DNA-TECHNOLOGY

GENETICS

EGFR

MUTATIONS REAL-TIME PCR
GENOTYPING KIT

CLINICAL SIGNIFICANCE

The epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein, the stimulation of which leads to the RAS-RAF-MEK-MAPK signaling pathway activation. It is involved in cell proliferation, apoptosis and angiogenesis.

The EGFR transmits a signal to the cell, activating receptor tyrosine kinase autophosphorylation and triggering cell proliferation and differentiation cascade.

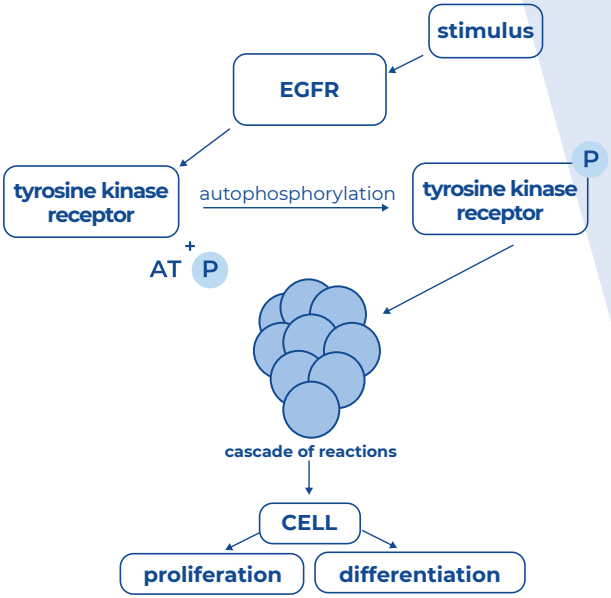


Fig. 1. EGFR activation.

Congenital and acquired mutations in the EGFR gene are associated with chromosomal instability, uncontrolled division and survival of transformed cells, that can lead to the development of cancer.

Lung cancer ranks second in new cancer cases and first in cancer-related deaths worldwide [1]. The 5-year survival rate for the lung cancer is 20–25% in average [2, 3].

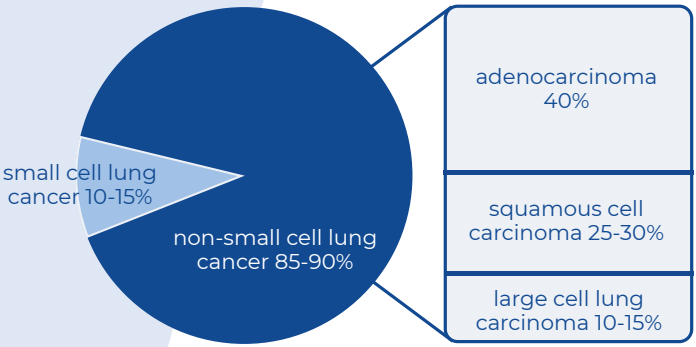


Fig. 2. Percentage distribution of major histological types of lung cancer [8].

The presence of activating mutations in the *EGFR* gene is an indication to prescribe target therapy with EGFR-tyrosine kinase inhibitors (TKI) of the 1st (gefitinib, erlotinib), 2nd (afatinib), or 3rd generation (osimertinib) [6]. Approximately 85-90% of activating mutations are in-frame deletions in exon 19 and the L858R point mutation in exon 21 [4].

The T790M mutation is rarely found in primary tumors (1-3%), but it often (41-62% of cases) develops secondary and leads to acquired resistance to 1st and 2nd EGFR-TKI [6]. This mutation dictates the need to switch to the 3rd generation EGFR-TKI, for example, osimertinib [7]. The frequency of other non-classic mutations in the EGFR gene is lower and ranges from 1% to 5% for G719X, L861Q, and S768I mutations in exon 20, and less than 1% for insertion mutations in exon 19 [5].

The molecular genetic testing determines the type of activating mutations in the *EGFR* gene for better selection of target therapy with TKI.

Differentiation of *EGFR* gene mutations and determination of *ALK* and *ROS1* translocations is included in the standards for the minimum «mandatory volume» of molecular genetic testing and determination of predictive biomarkers in non-small cell lung cancer.

Screening for mutations associated with non-small cell lung cancer makes it possible to increase clinical efficacy of the treatment and to reduce its cost by eliminating ineffective drugs.

EGFR kit

The EGFR mutations REAL-TIME PCR Genotyping Kit is intended for the detection of somatic mutations in the *EGFR* gene (deletions and insertions in exon 19, insertions in exon 20, mutations L858R, T790M, L861Q, S768I and G719X) by real-time PCR with DNA extracted from formaldehyde-fixed paraffin-embeded non-small-cell lung cancer samples in order to select the patients for targeted therapy with TKI.

EGFR 4

Semi-quantitative detection of 45 somatic mutations:

- 37 deletions in exon 19 (without distinction),
- 5 insertions in exon 19 (without distinction).

Mutations:

- L858R (without distinction),
- T790M

EGFR 8

Semi-quantitative detection of 53 somatic mutations:

- 37 deletions in exon 19 (without distinction),
- 5 insertions in exon 19 (without distinction),
- 3 insertions in exon 20 (without distinction).

Mutations:

- L858R (without distinction),
- G719X (X denotes A/S/C – without distinction),
- T790M,
- L861Q,
- S768I.

INDICATIONS FOR THE USE:

- histologically verified non-small cell lung cancer;
- newly diagnosed, recurrent and metastatic tumors.

