



ZytoMation

ERBB2/CEN 17 Dual Color FISH Probe

REF Z-2292-5.1ML  Up to 20 (5.1 ml)

For the qualitative detection of human ERBB2 gene amplifications and alpha satellites of chromosome 17 by fluorescence *in situ* hybridization (FISH)



In vitro diagnostic medical device
according to EU directive 98/79/EC

1. Intended use

The ZytoMation ERBB2/CEN 17 Dual Color FISH Probe (PL246) is intended to be used for the qualitative detection of human ERBB2 gene amplifications as well as the detection of chromosome 17 alpha satellites in formalin-fixed, paraffin-embedded specimens such as human breast cancer or gastric cancer tissues by fluorescence *in situ* hybridization (FISH). The probe is intended to be used in combination with the Bond FISH Kit (DS9636) on the automated Bond-MAX or Bond-III system by Leica Biosystems.

Interpretation of the results must be made within the context of the patient's clinical history with respect to further clinical and pathologic data of the patient by a qualified pathologist.

2. Clinical relevance

The ERBB2 gene (a.k.a. HER2 and NEU) is located in the chromosomal region 17q12 and encodes a 185-190 kDa transmembrane glycoprotein, p185, acting as a cellular growth factor receptor. The p185 protein belongs to the EGFR (epidermal growth factor receptor) subgroup of the RTK (receptor tyrosine kinase) superfamily also including EGFR (ERBB1), ERBB3 (HER3), and ERBB4 (HER4). Amplification of the proto-oncogene ERBB2, observed in approximately 20% of all breast cancer samples, has been correlated with a poor prognosis of the disease. Similar results have been obtained for a variety of other malignant neoplasms, e.g., ovarian cancer, stomach cancer, and carcinomas of the salivary gland.

3. Test principle

The fluorescence *in situ* hybridization (FISH) technique allows for the detection and visualization of specific nucleic acid sequences in cell preparations. Fluorescently-labeled DNA fragments, so called FISH probes, and their complementary target DNA strands in the preparations are co-denatured and subsequently allowed to anneal during hybridization. Afterwards, unspecific and unbound probe fragments are removed by stringency washing steps. After counterstaining the DNA with DAPI, hybridized probe fragments are visualized using a fluorescence microscope equipped with excitation and emission filters specific for the fluorochromes with which the FISH probe fragments have been directly labeled.

4. Reagents provided

The ZytoMation ERBB2/CEN 17 Dual Color FISH Probe is composed of:

- ZyGreen (excitation 503 nm/emission 528 nm) labeled polynucleotides (~5.0 ng/μl), which target sequences mapping in 17q12-q21.1* (chr17:37,572,531-38,181,308) harboring the ERBB2 gene region (see Fig. 1).
- ZyOrange (excitation 547 nm/emission 572 nm) labeled polynucleotides (~0.2 ng/μl), which target sequences mapping in 17p11.1-q11.1 specific for the alpha satellite centromeric region D17Z1 of chromosome 17.
- Formamide based hybridization buffer

*according to Human Genome Assembly GRCh37/hg19

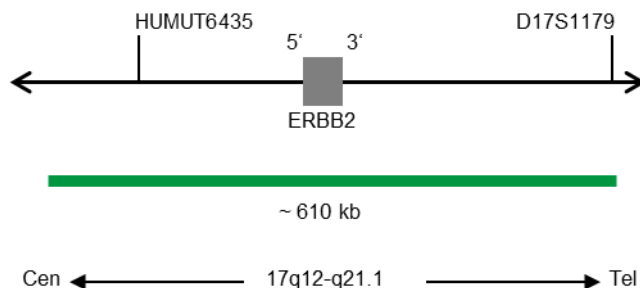


Fig. 1: ERBB2 Probe map (not to scale)

The ZytoMation ERBB2/CEN 17 Dual Color FISH Probe is available in one size:

- Z-2292-5.1ML: 5.1 ml (up to 20 reactions of 240 μl each)

