ERBB2/Cen17 DNA-FISH Probe

Two Color, Enumeration Probe

REF 22-003

IVD CE

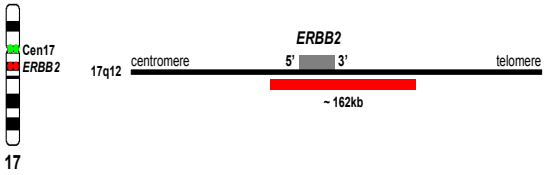
-20°C

ITALIA
Empowering Personalized Cancer Treatment

Instructions for Use

Intended use

The *ERBB2*/Cen17 DNA-FISH Probe is designed to detect the amplification of the *ERBB2* gene (also named *HER2/neu*) on chromosome 17q12 relative to the control Cen17 using fluorescence *in situ* hybridization (FISH) in formalin-fixed, paraffin-embedded (FFPE) breast cancer tissues. Overexpression of the *ERBB2* gene occurs in 25-30% of human breast carcinomas, and ~90-95% of these cases result directly from gene amplification.[1] Patients showing such an amplification are at high-risk for relapse and lower overall survival.[1-3] Amplification of the *ERBB2* gene predicts a favorable response to certain chemotherapy regimens and selective monoclonal antibody therapy with trastuzumab.[1-5] *ERBB2* amplification is also seen in other solid tumors such as gastric, esophageal, gynecologic, bladder, and non-small cell lung cancer and correlates with a poor prognosis.[6]



Schematic of the *ERBB2*/Cen17 DNA-FISH Probe:
The horizontal red bar indicates the region covered by the probe (approximate to scale, NCBI Build 36.1/Hg18/2006). The ideogram of chromosome 17 illustrates the respective locations of the hybridizations. The directly labeled Cen17 probe (green) hybridizes to the satellite DNA at 17p11.1-q11. The directly labeled *ERBB2* probe (red) spans the entire gene as indicated on the above schematic.

Storage

Storage of DNA-FISH Probe: Store at -20°C protected from light until the expiry date as indicated on the label.

Storage of Slides: Store hybridized slides at -20°C protected from direct light.

Note: The storage conditions apply to both opened and unopened products; vials stored under other conditions may not perform optimally and will affect the assay result. The number of freeze/thaw cycles should not exceed the recommended number of tests per vial. Store in original container.

Handling

» Handle all reagents as capable of transmitting infectious agents and dispose of according to current national law.

» Handle all reagents and slides containing fluorophores in reduced light to prevent photobleaching.

Warnings and Precautions

Please read instructions before proceeding.

» Improper transportation or storage can destroy or impair the performance of the product.

» If any of the following occurs: receipt of compromised package or vial, device failure (when used according to instructions for use), or injury of user, please contact the manufacturer.

» Any compromised vial should be discarded according to current national law and assays should not be performed from such reagent.

» Handle all reagents with care and wear appropriate personal protective equipment.

» Formamide, Saline-Sodium Citrate (SSC), and Sodium Dodecyl Sulfate (SDS) may have teratogenic and mutagenic effects: avoid inhalation, ingestion or contact with skin.

» DAPI is an irritant. See Material Safety Data Sheet (MSDS) for safety information.

Reagent provided

Ready-to-use DNA-FISH Probe: 100 µL per vial (10 tests); one test is defined as sufficient for a 22 x 22 mm area.

The DNA-FISH Probe is premixed in hybridization buffer (formamide, dextran sulphate, and SSC) and contains fluorophore-labeled probes for the *ERBB2* locus (red) and Cen17 locus (green).

Reagent, Material, and Equipment required but not provided

Equipment		Reagents
Coplin jars	Microfuge tube (0.5 mL)	100% Ethanol
Coverslip (22x22 mm & 25x25 mm)	Micropipette (1-200 µL)	10X PBS
	Parafilm	1N HCl
Epi-fluorescent microscope with appropriate filters	pH meter	1M $MgCl_2$
	Plus coated slides	1M NaOH
Forceps	Rubber Cement	1M NaSCN
Fume Hood	Slide tray	20X SSC
Gloves	Slide Warmer	10% Formalin
Humidified chamber	Thermometer, calibrated (37°C to 80°C)	Citrisolv™
Immersion oil		DAPI/Antifade
Incubator	Hot plate	Distilled Water
Mercury lamp (100 watt)	Water bath	Pepsin
Microcentrifuge		Tween 20

Citrisolv™ is a trademark of FisherBrand.

Reagent preparation

Note: Use distilled water for the preparation of all stock and working solutions.

Ethanol Series (70%, 85%, and 100%): Prepare v/v dilutions of 100% ethanol with distilled water (dH_2O). Store at RT.

0.01N HCl (Hydrochloric acid): Add 0.5 mL of 1N HCl to 49.5 mL (dH_2O) Store at RT. Pre-warm the solution to 37°C in a waterbath.

