ZytoLight

SPEC ERBB2/CEN 17 Dual Color Probe Kit

REF Z-2020-5 Σ 5

REF Z-2020-20 Σ 20

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

4250380N447S

CE 0124

IVD

In vitro diagnostic medical device

according to IVDR (EU) 2017/746

1. Intended purpose

The ZytoLight SPEC ERBB2/CEN 17 Dual Color Probe Kit is intended to be used for the qualitative detection of amplifications involving the human ERBB2 gene as well as the detection of chromosome 17 alpha satellites in formalin-fixed, paraffin-embedded specimens, such as breast cancer and gastric/gastroesophageal junction cancer, by fluorescence in situ hybridization (FISH).

The product is intended for professional use only. All tests using the product should be performed in a certified, licensed anatomic pathology laboratory under the supervision of a pathologist/human geneticist by qualified personnel.

The probe is intended to be used as an aid to the differential diagnosis of breast cancer and gastric/gastroesophageal junction cancer and therapeutic measures should not be initiated based on the test result alone.

2. 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.

3. Reagents provided

The ZytoLight SPEC ERBB2/CEN 17 Dual Color Probe Kit is available in two sizes and is composed of:

Code	Component	Quantity 5 Σ 20		Container
PT1	Heat Pretreatment Solution Citric	150 ml	500 ml	Screw-cap bottle (large)
ES1	Pepsin Solution	1 ml	4 ml	Dropper bottle, white cap
WB1	Wash Buffer SSC	210 ml	560 ml	Screw-cap bottle (large)
PL8	ZytoLight SPEC ERBB2/CEN 17 Dual Color Probe	0.05 ml	0.2 ml	Reaction vessel, red lid
WB2	25x Wash Buffer A	50 ml	2x50 ml	Screw-cap bottle (medium)
MT7	DAPI/DuraTect-Solution	0.2 ml	0.8 ml	Reaction vessel, blue lid
	Instructions for use	1	1	

Z-2020-5 (5 tests): Components **ES1**, **PL8,** and **MT7** are sufficient for 5 reactions. Component **WB2** is sufficient for 5x 3 staining jars of 70 ml each. Component **PT1** is sufficient for 2 staining jars of 70 ml each. Component **WB1** is sufficient for 3 staining jars of 70 ml each.

Z-2020-20 (20 tests): Components **ES1**, **PL8,** and **MT7** are sufficient for 20 reactions. Component **WB2** is sufficient for 11x 3 staining jars of 70 ml each. Component **PT1** is sufficient for 7 staining jars of 70 ml each. Component **WB1** is sufficient for 8 staining jars of 70 ml each.

The ZytoLight SPEC ERBB2/CEN 17 Dual Color Probe **(PL8)** is composed of:

- ZyGreen (excitation 503 nm/emission 528 nm) labeled polynucleotides (~10.0 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 (~1.5 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

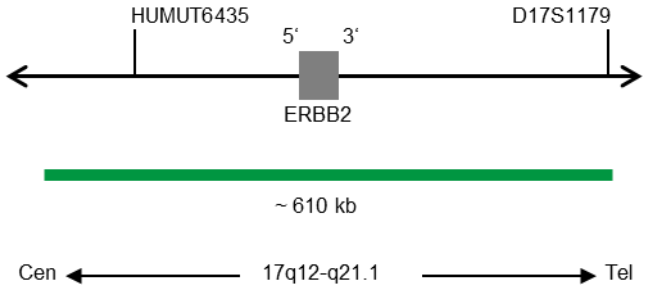

Fig. 1: SPEC ERBB2 Probe map (not to scale)

4. Materials required but not provided

- Positive and negative control specimens
- Microscope slides, positively charged
- Water bath (37 °C, 98 °C)
- Hybridizer or hot plate
- Hybridizer or humidity chamber in hybridization oven
- Adjustable pipettes (10 µl, 25 µl)
- Staining jars or baths
- Timer
- Calibrated thermometer
- Ethanol or reagent alcohol
- Xylene

- Deionized or distilled water
- Coverslips (22 mm x 22 mm, 24 mm x 60 mm)
- Rubber cement, e.g., Fixogum Rubber Cement (Prod. No.E-4005-50/-125) or similar
- Adequately maintained fluorescence microscope (400-1000x)
- Immersion oil approved for fluorescence microscopy
- Appropriate filter sets

5. Storage and handling

Store at 2-8 °C in an upright position protected from light. Use protected from light. 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.

6. Warnings and precautions

- Read the instructions for use prior to use!
- Do not use the reagents after the expiry date has been reached!
- 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)!
- Report any serious incident that has occurred in relation to the product to the manufacturer and the competent authority according to local regulations!
- If reagents come into contact with skin, rinse skin immediately with copious amounts of water!
- A material safety data sheet is available on request for the professional user.
- Do not reuse reagents, unless reuse is explicitly permitted!
- Avoid cross-contamination of samples as this may lead to erroneous results.
- **PL8** and **MT7** 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 for PL8:

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.

Special labelling of ES1:

EUH208	Contains Pepsin A. May produce an allergic reaction.
EUH210	Safety data sheet available on request.

Hazard and precautionary statements for PT1, WB1, and WB2:

The hazard determining component is a reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1).

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	IF skin irritation or rash occurs: Get medical advice/attention.
P362+P364	Take off contaminated clothing and wash it before reuse.

Hazards and precautionary statements for MT7:

This product is not classified as hazardous according to Regulation (EC) No. 1272/2008.