5. Materials required but not provided

- Bond-MAX or Bond-III system by Leica Biosystems
- Bond FISH Kit (DS9636)
- Bond Epitope Retrieval Solution 2 (AR9640)
- Bond Enzyme Pretreatment Kit (AR9551)
- DAPI/DuraTect-Solution (MT-0007-0.8)
- Bond Universal Covertiles (S21.4611)
- Bond Titration Kit (OPT9049)
- Dewax Solution (AR9222)
- Bond Wash Solution 10X Concentrate (AR9590)
- Positive and negative control specimens
- Microscope slides, positively charged
- Adjustable pipettes (20 μl, 1000 μl)
- Staining jars or baths
- Timer
- Ethanol or reagent alcohol
- Deionized or distilled water
- Coverslips (24 mm x 50 mm)
- Adequately maintained fluorescence microscope (400-1000x)
- Immersion oil approved for fluorescence microscopy
- Appropriate filter sets

For further information on materials required but not provided please refer to the instructions for use of the respective fully automated staining system.

6. Storage and handling

Store at 2-8°C in an upright position protected from light. Use protected from light. Prior to opening the vial, shake down liquid. Return to storage conditions immediately after use. Do not use reagents beyond expiry date indicated on the label. The product is stable until expiry date indicated on the label when handled accordingly.

7. Warnings and precautions

- Read the instruction for use prior to use!
- Do not use the reagents after the expiry date has been reached!
- The probe should not be used in manual FISH procedures!
- This product contains substances (in low concentrations and volumes) that are harmful to health and potentially infectious. Avoid any direct contact with the reagents. Take appropriate protective measures (use disposable gloves, protective glasses, and lab garments)!
- If reagents come into contact with skin, rinse skin immediately with copious quantities of water!
- A material safety data sheet is available on request for the professional user.
- Do not reuse reagents.
- Avoid cross-contamination of samples as this may lead to erroneous results.
- The probe should not be exposed to light, especially strong light, for a longer period of time, i.e., all steps should be accomplished, where possible, in the dark and/or using lightproof containers!

Hazard and precautionary statements:

The hazard determining component is Formamide.



Danger

| | |
|-----------|--|
| H351 | Suspected of causing cancer. |
| H360FD | May damage fertility. May damage the unborn child. |
| H373 | May cause damage to organs through prolonged or repeated exposure. |
| P201 | Obtain special instructions before use. |
| P202 | Do not handle until all safety precautions have been read and understood. |
| P260 | Do not breathe dust/fume/gas/mist/vapours/spray. |
| P280 | Wear protective gloves/protective clothing/eye protection/face protection. |
| P308+P313 | IF exposed or concerned: Get medical advice/attention. |
| P405 | Store locked up. |

8. Limitations

- For *in vitro* diagnostic use.
- For professional use only.
- For use on the fully automated Bond-MAX or Bond-III system (Leica) only.
- The clinical interpretation of any positive staining, or its absence, must be done within the context of clinical history, morphology, other histopathological criteria as well as other diagnostic tests. It is the responsibility of a qualified pathologist to be familiar with the FISH probes, reagents, diagnostic panels, and methods used to produce the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
- Specimen staining, especially signal intensity and background staining, is dependent on the handling and processing of the specimen prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other specimens or fluids may produce artefacts or false results. Inconsistent results may result from variations in fixation and embedding methods, as well as from inherent irregularities within the specimen.

- The probe should be used only for detecting loci described in 4. "Reagents provided".
- The performance was validated using the fully automated Bond-MAX system (Leica) and the procedures described in this instruction for use. Modifications to these procedures might alter the performance as CE-IVD and have to be validated by the user. This IVD is only certified as CE when used as described in this instruction for use within the scope of the intended use.

9. Interfering substances

Red blood cells present in the specimen might exhibit autofluorescence which hinders signal recognition.

The following fixatives are incompatible with FISH:

- Bouin's fixative
- B5 fixative
- Acidic fixatives (e.g., picric acid)
- Zenker's fixative
- Alcohols (when used alone)
- Mercuric chloride
- Formaldehyde/zinc fixative
- Hollande's fixative
- Non-buffered formalin

10. Preparation of specimens

Recommendations:

- Fixation in 10% neutrally buffered formalin for 24 h at room temperature (18-25°C).
- Sample size ≤ 0.5 cm³.
- Use premium quality paraffin.
- Embedding should be carried out at temperatures lower than 65°C.
- Prepare 2-4 μm microtome sections.
- Use positively charged microscope slides.
- Fix for 2-16 h at 50-60°C.