BIOMATERIAL:

- formaldehyde-fixed paraffin-embeded (FFPE) non-small-cell lung cancer samples

SPECIAL FEATURES OF THE KIT

Clinical:

- Determination of the *EGFR* mutations, including insertions and deletions in exon 19.
- Semi-quantitative assessment of *EGFR* mutation status in one of the ranges: <1%, 1-10%, 5-20%, 10-100%.

Technological:

- High sensitivity.
- Multiplex format – several DNA targets are detected simultaneously in one tube
- High speed of assay
- Sample intake control (SIC)
- Automatic generation of the results form when using the recommended Real-time PCR instruments of the DT series and RealTime_PCR software
- Availability of preset templates with test parameters, which automatically set the necessary settings and calculate the results

KIT SPECIFICATIONS

Sensitivity	Range of Cp value for SIC		
	Cp≤28	28<Cp≤31,5	31,5<Cp<34
Detection limit of the mutant allele proportion (MAP) in the sample	0,1% 0,3%	1%	5%

* 0,1% for deletions and insertions in exon 19,
0,3% for T790M, L858R, G719X, S768I, L861Q mutations, and exon 20 insertions.

The kit is designed for 48 tests, including negative and positive controls.

Hands-on time
(without sample preparation): from 2 hours.

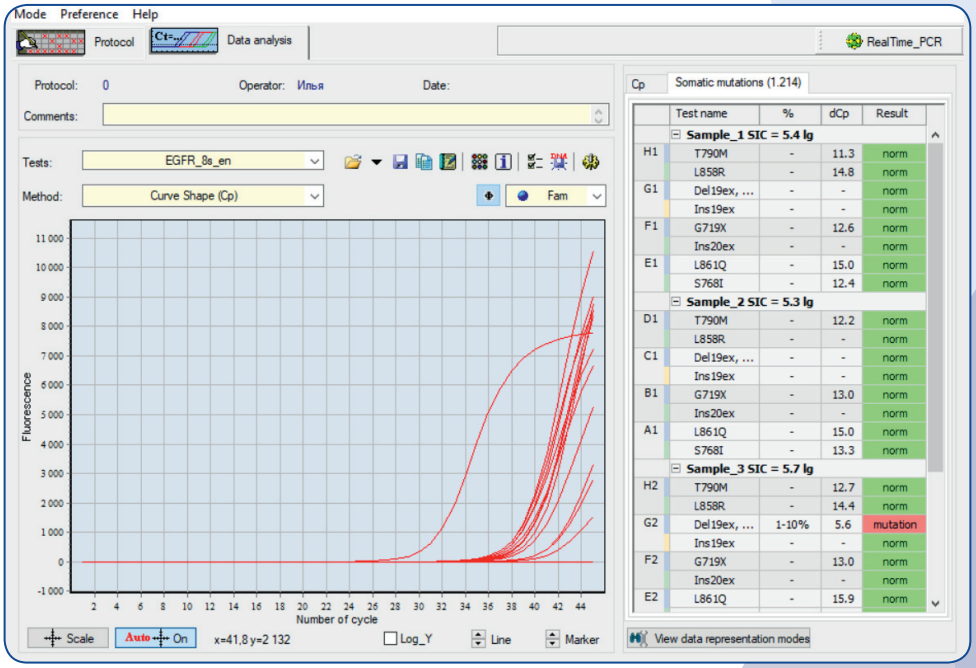
RECOMMENDED MATERIALS AND EQUIPMENT

DNA extraction kits	Real-time PCR instruments
• PREP-PK for primary processing • PREP-NA PLUS DNA/RNA Extraction Kit	• DTprime • DTlite
produced by DNA-Technology	

SOFTWARE

- RealTime_PCR Software;

Registration and interpretation of the reaction results are carried out automatically using the Real-Time PCR software for Real time PCR instruments of the «DT» series manufactured by «DNA-Technology».



AN EXAMPLE OF THE TEST RESULTS FORM

Screening for somatic mutations in the EGFR gene by real-time PCR

Data:
Tube number:
Patient:
Sex:
Age:
Physician:
Comment:

Sample ID:

logotype

information about laboratory

№	Test specification	Mutant allele proportion	Result
1	T790M	—	norm
2	L858R	—	norm
3	Del19ex, Ins19ex	1-10%	mutation
4	Ins19ex	—	norm
5	G719X	—	norm
6	Ins20ex	—	norm
7	L861Q	—	norm
8	S768I	—	norm

Conclusion:
A search was made for a deletion in exon 19, insertions in exon 19 and 20, and G719X, L858R, L861Q, S768I, T790M mutations in the EGFR gene. One deletion in exon 19 was found.

Study was carried out by:

Data:
Signatyre:

Results Form of EGFR PCR analysis was obtained using real-time PCR instrument of the «DT» series and related software.

TRANSPORT AND STORAGE CONDITIONS

The kit must be stored at temperatures from 2 °C to 8 °C during the storage period.

Transportation is allowed in thermoboxes with ice packs by all types of roofed transport at temperatures from 2 to 25 °C but no more than 5 days.

COMPOSITION OF THE KIT:

EGFR 4, EGFR 8 Package S:

- paraffin sealed PCR-mixes (strips);
- Taq polymerase solution;
- mineral oil;
- positive control;
- strip's caps.

EGFR 4, EGFR 8 package U (Universal)

- PCR-mix EGFR;
- PCR-buffer;
- Positive control;
- TechnoTaq MAX polymerase solution.

* Reagents for DNA extraction are not included in the Kit. Commercial kits for nucleic acid extraction from FFPE samples may be used for this purpose

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