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Epithelial Growth Factor Receptor Mutation Test Kit

(Fluorescent Probe-based Real-time PCR Assay)

Instructions For Use

English VER 2.0

REF **T071T012B0C0 12 Tests/Box**

PRODUCT NAME

Epithelial Growth Factor Receptor Mutation Test Kit (Fluorescent Probe-based real-time PCR assay)

INTENDED USE

This kit is intended for qualitative detection of 29 common mutations of EGFR (epithelial growth factor receptor) gene in human lung lesions in vitro (Table 1). It can be used for personalized molecular diagnosis of tumors, and then guide the clinical personalized treatment plan of tumor patients. For professional in-vitro diagnostic use.

Table 1 Details of 29 Somatic mutations in EGFR gene

The type of mutation	EGFR Mutation Group	Base change	The type of mutation	EGFR Mutation Group	Base change
Base deletion	19 del	2235-2249del15	Base deletion	19 del	2240-2251del12
		2235-2252>AAT(complex)			2240-2257del18
		2236-2253del18			2240-2254del15
		2237-2251del15			2239-2251>C(complex)
		2237-2254del18	Single base mutations	21 L858R	2573T>G
		2237-2255>T(complex)		21 L861Q	2582T>A
		2236-2250del15		20 S768I	2303G>T
		2238-2255del18		20 T790M	2369C>T
		2238-2248>GC (complex)		18 G719A	2156G>C
		2238-2252>GCA (complex)		18 G719C	2155G>A
		2239-2247del9		18 G719S	2155G>T
		2239-2253del15	Base insertion	20 ins9	GCCAGCGTG
		2239-2256del18		20 insCAC	CAC
		2239-2248TTAAGAGAAG>C(complex)		20 insGGT	GGT
		2239-2258>CA(complex)			

SUMMARY AND EXPLANATION

EGFR gene mutations in tumor cells can disrupt EGFR tyrosine kinase activity, leading to inappropriate activation. This aberrant activation promotes tumor angiogenesis, abnormal cell proliferation, and invasion. These mutations are varied and primarily found in exons 18-21. Notably, deletions of amino acids 747-750 in exon 19 constitute 45% of mutations, while exon 21 mutations make up 40-45%, exon 18 around 5%, and exon 20 insertion mutations about 1%. Clinical observations reveal differential responses to EGFR-tyrosine kinase inhibitors (EGFR TKIs) based on mutation types. Consequently, pre-screening for EGFR mutations has emerged as a pivotal step in tailoring individualized lung cancer therapy with EGFR-TKIs.

TEST PRINCIPLE

This kit uses polymerase chain reaction (PCR) technology based on fluorescent TaqMan probe to detect EGFR mutant genes in tissue samples obtained from lung cancer patients.

REAGENTS PROVIDED

Seq.	Labels	Main contents	*No.* (12 tests)
1	Primers/Probes 0 (EGFR)	Reference primers and probes.	1 tube (90 μL/tube)
2	Primers/Probes 1 (EGFR)	19del primers and probes.	1 tube (90 μL/tube)
3	Primers/Probes 2 (EGFR)	L858R primers and probes.	1 tube (90 μL/tube)
4	Primers/Probes 3 (EGFR)	L861Q primers and probes.	1 tube (90 μL/tube)
5	Primers/Probes 4 (EGFR)	G719X primers and probes.	1 tube (90 μL/tube)
6	Primers/Probes 5 (EGFR)	S768I primers and probes.	1 tube (90 μL/tube)

7	Primers/Probes 6 (EGFR)	20ins primers and probes.	1 tube (90 μL/tube)
8	Primers/Probes 7 (EGFR)	T790M primers and probes.	1 tube (90 μL/tube)
9	PCR Buffer (EGFR)	PCR buffer, *etc.*	1 tube (1300 μL/tube)
10	Enzyme (EGFR)	Taq DNA polymerase	1 tube (65 μL/tube)
11	Positive Control (EGFR)	Contains EGFR mutations, wild gene and internal control sequences	1 tube (200 μL/tube)
12	Negative Control (EGFR)	Contains internal control sequences	1 tube (200 μL/tube)

OTHER MATERIALS REQUESTED BUT NOT PROVIDED

The following materials are required for use but are not included in this kit:

- Nucleic acid extraction kit.
- Nuclease-free consumables, such as filter tips, 1.5mL tubes, PCR-well strips, or a 96-well plate.
- Experimental equipment, including a centrifuge suitable for 1.5mL tubes and PCR-well strips or a 96-well plate (if available) and a vortex.
- Real-time PCR instrument (thermocycler).
- Other consumables like micropipettes (ranging from 0.5-20μL, 10-100μL, 20-200μL, to 100-1000μL), powder-free disposable gloves, and microplate sealing film.