11. Preparatory treatment of the device

No reconstitution, mixing, or dilution is required. Bring probe to room temperature (18-25°C) before use, protect from light. Prior to opening the vial, mix by vortexing and shake down briefly.

12. Assay procedure

The ZytoMation ERBB2/CEN 17 Dual Color FISH Probe is intended to be used on fully automated Bond-MAX or Bond-III system in combination with the respective FISH kits and FISH protocols. Please refer to the respective instructions for use of the system used for further information.

12.1 Slide Setup on the fully automated Bond-MAX or Bond-III system

Set the following protocol steps in the Slide Setup Menu:

| | |
|----------------|-------------------------------------|
| Staining: | *FISH Protocol D |
| Preparation: | *Dewax |
| HIER: | set up as described in step 1 below |
| Enzyme: | set up as described in step 2 below |
| Denaturation: | *Denaturation (10min) |
| Hybridization: | *ISH Hybridization (12Hr) |

Specimen pretreatment

Perform specimen pretreatment (dewaxing, proteolysis, HIER) according to the respective instructions for use of the fully automated staining system.

Depending on the specimen, adjustments to the protocol might be needed. A validation of protocols deviating from recommended protocols must be performed by the user.

1. Pretreat specimens with the Bond Epitope Retrieval Solution 2 for 25 min at 100°C.

For the HIER protocol, create a new protocol as described in the respective instructions for use of the automated Bond-MAX/Bond-III system. Select the protocol for protocol step "HIER" in Slide Setup.

2. Pretreat specimens with BOND Enzyme Dilution at 37°C.

For enzyme digestion, choose a protocol according to conditions pre-validated by the user depending on the specimen and the conditions for *HIER*, denaturation and hybridization. Select the protocol for protocol step "Enzyme" in Slide Setup.

Denaturation and hybridization

1. Set the denaturation of the specimens to 10 min at 95°C.

Select the predefined protocol "Denaturation (10min)" for protocol step "Denaturation" in Slide Setup.

2. Set the hybridization of the specimens to 12 h at 37°C.

Select the predefined protocol "ISH Hybridization (12Hr)" for protocol step "Hybridization" in Slide Setup.

12.2 Staining run

1. Load slides, FISH probe, enzyme dilution, and the BOND FISH Kit onto the system according to the instructions for use.
2. When the staining run is complete, remove the slides from the instrument. Protect slides from light.

12.3 Post-hybridization and detection

1. Dehydrate with 70%, 90%, and 100% ethanol each for 1 min.
2. Air dry samples in the dark.
3. Pipette 20 µl DAPI/DuraTect-Solution (MT7) onto the slides. Avoiding trapped bubbles, cover the samples with a coverslip (24 mm x 50 mm). Incubate in the dark for 15 min.

Using a pipette tip which has been cut off to increase the size of the opening, can make the pipetting process easier. Avoid long exposure to light.

4. Store the slide in the dark. For longer storage periods, this should take place at 2-8°C.
5. Evaluation of the sample material is carried out by fluorescence microscopy.

13. Interpretation of results

With the use of appropriate filter sets, the hybridization signals of the probe appear green (ERBB2 gene region) and orange (CEN 17).

Normal situation: In interphases of normal cells or cells without an amplification involving the ERBB2 gene region, two green signals and two orange signals appear (see Fig. 2).

Aberrant situation: In cells with an amplification of the ERBB2 gene region an increased number of green signals or green signal clusters will be observed (see Fig. 2).

Overlapping signals may appear as yellow signals.

