primeFISH® *Her2/Neu (17q12)* LS DC Probe Kit

Product Code : 17-012
Spectrum : Red or Orange / Green
Storage : -20 °C
Ready to use : 10 tests
Content : 100 µl Probe and 100 µl DAPI

CE

DNA Probe

The ready-to-use HER2/Neu (17q12) DNA FISH Probe is used in formalin-fixed and paraffin-embedded (FFPE) tissues, in fresh solid tissues, in cultured tissues and in touch-material tissues. The HER2/Neu (17q12) DNA FISH probe, designed to detect the number of copies of the HER2/Neu (ERBB2) gene localized in the chromosome 17q12 region which includes the ERBB2 gene, and including the markers RH41770 on the centromere side of this gene and SHGC-130820 STS on the telomere side. The HER2/Neu (17q12) DNA FISH probe is marked in red. The kit's control probe is a DNA probe specific to the chromosome 17 centromere region (17p11.1-q11) and marked in green.

GENE	CHROMOSOME	BAND ZONE	DISEASE	COLOR	PROBE
ERBB2	Chr. 17	17q12	Breast Cancer	RED	833 kb
	Chr. 17	17p11.1-q11.1		GREEN	

Interpretation of signals

When the preparation is examined with an appropriate filter epifluorescent microscope, two red signals for normal HER2/Neu (ERBB2) genes and two green signals for normal centromere 17 are observed in both normal diploid interphase nuclei and metaphase chromosomes **(Image).**

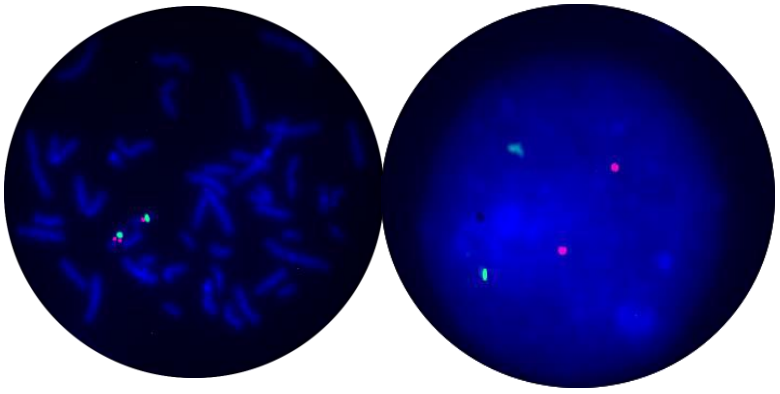

Image. Two red *Her2/Neu (ERBB2)* signals and two green chromosome 17 centomere signals in metaphase and interphase nuclei.

Depending on the amplification of the gene in the cells and the increase in the number of copies of the chromosomes, the number of ERBB2 (red) signals increases according to the number of control centromere 17 (green) signals. Accordingly, the number of copies of the HER2 gene and the number of copies of the chromosome 17 are deduced in order to separate the numerical anomaly and the increase in the number of HER2 gene copies from the amplification. In this context, a HER2 (Neu)/Cen17 ratio of 2.2 or greater is defined as amplification. Rates in the range of 1.8 to 2.2 are considered borderline **(Table).** Therefore, each laboratory must determine its own cut-off limit.

In addition, preparations obtained from paraffin blocks and solid tissues may affect the signal quality, and may lead to false positive results with cross-hybridizations and artifact signal formation. In this case, an optional FISH signal must be taken using optional preparation methods.

Table.

	Normal	Amplification
Expected Signals	**2R2G**	**R/G=2.2** or greater

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FISH Protocol

Kit content

The DNA FISH probe is prepared in 50 µl for 5 tests or 100 µl for 10 tests. Probes are mixed with hybridization buffer and ready to use. For each preparation, 10 µl of probe mix and 15 µl of DAPI should be used. The kit contents are given in the table below.

Tube	5 tests	10 tests	Color
Probe mix	50 µl	100 µl	Red Top
DAPI Counterstain	75 µl	150 µl	Blue Top

Storage conditions of the kit and preparations

The kit should be stored at -20°C and kept away from direct light. At the same time, the samples of the hybridized preparations should be kept at -20 oC. Both probe and hybridized preparations should not be frozen and thawed repeatedly.

