HER2 Gene Amplification Kit（FISH）
Instruction For Use

[Product Name]

HER2 Gene Amplification Kit（FISH）

[Specification]

10 tests/kit, 20 tests/kit.

[Intended Use]

It was used to qualitatively test the amplification of HER2 (human epidermal growth factor receptor 2) gene in the paraffin-embedded breast cancer tissue section fixed by 10% neutral buffered formalin in vitro. Breast cancer specimens generally detected by IHC first. The results of IHC (3+) are considered to be positive for HER2, while the results of IHC (0) and IHC (1+) are generally considered to be negative for HER2. The results of IHC (2+) are considered to be uncertain cases, and the amplification state of HER2 gene should be further determined by in situ hybridization.

This kit is only used for the detection of HER2 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.

[Test Principle]

Based on the principle of complementary base pairing, marked the fluorescent stain in DNA fragments (probe) matching with the target DNA.The probe and the corresponding DNA fragments in material waiting for test specificaly combined under certain conditions (hybrid), to form double-stranded nucleic acid, by means of fluorescence microscope to observe and record the hybrid double chain type, quantity, to judge target DNA in inspected samples normal or not.

The samples were fixed by 10% neutral formalin, paraffin-embedded and sliced, then placed on a slide. The denatured DNA formed a single strand state and hybridized with the probe. After hybridization, unbound probes are washed away. Use DAPI (4,6- diimiyl -2- diphenylindoles) to

restain the nucleus. DAPI is a nucleic acid stain used as a specific DNA stain. The kit's probe hybridization signals can be observed by fluorescence microscopy equipped with corresponding filters (blue, red and green fluorescent signals).Fluorescence signals in the nucleus were observed under a microscope, and HER2 gene (red) and chromosome 17 (CEP17) (green) signals were counted. According to relevant guidelines and normative documents, the interpretation results were determined in the form of HER2 copy number and/or the ratio of total copy number of HER2 to total copy number of CEP17 (double signal).

[Main Components]

The kit is mainly composed of HER2/CEP17 probe and DAPI counterstain solution.

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

Other reagents not provided: HER2 positive contrast photo; Xylene; Ethanol (anhydrous, 85%, 70%);Purified water; Sealing gum.

[Storage and Validity]

Stored at -20℃±5℃ 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.

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

[Sample Requirements]

The paraffin tissue sample of breast 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 (3-5μm) 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. If the tissue sections are stored at room temperature, the test should be completed within 7 days;If the tissue sections are stored in cold condition (2℃ ~ 8℃), the test should be completed within 1 month.

[Test Method]

1. Testing instruments and equipment: in situ hybridization system, fluorescence microscope, slice broiler, centrifuge, electric thermostatic incubator, water bath, staining cylinder, induction cooker, pressure cooker, diamond pen.

2. 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.

3. Detection method

3.1 deparaffining

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

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

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

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

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

3.2 Retrieval

Add an appropriate amount of pre-treatment buffer into the pressure cooker and put the slices into the cooker, boil them for 20min to retrieval, or repair with high pressure for 5min (high-pressure air injection for 5min).

3.3 enzyme digestion

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

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

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

3.4 dehydration

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

3.5 Denaturation and hybridization

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

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

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

3.5.4 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.

3.6 washing

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

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

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

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

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

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

3.7 counterstaining

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

3.8 Microscope examination

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

4. Quality control

4.1 It is recommended to set internal and external controls for each test to control the quality of the whole operation procedure.

4.1.1 The internal control can be paracancer normal tissue. If hybridization signals are present in more than 75% of tumor nucleus, the detection is deemed as successful.

4.1.2 Tissue sections or cell strains with known amplified status of HER2 gene can be selected for external controls, including at least critical value and non-amplified samples. When each batch of patient samples is tested and new kit batches are used, quality control testing should be performed simultaneously to monitor test performance and evaluate the accuracy of signal counting.

[Positive judgment value]

HER2/CEP17 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 HER2 DNA probe was hybridized to the long arm of human chromosome 17 (17q11.2-q12), and the fluorescence signal was red. The control probe is CEP17, of which the probe hybridization signal is located on human chromosome 17 (17p11.1-q11.1), covering the centromeric region, and the fluorescence signal is green.