STORAGE CONDITIONS AND SHELF LIFE

The shelf life of this kit is 12 months when stored in the freezer at -20±5℃. During transportation, it is recommended to use a sealed foam box with ice packs, and the shipping process should not exceed 7 days. Additionally, avoid leaving the kit for more than 7 days at 2-8℃ while also keeping it away from direct light. At 37℃, do not leave the kit for more than 3 days. Finally, refrain from repeating the kit to more than 6 freeze-thaw cycles.

APPLICABLE EQUIPMENT

Applicable to ABI 7500 Real-Time PCR thermocycles. For other Real-Time PCR thermocycles, please consult the manufacturer before use.

ACCEPTABLE SPECIMENS

The specimens to be collected for analysis include lung cancer lesions, which encompass fresh frozen tissues, paraffin-embedded tissues, and sectioned samples.

SPECIMENS COLLECTION AND STORAGE

Preference of Sample Types: Fresh diseased tissue > frozen pathological tissue (or section) > paraffin-embedded pathological tissue (or section).
Sample Quality Confirmation: Ensure that tissue samples used for extraction contain more than 1% tumor cells. This confirmation should be conducted by an experienced pathologist. To maintain sample quality, it's important to select paraffin-embedded tissue or section samples that have not been stored for more than 3 years.
Clinical Information Attachment: Attach accurate clinical information to the specimens, including specimen number, date of onset, and date of collection. This information is essential for proper tracking and analysis.
Avoid Repeated Freeze-Thaw: During transportation and storage, prevent repeated freezing and thawing of clinical specimens, as it can compromise sample integrity.
Transportation Temperature: If transportation cannot be guaranteed at -20±5℃, transport the samples at least at 0-8℃. Ensure that the transport process does not exceed 2 days.
Shelf Life at -20±5℃: The shelf life of the samples at -20±5℃is 4 months. For longer-term storage, samples should be kept at -70℃.

TEST METHODOLOGY

1. Nucleic Acid Extraction (Pre-PCR)

Extract the nucleic acids from the clinical samples according to the instructions of the nucleic acid extraction kit. The concentration of the extracted nucleic acids need to be determined by UV spectrophotometer, and the concentration should be 1~10ng/μL. If the concentration of a substance is excessively high, it is necessary to dilute it.
Positive and negative controls no require extraction.
* We have validated the following kits for extracting nucleic acids from lung cancer lesion specimens:

- QIAamp DNA FFPE Tissue Kit (QIAGEN, Cat No.56404). recommended*.
- DNeasy Blood & Tissue Kit (QIAGEN, Cat No.69506). recommended*.
- Before using nucleic acid extraction kits from other suppliers, please perform verification.

2. Amplification Processes (PCR)

2.1 Preparation of Amplification Reagent (PCR Room I)
Retrieve the tubes containing buffer, primers, and probes from the kit and thaw them on ice or at 2-8℃.
Remove the enzyme tube, shake it thoroughly, and briefly centrifuge all reagent tubes at low speed.
Prepare the amplification PCR mixture according to the specified ratios:

Tubes	PCR Buffer (EGFR)	Enzyme (EGFR)	Primers/ Probes0 (EGFR)	Primers/ Probes1 (EGFR)	Primers/ Probes2 (EGFR)	Primers /Probes 3 (EGFR)	Primers/ Probes4 (EGFR)	Primers/ Probes5 (EGFR)	Primers/ Probes6 (EGFR)	Primers/ Probes7 (EGFR)
PCRmix0	12.5 μL	0.6 μL	6.9 μL	/	/	/	/	/	/	/
PCRmix1	12.5 μL	0.6 μL	/	6.9 μL	/	/	/	/	/	/
PCRmix2	12.5 μL	0.6 μL	/	/	6.9 μL	/	/	/	/	/
PCRmix3	12.5 μL	0.6 μL	/	/	/	6.9 μL	/	/	/	/
PCRmix4	12.5 μL	0.6 μL	/	/	/	/	6.9 μL	/	/	/
PCRmix5	12.5 μL	0.6 μL	/	/	/	/	/	6.9 μL	/	/
PCRmix6	12.5 μL	0.6 μL	/	/	/	/	/	/	6.9 μL	/

PCRmix7	12.5 μL	0.6 μL	/	/	/	/	/	/	/	6.9 μL

Determine the required volume for each reagent, combine them in a suitable centrifuge tube, mix thoroughly, and briefly centrifuge.