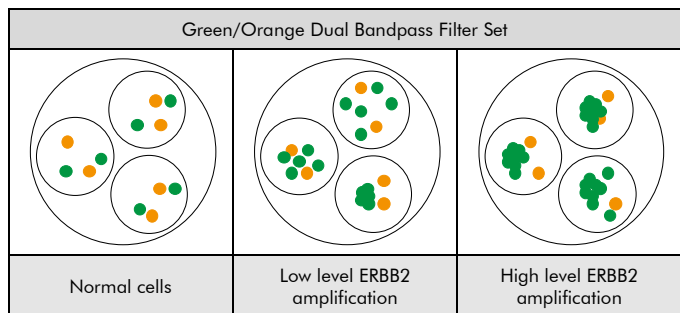


Fig. 2: Expected results in normal and aberrant interphase nuclei

Other signal distribution may be observed in some abnormal samples which might result in a different signal pattern than described above, indicating variant rearrangements. Unexpected signal patterns should be further investigated.

Please note:

- Due to decondensed chromatin, single FISH signals can appear as small signal clusters. Thus, two or three signals of the same size, separated by a distance ≤ 1 signal diameter, should be counted as one signal.
- Do not evaluate overlapping nuclei.

- Do not count over-digested nuclei (recognized by dark areas visible inside of the nuclei).
- Do not count nuclei with strong auto-fluorescence, which hinders signal recognition.
- A negative or unspecific result can be caused by multiple factors (see chapter 17).
- In order to correctly interpret the results, the user must validate this product prior to use in diagnostic procedures according to national and/or international guidelines.

14. Recommended quality control procedures

In order to monitor correct performance of processed specimens and test reagents, each assay should be accompanied by internal and external controls. If internal and/or external controls fail to demonstrate appropriate staining, results with patient specimens must be considered invalid.

Internal control: Non-neoplastic cells within the specimen that exhibit normal signal pattern, e.g., fibroblasts.

External control: Validated positive and negative control specimens.

15. Performance characteristics

The performance of the probe was determined by comparison against the corresponding IVD approved FISH probe. The concordance was 100%.

Accuracy: The accuracy was calculated as 100%.

Analytical sensitivity: The analytical sensitivity was calculated as 100%.

Analytical specificity: The analytical specificity was calculated as 100%.

16. Disposal

The disposal of reagents must be carried out in accordance with local regulations.

17. Troubleshooting

Any deviation from the operating instructions can lead to inferior staining results or to no staining at all.

Weak signals or no signals at all

| Possible cause | Action |
|---|--|
| No target sequences available | Use appropriate controls |
| Cell or tissue sample not fixed properly | Optimize fixing time and fixative |
| Proteolytic pretreatment not carried out properly | Optimize enzyme concentration and incubation time, increase or decrease if necessary |
| Old dehydration solutions | Prepare fresh dehydration solutions |
| Fluorescence microscope adjusted wrongly | Adjust correctly |
| Inappropriate filter sets used | Use filter sets appropriate for the fluochromes of the probe. <i>Triple-bandpass filter sets provide less light compared to single or dual-bandpass filter sets. Consequently, the signals may appear fainter using these triple-bandpass filter sets</i> |
| Photo-damage of the probes/fluorophores | Accomplish hybridization and washing steps in the dark |

Cross hybridization signals; noisy background

| Possible cause | Action |
|-------------------------------------|--|
| Proteolytic pretreatment too strong | Reduce enzyme concentration or incubation time |

Morphology degraded

| Possible cause | Action |
|---|---|
| Cell or tissue sample not fixed properly | Optimize fixing time and fixative |
| Proteolytic pretreatment not carried out properly | Optimize enzyme concentration or incubation time, decrease if necessary |
| Heat pretreatment not carried out properly | Optimize heat pretreatment |

Overlapping nuclei

| Possible cause | Action |
|--|--|
| Inappropriate thickness of tissue sections | Prepare 2-4 μm microtome sections |

Specimen floats off the slide

| Possible cause | Action |
|---|---|
| Unsuitable slide coating | Use appropriate slides |
| Insufficient drying of the tissue section | Adjust the time to dry the tissues sufficiently before staining |
| Fixation in formalin that was not properly neutral buffered | Use appropriate neutral buffered formalin of high quality |

Weak counterstain

| Possible cause | Action |
|--------------------------------|---|
| Low concentrated DAPI solution | Use <u>DAPI/DuraTect-Solution (ultra)</u> (Prod. No. MT-0008-0.8) instead |
| DAPI incubation time too short | Adjust DAPI incubation time |

18. Literature

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Our experts are available to answer your questions.
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