1. When the HER2/CEP17 ratio ≥2.0, the mean copy number of HER2/ cells ≥4.0 or part of the cells were in clusters of red signals, which were interpreted as positive results.

2. When the HER2/CEP17 ratio > 2.0 and the mean copy number of HER2/ cell < 4.0, it is suggested to increase the count cells and recalculate ,if the result remain unchanged, which should be deemed as negative.

3. When the HER2/CEP17 ratio ＜2.0 and the mean copy number of HER2/ cells ≥6.0, it is suggested to increase the count cell and recalculate, if the result remains unchanged, which should be deemed positive.

Note: The amplified cells should be homogeneous and continuous, and account for more than 10% of the infiltrating carcinoma.

4. When the HER2/CEP17 ratio < 2.0 and the mean copy number of HER2/ cell is < 6.0, but > 4.0, it is suggested to increase the count cell and recalculate, if the result remains unchanged, IHC 3+ is considered positive, and IHC 0, 1+, 2+ is considered negative, if the results have been changed, comprehensively analyse the two results to make the final decision.

5. When the HER2/CEP17 ratio < 2.0, the mean copy number of HER2/ cell < 4.0, the HER2 was interpreted as negative.

Note: HER2/CEP17 = total red signals/total green signals in at least 20 cell nucleus.

[Result Interpretation]

1. Evaluation of the effectiveness of slides

1.1 The nuclear structure should be complete, with clear edges and no overlap.

1.2 The probe signal should be bright, clear and easy to observe.

1.3 The background shall be black with no fluorescent particles and a blurry fluorescent background.

2. Attentions in result analysis

2.1 Tumor cells should be randomly counted in the samples.

2.2 Counting cells must have clear and recognizable signals in each channel.

2.3 Do not analyze the heterogeneous regions.

2.4 Do not analyze the area where the background is so deep that affects the signal judgment.

2.5 Counting results shall be independently completed by two participants, and the results should be consistent before confirmation.

2.6 If HER2 expression is heterogeneous, it should be explained in detail in the test results.

2.7 If the signal in over 25% of the nucleus is too weak, the region should not be analyzed.

2.8 If signals are present in more than 10% of the cytoplasm, the region should not be analyzed.

[Method Limitations]

1. This kit is an in vitro diagnostic reagent. The clinical evaluation of the test results should be combined with the patient's medical history and other clinical diagnosis results and should not be used as the sigle basis for clinical diagnosis and treatment.

2. The results are affected by the sample source, sample collection procedure, sample quality, sample transport conditions, sample pretreatment and other factors. Meanwhile, due to the subjective judgment, false positive or false negative results may be obtained, so users should aware of limitations such as inaccurate results caused by potential errors in the test process.

3. If the test results are inconsistent with the histopathological characteristics, the pathological diagnosis should be verified or retested.

4. The performance of the product are obtained based on the test procedure described in the specification. Changes to this procedure may change the results .

5. This kit is only suitable for detection of HER2 gene amplification in breast cancer patients by fluorescence in situ hybridization, not for detection of other gene mutation modes.

[Product Performance]

1. Intensity of fluorescence signal

After hybridization of the probe with peripheral blood cultured lymphocytes and breast/cancer tissue sections, fluorescence signals should be generated, and recognized by the naked eye under the fluorescence microscope.

2. Sensitivity

Choose 20 Tissue samples of invasive breast cancer/gastric cancer and 20 adjacent normal or benign tissue samples. Each sample is analyzed randomly. At least 20 cells should be detected in each sample, with 98% of the cells showing fluorescence signal both of HER2 locus marker and CEP17 locus marker.

3. Specificity

The peripheral blood lymphocytes of metaphase phase were used for smear analysis. Choose At least 5 smear samples of peripheral blood culture cells from healthy people, and 100 chromosomes 17 of 50 cells in metaphase phase were analyzed in each sample. At least 98 chromosomes showed their specific fluorescence signals in the centromere region (17p11.1-q11.1) and HER2 gene locus region (17q11.2-q12), respectively.

[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
	Batch code		In vitro diagnostic medical device
	Temperature limit		Manufacturer
	CE mark		Authorized representative in the European Community
	Avoid overexposure to the sun		Date of manufacture

[Basic Information]

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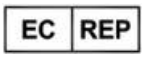

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[INSTRUCTION APPROVAL AND REVISION DATE]

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