 ml) microtubes (e.g., InterLabService, Russia).
3. An individual coat and disposable gloves.
4. Container with a cover for disinfecting solution.
5. Disposable polypropylene tubes for PCR:

a) 0.2 ml tubes (flat cap, not stripped), (e.g., Axygen, USA) for placing in a rotor for 36 tubes – for devices for real-time PCR with detection through the tube bottom (e.g., Rotor-Gene).

b) 0.2 ml tubes (domed cap) (e.g., Axygen, USA) for devices for real-time PCR with detection through the cap (e.g., CFX96, DT-Prime).

6. PCR plates (may be used instead of the tubes identified in p.5).
7. Optically transparent film for sealing the plates.

8. Test samples

Material for PCR reaction is human genomic DNA from samples of paraffin-fixed tissue.

8.1 Obtaining of human genomic DNA from samples of paraffin-fixed tissue

For isolation of human genomic DNA from paraffin-fixed tissue which is necessary for PCR purity testing, it is recommended to use the following kits of reagents:

- Kit for human genomic DNA isolation from formalin-fixed paraffin-embedded (FFPE) tissue (DNA-Tissue-F) by TS 21.20.23-009-97638376-2016 in the versions: 1) «DNA-Tissue-F-50» for 50 tests, 2) «DNA-Tissue-F-100» for 100 tests (TestGene, Russia).
- NucleoSpin FFPE DNA (MACHEREY-NAGEL, Germany)
- QIAamp DNA FFPE Tissue Kit (QIAGEN, Germany)

or equivalent for DNA isolation from tissue with the following characteristics:

- DNA purity expressed in ratio A260/280 nm must be not less than 1,4.
- DNA concentration enough for testing must be 1-100 ng/μl.

8.2 Interfering substances and limitations of sampling material use

For isolation from clinical sample of sufficient DNA amount for PCR-testing and necessary purity, it is recommended to use kits for isolation specified in p. 8.1.

For DNA isolation effectiveness and possible inhibition in the probe that may cause false-negative results, all reaction mixtures contain internal control sample. Internal control is HEX-labeled to distinguish internal control signal from signal of FAM-labeled primers in reactions of *EGFR* gene mutations. Reaction passage is indicative of effective nucleic acids isolation and absence of PCR-inhibitors. Non-reactiveness result should be considered as inaccurate. In this case, it is recommended to make additional DNA isolation for this sample for PCR-testing (see Section 11 «Recording and interpretation of results»).

The effect of potentially interfering substances on the functioning of «Test-EGFR-tissue» was tested for potentially interfering substances that can remain in the human genomic DNA sample after the DNA isolation, inhibit the PCR reaction and affect the ability of the «Test-KRAS-tissue» to differ between mutant and normal variants of the *EGFR* gene.

For evaluating the impact of potentially interfering substances it was studied by analyzing the effect of each substances on C_t values and identification of mutations status in the test sample, at two concentrations (maximum and minimum). Potentially interfering substances and their concentrations are shown in Table 4.

Table 4 – Concentration of interfering substances tested during study

Interfering substances	Maximum concentration (µl / 200 µl OF DNA solution)	Minimal concentration (µl / 200 µl of DNA solution)
Paraffin (in xylol)	$2{,}00*10^{-4}$	$5{,}00*10^{-5}$
Xylol	$2{,}00*10^{-4}$	$5{,}00*10^{-5}$
Ethanol (95%)	$1{,}35*10^{-3}$	$3{,}38*10^{-4}$
DNA Binding buffers	$5{,}40*10^{-4}$	$1{,}35*10^{-4}$
Proteinase K	$1{,}32*10^{-5}$	$3{,}30*10^{-6}$
Eluent	$1{,}33*10^{-3}$	$3{,}33*10^{-5}$
Wash buffer №1	0,50	$1{,}25*10^{-1}$
Wash buffer №2	5,00	1,25

None of the potentially interfering substances, estimated at the concentrations expected to be detected in normal use, impacts the ability of the kit «Test-EGFR-tissue» to distinguish between mutation-positive and mutation-negative samples of *EGFR* gene.

