# EGFR Gene Amplification Kit（FISH）Instruction For Use

## [Product Name]

EGFR Gene Amplification Kit（FISH）

## [Specification]

5 tests/kit, 10 tests/kit, 20 tests/kit.

## [Intended Use]

It was used to qualitatively test the amplification of EGFR gene in the paraffin-embedded human lung cancer tissue section fixed by 10% neutral buffered formalin in vitro. This kit is only used for the detection of EGFR gene amplification in specific tumor patients, and the test results are for clinical reference only. The interpretation of any positive or negative results should be carried out by the pathologist in combination with pathomorphology, clinical signs and other detection methods, rather than a separate diagnostic indicator.

EGFR amplification is one of the best indications for targeted therapy, which can bring significant curative effects. For example, based on Erbitux and Centuximab the therapy has an effective rate of 45%. Other targeted drugs for EGFR include Iressa, Geftinib, Tarceva, Erlotinib, etc. According to this, the individualized treatment plans can significantly prolong the median survival of patients (more than double that of patients without amplification).

Without above treatments, the prognosis is worse than without amplification.

## [Test Principle]

In situ hybridization is a method that can observe specific nucleotide sequences of cells, especially DNA fluorescence in situ hybridization (FISH) technology. FISH technology can accurately identify annealed target sequences with single-stranded fluorescently labeled DNA probes, and directly observe the hybridization of probes and intracellular DNA sites using a fluorescence microscope with corresponding filters.

The lung cancer samples were fixed by 10% neutral formalin, paraffin-embedded and sliced, then placed on a slide. It can  be used for fluorescence in situ hybridization analysis.

The hybridization operation process includes: the nucleic acid DNA in the nucleus and the probe site-specific fluorescence in situ hybridization probe are denatured into single-stranded DNA at high temperature. Based on the principle of complementary base pairing, the samples that have been denatured into single strands and probe nucleic acids are specifically bound to form hybrid double-stranded that can be detected. After washing the non-specific hybridization, the fluorescent signal labeled on the probe can be detected under a fluorescence microscope.

Normal cell signal: Each cell shows two red and two green signals.

Abnormal cell signal: there are three or more red signals and no less than two green signals in the cells.

## [Main Components]

The kit is mainly composed of CBFB/MYH11 probe and DAPI counterstain solution.

The reagent does not contain but necessary for the test: FISH Sample Pretreatment Kit.

Other reagents not provided: positive contrast photo; Xylene; Ethanol (anhydrous, 85%, 70%);Purified water; Sealing gum.

## [Storage and Validity]

Stored at -20℃ in dark, sealed conditions, valid for 12 months.

## [Applicable Instrument]

Fluorescence in situ hybridization system;

Fluorescence microscope, in which:

Objective lens: It is recommended to use 100 times achromatic objective lens of immersion type, which can obtain satisfactory results.

Immersion Oil: Low level self-fluorescence Immersion Oil exclusive use for fluorescence microscopy.

Optical Filters: It is recommended to select filters suitable for labeled fluorescent stains.

Green fluorescence: excitation wavelength is 490nm , and emission wavelength was 516nm.

Orangered fluorescence: excitation wavelength was 551nm ,and emission wavelength was 572nm.

## [Sample Requirements]

The paraffin tissue sample of lung cancer patients. Fix the fresh tissue sample with 10% neutral formalin for 6-48h. According to the requirements of pathological technical specifications, sampling, dehydration, paraffin embedding, then make into paraffin block. Place the tissue sections (3-5 μm) in purified water at 40℃ to float and spread. Then stick the tissue sections to a glass slide, air-dried and baked for at least 3h (or overnight) at 56℃ to 65℃, so that the tissue sections could adhere more closely to the glass slide to prevent peeling.

Paraffin block should avoid contact with acid, strong alkali, and avoid overheating. After the tissue sections are prepared, the FISH test should be completed within 6 weeks, otherwise the result may be affected.

## [Test Method]

1. Reagent preparation: ethanol solution (70% ethanol, 85% ethanol)

Dilute 700mL and 850mL anhydric ethanol with purified water to 1000mL, respectively, and store at 2℃ to 8℃ during use. Discard the reagents 6 months after preparation or after been used for the hybridization of 20 glass slides. Discard the reagents if they become cloudy or contaminated.

2. Detection method

2.1 deparaffining

2.1.1 Circle the tissue area on the back of the slide with a diamond pen.

2.1.2 Immersed the glass slides in xylene solution for three times at room temperature,10min/ time.

2.1.3 Immersed the glass slides in anhydrous ethanol at room temperature, twice ,5min/ time.

2.1.4 glass slides were processed in 85% ethanol and 70% ethanol for 3min at room temperature.

2.1.5 Rinse with purified water for 3 times, 2min/ time.

2.2 Retrieval

Add an appropriate amount of pre-treatment buffer into the pressure cooker, repair with high pressure for 4min.