0.4% (4 mg/mL) Pepsin Stock Solution: Dissolve 100 mg of pepsin in 25 mL 0.2N HCl. Store 500 µL aliquots at -20°C.

1% Formaldehyde: Add 12.5 mL of 10% formalin (4% formaldehyde) to 37 mL of 1X PBS. Add 500 µL of 100X $MgCl_2$. Store up to 1 week at 4°C.

0.5X SSC (Saline-Sodium Citrate)/0.1% Tween 20: Add 25 mL of 20X SSC and 1 mL of Tween 20 to 974 mL dH_2O. Mix well by swirling. Store at RT.

1X PBS (Phosphate Buffer Saline): Mix 100 mL of 10X PBS to 900 mL dH_2O. Adjust pH to 7.0. Store at RT.

2X SSC: Mix 100 mL of 20X SSC and 900 mL of dH_2O. Adjust pH to 7.0. Store at room temperature (RT).

2X SSC/0.1% Tween 20: Add 100 mL of 20X SSC and 1 mL of Tween 20 to 899 mL dH_2O. Mix well by swirling. Store at RT.

100X $MgCl_2$ (Magnesium Chloride) in 1X PBS: Add 50 µL of 1M $MgCl_2$ to 450 µL of 1X PBS.

Formalin-Fixed, Paraffin-Embedded (FFPE) Procedure

Note: Products ready-to-use. Do not reconstitute or dilute with hybridization buffer. For professional use only.

» Only a technologist familiar with cytogenetic methods and trained for the FISH technique can perform the assay. All equipment should be calibrated prior to performance of the assay.

» The intended tissue is FFPE sections that are 4-5 µm in size. The slides should be prepared according to the guidelines for standard cytogenetic methods of the laboratory performing the assay.

Slide Preparation

Procedure Note: Sections should be prepared for staining with hematoxylin and eosin (H&E) in conjunction with sections prepared for FISH analysis.

1. Ensure that sections are 4-5 µm and mounted on positively charged slides.
2. Bake overnight (12-18 hours) on slide warmer at 55°C; use within 3 days.

Slide Pretreatment (to be performed in a fume hood)

1. Deparaffinize the slide in 3 changes of Citrisolv™ for 10 min each at RT.
   Procedure Note: Jars of Citrisolv™ may be used twice; however, the third jar should not contain previsouly used reagent.
2. Dehydrate the slide in two changes of 100% ethanol for 5 min each at RT, then allow to air dry.
   Procedure Note: The slide may remain dry at RT for several hours.
3. Incubate the slide in 0.2N HCl at RT for 20 min.
4. Rinse the slide in one change of dH_2O for 1 min at RT, then in two changes of 2X SSC for 5 min each at RT.
5. Incubate slide in prewarmed 1M NaSCN solution for 10 min at 80°C.
   Procedure Note: Certain tissue types, such as breast, require a longer incubation time (30-60 mins).
6. Rinse the slide in one change of dH_2O and two changes of 2X SSC for 5 min each at RT.
7. Place the slide in either a humid chamber or in a thermobrite. Cover the target area with at least 100 mL of 0.4% pepsin, maintain humidity and moisture conditions on the slide during incubation. Do not allow the specimen to dry out.
8. Incubate for 10 min at 37°C.
   Procedure Note: Depending on the fixation conditions and the age of the section, the time may need to be adjusted.
9. Rinse the slide in dH_2O for 5 min at RT.
10. Rinse the slide in two changes of 2X SSC for 5 min each at RT; dip briefly in dH_2O and air dry.
11. Incubate slide in 10% neutral-buffered formalin for 15 min at RT.
12. Rinse the slide two changes of 2X SSC for 5 min each at RT.
13. Dip briefly in dH_2O and air dry.

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FFPE Procedure (con't)

DNA-FISH Denaturation/Hybridization

1. Vortex DNA-FISH Probe briefly, then spin tube in a microcentrifuge.
2. Apply 10 µL of the DNA-FISH Probe to the target area and cover with a coverslip (22x22 mm).

   Procedure Note: Care should be taken to avoid air bubbles. Smaller or larger coverslips may be used with proportional change in DNA-FISH Probe volume.
3. Seal edges of coverslip thoroughly with rubber cement.
4. Co-denature the slide and the DNA-FISH Probe for 5 min at 90°C on a temperature controlled hot plate or on an automated hybridization device.
5. Incubate for 12-18 hours at 37°C in a humidified chamber protected from light.

Post Hybridization Wash

Procedure Note: Do not allow the slide to dry before washes are complete.

1. Remove rubber cement with forceps.
2. Remove coverslip by soaking in 2X SSC at RT.
3. Wash slide 2x 5 min in 2X SSC/0.1% Tween 20 at 45°C.
4. Briefly rinse slide in dH_2O and air dry out of direct light.
5. Apply 20 µL of DAPI/Antifade solution to the hybridized area and cover with a coverslip (25x25 mm).