In addition to the interfering substances study, it was evaluated the impact of necrotic tissue in tumor samples on the ability of «Test-EGFR-tissue» to generate valid data. The study of the impact of necrosis was carried out on 10 samples that had necrosis at a level >50%, as determined by pathology review. After testing using «Test-EGFR-tissue» and

interpretation of the results, the obtained data were compared with the results of bidirectional Sanger sequencing. A single result was false-negative due to insufficient DNA amount.

Limitations for testing material use:

- Testing material is not suitable for use if storage and transportation conditions are violated (temperature, duration, multiple freezing and thawing). Testing DNA must be stored at 2 °C to 8 °C and used during 24 hours. It is recommended to store DNA solution at temperature -20 °C for storage more than 24 hours.
- Purity of isolated DNA expressed in ratio to optical dense (A260/280nm) should be no less than 1,4.
- DNA amount sufficient for testing must be 1-100 ng.
- Samples contaminated with outside biological material are not allowed for use.
- For testing it is necessary to use probes of genomic DNA isolated from histologically proven tumor tissue.

8.3 Storage conditions of testing samples

Storage conditions of the probe of genomic DNA isolated from paraffin-fixed tissue:

Testing DNA must be stored at 2 °C to 8 °C and used during 24 hours. It is recommended to store DNA solution at temperature -20 °C for storage more than 24 hours.

Storage conditions of initial clinical material:

The most available clinical material for DNA isolation is formalin-fixed paraffin-embedded tissue (FFPE-blocks). FFPE-blocks may be stored at room temperature.

Paraffin sections may be stored at room temperature during 4 weeks before DNA isolation.

Storage conditions of the biopsic specimen for DNA isolation:

- at room temperature — during 6 hours;
- at temperature 2–8 °C — during 3 days;
- at temperature minus 20 °C — during 1 week;
- at temperature minus 70 °C — for a long storage.

9. Preparation of the components for testing

Installation, mounting, setup, calibration of the medical product is not required for putting into operation.

Mix thoroughly tubes contents by turning over each tube 10 times or then vortex on low speed during 3-5 seconds. Precipitate drops from tube caps by short centrifugation.

ATTENTION! Do not vortex Taq-polymerase (Taq) because it may inactivate the ferment.

10. Testing procedure

The PCR-testing consists of the following stages:

A) PCR preparation;

B) DNA PCR-amplification and «real-time» hybridization-fluorescent detection of amplification products;

C) interpretation of results (specified in Section 11).

A) PCR preparation

(performed in the pre-amplification area – room for reagents dropping and PCR-amplification)

Total reaction volume – 20 µl.

ATTENTION! It is prohibited to change the reaction volume. If the volume is changed, the method sensitivity will decrease greatly!!!

Before testing it is necessary to prepare reaction mixes (master mixes) for testing DNA, PC and NTC. Mix all necessary components in separate sterile tubes assuming that it is necessary to take 4 µl of PCR mix and 10 µl of Taq-polymerase for one reaction. Use separate tip with an aerosol barrier for each reaction component of each sample.

Prepare master mixes according to Table 5. In the table it is taken into account the stock of reagents (+1 volume of each kind) for compensation of possible losses during pipetting.

ATTENTION! When working with Taq-polymerase take the required volume from the tube while not sinking the tip deeply in the

reagent so that not to take excessive enzyme volume because of its getting on the external tip surface.

Table 5 – Preparation of master mixes (calculated for one specimen).

Samples quantity	PCR mix, µl	Taq, µl	Total, µl
1	16	40	56
2	20	50	70
3	24	60	84
4	28	70	98
5	32	80	112
6	36	90	126
7	40	100	140
8	44	110	154
9	48	120	168
10	52	130	182
11	56	140	196
12	60	150	210
13	64	160	224
14	68	170	238
15	72	180	252
16	76	190	266
17	80	200	280
18	84	210	294
19	88	220	308
20	92	230	322
21	96	240	336
22	100	250	350
23	104	260	364
24	108	270	378

1. Put 14 µl of each master mix in the relevant tubes as per the recommended arrangement of reactions (see table 6).

2. Put 6 µl of deionized water in «NTC» tubes.

3. Put 6 µl of PC standard mixture in «PC» tubes.

4. Put 6 µl of DNA samples to «S» tubes.

5. Seal the PCR-plate/close the tubes; make sure that all the covers or the film fit tightly.

6. Remove the PCR-plate/ tubes in order to collect the reaction mixture on the well bottom, while preserving the correct orientation of the plate or tube series.