The total number of PCR mixtures should = n number of clinical samples + 1 positive control + 1 negative control, n= sample number.

Dispense 20.0 μL of the PCR mixture into each PCR well or tube, and subsequently transfer the plates or tubes to PCR room II.

2.2 Add the Templates (PCR Room II)

Add 5.0 μL of nucleic acid extracted from each sample (prepared in the first step: Pre-PCR) into each PCR well/tube with PCR mixture solution.

***Example:** Extract 10 nucleic acids from 10 clinical samples according to the instructions of Nucleic acid extraction kit, and label them as S1~S10, respectively. Label Positive Control (EGFR) and Negative Control (EGFR) as PC and NC, respectively. Take a new 96-well plate, add 20.0μL PCR mix 0~7 into each well in each row respectively in PCR room I, *e.g.*, add PCRmix0 to each well in A1~A12, add PCRmix1 to each well in B1~B12, etc. Add 5.0μL nucleic acids into each well in each column respectively in PCR room II, e.g., add S1 to each well in A1~H1, add S2 to each well in A2~H2, etc. Centrifuge after sealing the plates/tubes.

Tubes	Location	1	2	3	4	5	6	7	8	9	10	11	12
PCRmix0	A	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NC
PCRmix1	B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NC
PCRmix2	C	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NC
PCRmix3	D	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NC
PCRmix4	E	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NC
PCRmix5	F	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NC
PCRmix6	G	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NC
PCRmix7	H	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	PC	NC

2.3 Amplification (Detection Area)

Put the reaction tubes/plates into the fluorescent real time PCR thermocycler and set the cycles program as follows:

Step	Cycles	Temperature (℃)	Time(min:sec)
1	1	95	5:00
2	45	95	00:10
		62*	00:30

Fluorescent dye signals assigning: FAM (EGFR), HEX/VIC (internal control). *The signal data is collected at 62℃. When using the ABI7500, select the 'Quencher' and 'Passive reference' columns as "none". Set the reaction volume of per tube/well to 25 μL.

CUT-OFF VALUES

The cut-off value of HEX/VIC is 36.00.

EXPLANATION OF THE TEST RESULTS

After the reaction completed, the instrument saves the result automatically and adjusts the Baseline and Threshold values.

1. The difference Ct values (ΔCt) in FAM channel between reference (Ct value corresponding to mix0) and mutation (Ct value corresponding to mix1-7) tubes are calculated as followed formula:

   ΔCt = Ct value of mutation -Ct value of reference.
2. Each mutant well of the positive control is ΔCt ≤ 8, indicating that the detection reagent is sensitive and effective, and the purpose is to exclude false negatives.
3. There should be no typical amplification curve in FAM channel of negative control, indicating that the detection reagent is effective and free of contamination.
4. The Ct value in HEX/VIC channel in all reaction wells should be ≤ 36.00, with a typical S-type amplification curve. Otherwise, re-extraction or sample usage increasing is needed.
5. The Ct value in FAM channel in the reference tube should in the range of 21 ≤ Ct ≤ 31. If Ct < 21, the sample should be diluted, if Ct > 31, the amount of extracted DNA is too low, and to increase the sample size for re-extraction is recommended.