   Procedure Note: Depending on fixation, age of the section, and the pretreatement conditions, a green background may be observed. If the green background is excessive or interferes with scoring, the slides can be re-washed at a higher stringency.

   Before re-washing, remove the Antifade by removing the coverslip and washing in two changes of 2X SSC/0.1% Tween 20 for 5 min each with agitation at RT; proceed immediately with re-washing, do not allow the slide to dry out.

   The stringency can be increased by an additional wash step of 2 x 5 min in 0.5X SSC/0.1% Tween 20 at 45°C; further increase in stringency may be obtained by increasing the wash time and/or temperature (up to 65°C).

Microscope accessories

» Objectives

A 10X objective is suitable for scanning the target area. Higher magnification is required for signal analysis and should be performed with a 63X or a 100X oil immersion objective.

» Immersion oil

The immersion oil should be suitable for fluorescence microscopy.

» Lamp

A 100 watt mercury lamp with a maximum life of 200 hours is recommended. Replace the lamp before it exceeds 200 hours.

» Filter Requirements

Fluorophore	Excitation $_{max}$	Emission $_{max}$
Green	496 nm	520 nm
Red	580 nm	603 nm
DAPI	360 nm	460 nm

Signal visualization and interpretation

The signal should be visualized with an epi-fluorescence microscope equipped with the appropriate filters.

Procedure Note: The signals can be at different focal plane so it is important to focus up and down on the specimen to ensure that all the signals are counted.

» In normal diploid metaphase chromosomes and interphase nuclei the probes generate two green and two red signals corresponding to the two normal homologous chromosomes 17.

» In cells with amplification or copy number increases, the number of red (*ERBB2*) signals is increased relative to the number of green (Cen17) signals.

» Amplification may also be present in the form of an hsr (homogenously staining region), observed as a brightly fluorescing mass of red signals. An *ERBB2*:Cen17 ratio with a value of 2.2 or more is defined as amplification; ratios with a value between 1.8 and 2.2 are considered borderline and should be subjected to discussion between the pathologist and the clinician.[6]

» An H & E stained slide should be prepared from a section adjacent to those used for FISH, and the region of invasive cancer must be determined by a qualified pathologist. Only tumor nuclei within invasive region should be scored – do not score inflammatory cells, muscle cells, fibroblasts or other stromal cells. Score a minimum of 20 nuclei within the invasive region, record the number of green and red signals seen in each nucleus.[2,4,6] Calculate the ratio of *ERBB2*/Cen17 signals by dividing the total number of *ERBB2* signals (red) by the total number of Cen17 signals (green).

» Non-continuous invasive regions may be identified by the pathologist, and these may all be scored and combined in the analysis. Amplification may also be present in the form of a homogenously staining region (hsr), observed as a brightly fluorescing mass of red signal. In recording this pattern, estimate the size/brightness of the signal mass as a multiple of the normal signal size, e.g. a mass ten times the normal signal size, plus a single normal red signal in the same cell, would be recorded as 11 reds.

» According to published guidelines, an *ERBB2*/Cen17 ratio above 2.2 is associated with an amplification of the *ERBB2* gene.[6] A ratio less than 1.8 is considered negative for *ERBB2* gene amplification. A ratio in the range 1.8-2.2 is considered borderline. In this case, 20 additional nuclei should be counted and the ratio calculated for 40 nuclei. If the doubt subsists, supplementary testing must be performed to define the *ERBB2* status and dialogue between the pathologist and the physician is warranted.[6]

Recommendations and limitations

» The *ERBB2* and Cen17 DNA-FISH Probe is not intended for the diagnosis of human breast cancer. The information gained from the FISH analysis must be interpreted in the full context of the patient's clinical history. A medical decision cannot be made based on the result of the FISH assay alone.

» This product has been optimized for use on slides prepared from FFPE tissue specimens. The manufacturer ensures that this product meets the analytical performance characteristics (sensitivity, specificity, reproducibility, and reportable range) established on the intended tissue.

» Normal cells within the specimen should be used as an internal control of the FISH assay. It is the responsibility of the laboratory to establish the reportable ranges using positive and negative control specimens of the intended tissue.

» Use of filters with spectral characteristics other than specified may adversely affect the strength of the signal. For example, the red fluorophore is visible through an orange filter, but the signals appear dim.

Symbol Glossary

LOT Batch Code

Biological risks

REF Catalogue number

Caution, consult accompanying documents

CE marking of conformity

Contains sufficient for 10 tests

IVD *In Vitro* Diagnostic medical device

Keep away from sunlight

Manufacturer

Upper limit of temperature

Use By

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DNA-FISH Probe manufactured in EU by Cancer Genetics Italia S.r.l.

References

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