2.3 enzyme digestion

2.3.1 Preheat the pepsin solution to 37℃ in a water bath.

2.3.2 Put the slices into the preheated pepsin solution and digest for 15±5 minutes.

2.3.3 Rinse with purified water twice, 1min/ time.

2.4 dehydration

At room temperature, the slides were successively treated in 70%, 85% and anhydrous ethanol solution for 2 min, and then dried.

2.5 Denaturation and hybridization

2.5.1 Remove the probe from the refrigerator and centrifuge for 10-15s.

2.5.2 Take 10 μL probe and drop it on the target area.

2.5.3 Cover the coverslip and seal it with the sealing gum.

2.5.4 Put the slides into a hybridization instrument, denaturate at 83℃ for 6min, and hybridize at 40℃ for 18h.

Note: Specimens should be kept moist during hybridization.

2.6 washing

2.6.1 Put the washing buffer I into the water bath and heat it to 72℃.

2.6.2 Gently remove the sealing gum from the slide with tweezers.

2.6.3 Soak the glass slides in the washing buffer II solution at room temperature, placed for about 5 minutes, and remove the coverslip.

2.6.4 Remove the coverslip and wipe the excess liquid off the glass.

2.6.5 immerse the slides again in 72℃ washing buffer I for 3min.

2.6.6 wash the slides in 70% and 85% ethanol solution for 2min in turn and dry them.

2.7 counterstaining

Add 10μL DAPI to the target area of the slide and cover with a coverslip.

2.8 Microscope examination

The slides were placed in -20℃ condition for 15 minutes and then taken out for observation and analysis under a fluorescence microscope.

## [Analysis of FISH test results]

1. Normal cells: Normal cells: 2 red and 2 green signals are counted in a single interphase nucleus.
2. EGFR gene amplification abnormal cells: In a single interphase nucleus, the red signal is greater than 2 and the green signal is not less than 2.

At least 100 tumor cells were counted, and the ratio value was calculated (Ratio value = total number of red signals in 100 tumor cell nuclei/total number of green signals in the 100 nuclei).

When the ratio ＜2.0, it is interpreted as negative results. It suggests that the EGFR gene in this sample is not amplified.

When the ratio ≥2.0 and the CSP7 signal in the counting nucleus ≥2, it indicates that the EGFR gene is amplified in the sample, which were interpreted as positive results.

In addition, if >10% of cells exist in more than or equal to 15 signals or there are clustered red signals or >40% of cells are greater than or equal to 4 red signals, which were interpreted as positive results. It suggests that the EGFR gene is amplified.

## [Attentions in result analysis]

1. Tumor cells should be randomly counted in the samples.
2. Counting cells must have clear and recognizable signals in each channel.
3. Do not analyze the heterogeneous regions.
4. Do not analyze nucleus with unclear outline or overlap.
5. Do not analyze the area where the background is so deep that affects the signal judgment.
6. Counting results shall be independently completed by two participants, and the results should be consistent before confirmation.
7. If the signal in over 25% of the nucleus is too weak, the region should not be analyzed.

## [Positive judgment value]

EGFR/CEP7 probes contain two kinds of DNA probes, which can hybridize on metaphase chromosomes and interphase nuclei to produce bright signals that can be clearly recognized by the naked eye. The EGFR DNA probe was hybridized to the long arm of human chromosome 7 (7q11.2), and the fluorescence signal was red. The control probe is CEP7, of which the probe hybridization signal is located on human chromosome 7 (7p11.1-q11.1), covering the centromeric region, and the fluorescence signal is green.

1. When the EGFR/CEP7 ratio ≥2.0, it is interpreted as positive results. It suggests that the EGFR gene in this sample is amplified.
2. When the EGFR/CEP7 ratio < 2.0, but the mean copy number of EGFR/ cells ≥4.0 accounted for 40% of the total number of cells , which was interpreted as positive results.

3. When EGFR cluster signal is present in ≥10% of tumor cells (this cluster signal means the presence of ≥15 signals in each cell ), which was interpreted as positive results.
4. When the EGFR/CEP7 ratio < 2.0 and the mean copy number of EGFR/ cell is < 4.0, which was interpreted as negative.

## [Method Limitations]

This kit is only suitable for detection of EGFR/CEP7 gene amplification in lung adenocarcinoma patients by fluorescence in situ hybridization, not for detection of other gene mutation modes.

## [Cautions]

1. This kit is only used for in vitro diagnosis and must be used by strictly trained professionals.
2. In order to obtain ideal results, it is necessary to ensure that the reagents are correctly prepared and stored in accordance with the instructions.

## [Symbols]

| Symbol | Used for | Symbol | Used for |
|---|---|---|---|
| | Use-by date | | Consult instructions for use |
| LOT | Batch code | IVD | In vitro diagnostic medical device |
| | Temperature limit | | Manufacturer |
| | Avoid overexposure to the sun | | Date of manufacture |

## [Basic Information]

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