Table 6 - Recommended arrangement of reactions

96-well plate												
Тест	1	2	3	4	5	6	7	8	9	10	11	**12**
del	NTC	PC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
L858R	NTC	PC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
del	S11	S12	Etc.									
L858R	S11	S12	Etc.									

S1 – DNA isolated from testing sample №1 etc.

B) DNA PCR-amplification and «real-time» hybridization-fluorescent detection of amplification products

(performed in area for PCR – room for PCR-amplification)

1. Place the tubes into the reaction module of a device for «real-time» PCR. Pay attention to the fact that the devices for «real-time» PCR shall be maintained, calibrated and used in accordance with the manufacturer's recommendations. The use of this kit in a non-calibrated device may affect the test performance.

2. Program the device for fulfillment of a relevant amplification program and fluorescent signal detection as per the description for this device (see tables 7, 8).

Table 7 – Amplification program for devices produced by «DNA-Technology»

Stage	Temperature, °C	Time	Total cycle number
1	95	2 min	1
2	95	5 s	50
3	64	15 s	

Table 8 – Amplification program for other devices

Stage	Temperature, °C	Time	Total cycle number
1	95	2 min	1
2	95	5 s	50
3	62	15 s	

3. Start amplification program with fluorescent signal detection at stage 3.

4. After completing the program, analyze the results.

11. Recording and interpretation of results

Record the results with the use of software of the device used for performing PCR with detection in «real-time» mode. Analyze fluorescent signal accumulation curves by two channels:

– the channel **FAM** records a signal evidencing the accumulation of DNA amplification products of mutant *EGFR* gene variants and deletions in exon 19 (detects 27 deletions but not differs).

– the channel **HEX** records a signal evidencing the accumulation of DNA amplification products of normal *EGFR* gene variants *(used as internal control sample).*

The results are interpreted based on the presence (or absence) of intersection of the fluorescence curve with a threshold line set.

Interpretation of results in the test samples and control samples is presented in the Table 9 and Table 10.

Table 9 – Interpretation of results in the test samples

Tubes	Mutant DNA of *EGFR* gene is detected	Mutant DNA of *EGFR* gene is not detected	Doubtful result	Invalid result
del L858R	Channel **FAM:** Ct≤ 35 Channel **HEX:** amplification curve rising and (any Ct) or absence of curve rising.	Channel **FAM:** absence of curve rising. Channel **HEX:** Ct≤ 35	Channel **FAM:** amplification curve rising, Ct > 35 Channel **HEX:** Ct≤ 35	Absence of amplification curve over two channels **HEX** and **FAM**

Table 10 – Interpretation of results in the control samples

Tubes	Correct result	Invalid result
PC	Channel **FAM** and **HEX**: Ct≤ 35	Absence of amplification curve rising at **HEX** and/or **FAM**
NTC	Channel **FAM** and **HEX**: absence of amplification curve rising	Amplification curve rising at channel **FAM** and/or **HEX** (any Ct)

Interpretation of results in control samples

The result of the PCR-testing is considered to be correct if reactions del, L858R are held in tubes with relevant PC not later than 35 cycle and there is absence of amplification curve rising in NTC tubes on the channels **FAM** *и* **HEX**.

Interpretation of results in test samples

Interpretation of results for test samples is performed only if results for NTC and PC are correct.

The interpretation is performed using software of the device used. Threshold line is set at the level of curves transition into exponential growth phase.

Mutant DNA of *EGFR* gene is detected if amplification curve at FAM channel rises above the established threshold line, and Ct≤35. Channel HEX – amplification curve rising (any Ct) or absence of curve rising.