The above requirements must be met in the same test at the same time, otherwise the PCR reaction is considered invalid and should be re-performed. The interpretation and judgment of test results are as following when met all above requirements:

Tubes	ΔCt value	Interpretation
PCR mix 1(19del)	≤16	19del mutation positive
	>16	No 19del mutation detected, or concentration is below detection limit
PCR mix 2(L858R)	≤16	L858R mutation positive
	>16	No L858R mutation detected, or concentration is below detection limit
PCR mix 3 (L861Q)	≤15	L861Q mutation positive
	>15	No L861Q mutation detected, or concentration is below detection limit
PCR mix 4 (G719X)	≤10	G719X mutation positive
	>10	No G719X mutation detected, or concentration is below detection limit
PCR mix 5 (S768I)	≤15	S768I mutation positive
	>15	No S768I mutation detected, or concentration is below detection limit

PCR mix 6 (20ins)	≤14	20ins mutation positive
	>14	No 20ins mutation detected, or concentration is below detection limit
PCR mix 7 (T790M)	≤11	T790M mutation positive
	>11	No T790M mutation detected, or concentration is below detection limit

LIMITATIONS OF THE TEST METHOD

The test results serve solely as clinical references. The choice of personalized treatment for patients should be made comprehensively, considering their symptoms, medical history, other laboratory tests, and treatment responses. Negative results do not definitively rule out the presence of mutations.

Tumor tissue (cell) heterogeneity may exist, leading to different detection results when sampling from various areas.

The detection process is confined to specified sample types and detection systems, including relevant equipment, nucleic acid extraction reagents, and detection methods.

The following factors can also contribute to false positive or false negative test results:

a. Insufficient tumor cells in the sample, tumor tissue heterogeneity, excessive degradation, or target gene concentration below the detection limit in the amplification reaction system can result in negative outcomes.

b. Inappropriate sample collection, handling, and transportation, as well as errors in test procedures and experimental conditions, may lead to erroneous positive or negative findings.

PRODUCT PERFORMANCE

1. Using 200ng of genomic DNA as a template yielded no false positive results.
2. We successfully detected mutations in 10ng DNA samples with mutation ratios as low as 1%, achieving a 100% mutation detection rate. However, when sample concentration below 1ng/μL and the mutation rate is also less than 1%, repeated tests may not consistently yield positive results.
3. Precision: Both intra-batch and inter-batch coefficient of variation for Ct values are <10%.

PRECAUTIONS

1. Prior to genomic DNA extraction from tissue samples, a qualified pathologist must confirm the presence of tumor cells to ensure sample quality.
2. This test requires manual operation by personnel trained in gene amplification or molecular biology diagnostics. Laboratories conducting this test must adhere to relevant Biosafety Regulations for Microbiological and Biomedical Laboratories, implementing appropriate biosecurity measures and safety protocols.
3. The entire detection process should be conducted in 3 designated areas: the first for reagent preparation, the second for specimen processing and reaction system preparation, and the third for amplification, fluorescence detection, and results analysis. Instruments, equipment, and lab coats must be used exclusively within each area to prevent contamination.
4. Throughout the testing process, precautions must be taken to avoid RNase contamination. Disposable gloves without fluorescent substances should be worn (with regular replacement recommended). Utilize disposable thin-walled 200μL PCR tubes (or 96-well PCR plates with optical film) and pipette tips with filters; never touch the reaction tube directly with bare hands.
5. Handling of clinical specimens should be carried out within a biosafety cabinet to ensure laboratory staff safety and prevent environmental contamination. Harmful or toxic specimens and reagents must be appropriately stored and managed by assigned personnel. Waste should be disposed of in dedicated containers. Lab benches and equipment, such as operator's stations, pipettes, centrifuges, and PCR thermocyclers, should be regularly cleaned and disinfected with 1.0% sodium hypochlorite and/or 70% ethanol. Laboratory rooms and ultra-clean benches should be periodically disinfected with ultraviolet lamps, both before and after each experiment.
6. Prior to commencing the experiment, ensure that reagents are fully thawed, thoroughly mixed, and briefly centrifuged to settle all liquid to the bottom of the tubes. When preparing the reaction solution, pay close attention to mixing all liquids using a vortex mixer, avoid pipette-induced bubbles, and briefly centrifuge the reaction mixture. Use reagents before their expiration date, and avoid combining reagents from different batch numbers, which can be found on the label along with the manufacturing and expiration dates.

Manufacturing date and expiration date: view on label

INDEX OF SYMBOLS

	Consult Instructions for Use	Σ	Contain <n> tests	EC REP	Authorized Representative in the European Community
IVD	In vitro diagnostic medical device		Use-by date	-25℃ -15℃	Temperature limit -25 to -15℃
REF	Catalogue #	LOT	Lot Number	CN	Country of manufacture
	Manufacture Date		Manufacturer	UDI	Unique device identifier
	Keep dry		Keep away from sunlight	CE	CE conformity marking

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