Necessary Solutions *(Not provided with Kit)*

- 20xSSC
- 0.4xSSC
- 2xSSC-Tween20 (%0.05)
- İmmersiyon oil

Required devices and materials *(Not provided with Kit)*

- ✓Slide
- ✓Coverslip (22x22mm) / (24x24mm)
- ✓Pens
- ✓Epifluorescent microscope (necessary filters including)
- ✓Fume Hood
- ✓Microcentrifuge
- ✓pH meter
- ✓Chalet (At least 4 pcs)
- ✓ Water bath (2 units) or hot plate or hybridization oven
- ✓ Thermometer
- ✓ Micropipette set
- ✓ (10, 100, 200, 1000 µl)
- ✓ Micropipette tips
- ✓ (10, 100, 200, 1000 µl)
- ✓ hybridization vessel
- ✓ Microcentrifuge tubes

Cautions

- The kit should be used by persons trained to operate FISH.
- The kit should only be used with appropriate laboratory equipment.
- Preparations and cells and tissues fixed in the preparations must comply with FISH study quality standards.
- Improper storage and handling conditions quickly degrade the probe quality and cause test failure.
- Studies should be performed according to the kit's protocol.
- Laboratory coat and gloves should be used during the work.
- The contents of the kit should be avoided from direct skin contact.
- Kit waste should be disposed of in accordance with laboratory rules.
- Worked samples should be considered as potential sources of infection and necessary precautions should be taken for laboratory risk factors.
- Products which are expired should not be used.
- Repeated freezing and thawing as well as inappropriate storage conditions should be avoided..

FISH protocol

The FISH study basically consists of the steps of preparing a preparation from the samples, using the ready kit and interpreting the results. For a successful FISH analysis, the quality of the material to be examined and its preparation are as important as the quality of the probe

Preparation

Freshly prepared preparations for cytogenetic purposes are used. After the preparations are prepared for cytogenetic purposes, they are dried at room temperature for about 30 minutes. Then, under the light microscope, the region to be hybridized on the slide is marked with a pen on the glass, taking into account the cell frequency and distribution. Care should be taken to ensure that the preparation is free from tissue and cytoplasmic debris.

Probe application, denaturation ve hybridization

In the following steps, both the FISH probe and the hybridization preparations should be avoided from direct contact with light.

1. The DNA FISH probe is taken from -20 C and left for 3-5 minutes at room temperature to reduce the viscosity. It is then briefly vortexed and briefly centrifuged in a microcentrifuge.
2. 10 µl probe is taken with a micropipette and placed on the coverslip.
3. A coverslip with 10 µl probe is placed on the pre-prepared preparation with a designated hybridization zone.
4. Note: At this stage, care should be taken to avoid air bubbles and the direction of the slide.
5. Slides are placed on a metal tray and kept in a water bath brought to 65-70 °C for 6-8 minutes. Here, the probe and target DNA sequences are denatured together.
6. Note: When the denaturation is carried out in a humid environment such as a hotplate, oven, etc., the coverslips are covered with coverslip adhesive to prevent evaporation of the probe mixture.
7. After denaturation, the preparations are left to hybridize for 12-16 hours in a water bath prepared at 37-38 °C or in a moist container.

Washing and Counter stain

After completing the hybridization period, the following washing and staining steps are applied to the preparations and protected from light.

1. The adhesive around the coverslip in the preparations is gently lifted with a forceps and the coverslip is carefully removed.
2. The preparations are kept for 10-15 seconds in a chalet containing a preheated 0.4xSSC solution at 65-70°C.
3. The preparations are kept in 2xSSC-Tween20 (0.05%) at room temperature for 30-45 seconds. Afterwards, the preparations are allowed to dry in an upright position for 3-5 minutes at room temperature.
4. For each hybridization area, 22x22 or 24x24 mm clean, transparent coverslips are placed on a flat surface and 10 µl of counterstaining solution is added. The marked region of the slides applied into hybridization is placed on the counterstaining solution on the coverslip.

The preparations are wrapped in aluminum foil to protect them from light or placed in an opaque box. The preparations are kept at -20°C for 5-10 minutes before being analyzed.

Examination under the microscope

After removing the excess counterstaining solution from the preparation with a paper, the entire preparation is examined under an epifluorescent microscope with a 10x magnification objective and a DAPI filter. Then, according to the wavelength of the marked probe, green, red and bicolor (green-red) filters and the preparation are analyzed with a 100x objective.

Fluorescent microscope objectives and fluorescent filters should be compatible with the fluorescent dyes of the probes used.

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