Mutant DNA of *EGFR* gene is not detected if amplification curve at FAM channel doesn't rise above the established threshold line, and amplification curve at HEX channel rises above the established threshold line, Ct≤ 35 (Internal control sample).

Test result is doubtful if amplification curve at channel FAM rises above established threshold line, and Ct>35. Amplification curve at HEX channel rises above than established threshold line and Ct≤35.

Result is invalid, if amplification curves do not rise neither at channel FAM, nor channel HEX above established threshold line. It indicates that neither normal DNA nor a mutant reaction has been tested.

If an invalid result is obtained for the sample, it is necessary to repeat PCR-testing of the respective test sample starting from DNA isolation from the tissue, or reject the sample as unsuitable for this type of testing.

If a doubtful result is obtained for the sample, it is required to repeat PCR-testing of the respective test sample starting from DNA isolation from the tissue.

The kit is unsuitable for further use if amplification curves at channels FAM and HEX in PC tubes are lower than the established threshold line and such result is steadily reproduced.

12. Storage, transportation and usage conditions

Storage.

«Test-EGFR-tissue» kit must be stored at 2 °C to 8 °C in the manufacturer packing during all shelf life.

After packing opening kit components should be stored under the following conditions:

- Kit components must be stored at 2 °C to 8 °C during all shelf life;
- PCR mixes del, L858R must be stored in a light-proof place during all shelf life.

Kit stored with violation of storage conditions are not to be applied.

Transportation.

«Test-EGFR-tissue» kit must be transported by all kinds of transport at the covered vehicles in accordance with rules of transportation acting on the transport of this type.

Kit must be transported at temperature from 2 °C to 8 °C during all shelf life. Transportation at room temperature (15–25°C) is acceptable but no longer than 5 days.

Atmosphere pressure is not controlled because it does not influence the sample quality.

For ensuring of transportation conditions during all transportation period the kit is placed into reusable polyurethane-foam thermal container with ice pack for temporary storage and transportation. Type, volume, ice pack amount at transported kits and thermal container volume are selected depending on duration and transportation conditions.

Kits transported with violations of temperature conditions are not to be used.

Shelf life. Shelf life of the kit «Test-EGFR-tissue» is 12 months. The kit shall not be used after the expiry date.

Shelf life of opened kit components. 12 months if stored at 2 °C to 8 °C.

Shelf life of kit components ready for operation. 1 hour if stored in an ice bath and complied with conditions that prevent components drying and contamination by outside biological material.

13. Utilization

Kits that have become unusable including because of the expiration of the expiry date, are subject to utilization in accordance with the requirements of SanPiN 2.1.7.2790-10 «Sanitary and epidemiologic requirements to the address with medical waste».

In accordance with classification of medical waste the kits refer to class A (epidemiologically safe waste approached on structure to municipal solid waste). Unused reagents in accordance with p. 4.28 of

SanPiN 2.1.7.2790-10 «Sanitary and epidemiologic requirements to the address with medical waste» are not subject to use gather in the one-time marked packaging of any color (except yellow and red).

Residual tubes and materials are utilized in accordance with MU 287-113 (Methodological instructions for disinfection, pre-sterilization purification and sterilization of medical devices).

Liquid components are eliminated by draining into the sewage system with a preliminary watering of the reagent with tap water 1: 100 and removal of the rest of the packages as industrial or household garbage.

Consumer package of «Test-EGFR-tissue» kit is subject to mechanical destruction with removal of residues as industrial or household garbage.

Personnel carrying out the destruction of the kit must comply with the safety rules for carrying out a particular method of destruction.

14. Warranty obligations, contact information

The manufacturer guarantees the conformity of «Test-EGFR-tissue» kit to technical requirements under transportation, storage and operation conditions established by technical specification.

If there are any complaints regarding the quality, undesired events that may cause adverse event (incident), send the information to the address:

Limited liability company «TestGene»
(TestGene LLC),
9, 44th Inzhenerny Proyezd, office 13, Ulyanovsk, 432072, Russia
Tel.: +7 499 705-03-75
www.testgen.ru

Technical support service:
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