7. Limitations

- For *in vitro* diagnostic use.
- For professional use only.
- For non-automated use 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/human geneticist 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/human geneticist 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 chapter 3. "Reagents provided".
- The performance was validated using the procedures described in these instructions for use. Modifications to these procedures might alter the performance 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.

8. 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

9. Preparation of specimens

Recommendations:

- Fixation in 10% neutrally buffered formalin for 24 h at room temperature (18-25°C).
- Sample size ≤ 0.5 cm^3.
- 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.

10. Preparatory treatment of the device

25x Wash Buffer **(WB2)** is to be pretreated according to the instructions in 11.2 "Assay procedure - Day 2". All other kit reagents are ready-to-use. 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 spin down briefly.

11. Assay procedure

11.1 Day 1

Preparatory steps

(1) *Prepare two ethanol series (70%, 90%, and 100% ethanol solutions):* Dilute 100% ethanol with deionized or distilled water. These solutions can be stored in suitable containers and can be re-used.
(2) *Heat Pretreatment Solution Citric* **(PT1)**: Warm to 98°C.
(3) *Wash Buffer SSC* **(WB1)**: Bring to room temperature (RT). **WB1** may form precipitates at 2-8°C, which do not affect the quality and should dissolve when heated.
(4) *ZytoLight FISH Probe*: Bring to RT before use, protect from light.

Optional, when performing post-fixation step:
(strongly recommended if tissue fixation is not optimal)
Prepare a 1% Formaldehyde solution using the Formaldehyde Dilution Buffer Set (PT-0006-100)

Pretreatment (dewax/proteolysis)

(1) Incubate slides for 10 min at 70°C (e.g., on a hot plate).
(2) Incubate slides for 2x 10 min in xylene.
(3) Incubate in 100%, 100%, 90%, and 70% ethanol, each for 5 min.
(4) Wash 2x 2 min in deionized or distilled water.
(5) Incubate for 15 min in pre-warmed Heat Pretreatment Solution Citric **(PT1)** at 98°C.

We recommend not to use more than eight slides per staining jar.

(6) Transfer slides immediately to deionized or distilled water, wash for 2x 2 min and drain off or blot off the water.
(7) Apply (dropwise) Pepsin Solution **(ES1)** to the specimens and incubate for 15 min at 37°C in a humidity chamber.

ES1 *may form precipitates, which do not affect the quality.*

Depending on multiple factors, e.g. nature and duration of fixing, thickness of sections, and nature of tissue/cells, different incubation times may be required. As an incubation guideline, we recommend an incubation time of 2-30 min for tissue samples and 2-15 min for cell samples. As a general rule, we recommend to ascertain the optimum time for proteolysis in pre-tests.

(8) Wash for 5 min in Wash Buffer SSC **(WB1)**.

Optional, when performing post-fixation step:
Incubate slides for 15 min in 1% Formaldehyde solution and wash subsequently for 5 min in Wash Buffer SSC (WB1)

(9) Wash for 1 min in deionized or distilled water
(10) Dehydration: in 70%, 90%, and 100% ethanol, each for 1 min
(11) Air dry sections.

Note: Make sure to completely dry sections prior to probe application since residual moisture may reduce signal intensity and/or affect tissue morphology.

Denaturation and hybridization

(1) Pipette 10 µl of the ZytoLight SPEC ERBB2/CEN 17 Dual Color Probe **(PL8)** onto each pretreated specimen.

Avoid long exposure of the probe to light.

(2) Cover specimens with a 22 mm x 22 mm coverslip (avoid trapped bubbles) and seal the coverslip.

We recommend using rubber cement (e.g., Fixogum Rubber Cement) for sealing.

(3) Place slides on a hot plate or hybridizer and denature specimens for 10 min at 75°C.
(4) Transfer the slides to a humidity chamber and hybridize overnight at 37°C (e.g., in a hybridization oven).

It is essential that the tissue/cell samples do not dry out during the hybridization step.

11.2 Day 2

Preparatory steps

(1) *Preparation of 1x Wash Buffer A*: Dilute 1 part 25x Wash Buffer A **(WB2)** with 24 parts deionized or distilled water. Fill three staining jars with the 1x Wash Buffer A and pre-warm it to 37°C.

Diluted 1x Wash Buffer A is stable for one week when stored at 2-8°C.

(2) *DAPI/DuraTect-Solution* **(MT7)**: Bring to room temperature before use, protect from light.

Post-hybridization and detection

(1) Carefully remove the rubber cement or glue.
(2) Remove the coverslip by submerging in 1x Wash Buffer A at 37°C for 1-3 min.
(3) Wash using 1x Wash Buffer A for 2x 5 min at 37°C.

The 1x Wash Buffer A should be pre-warmed. Check with a thermometer if necessary.

(4) Incubate the slides in 70%, 90%, and 100% ethanol, each for 1 min.
(5) Air dry the samples protected from light.
(6) Pipette 25 µl DAPI/DuraTect-Solution **(MT7)** onto the slides. Avoiding trapped bubbles, cover the samples with a coverslip (24 mm x 60 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.

(7) Store the slide in the dark. For longer storage periods, this should take place at 2-8°C.
(8) Evaluation of the sample material is carried out by fluorescence microscopy. Filter sets for the following wavelength ranges are required:

Fluorescent dye	Excitation	Emission
ZyGreen	503 nm	528 nm
ZyOrange	547 nm	572 nm

12. 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.

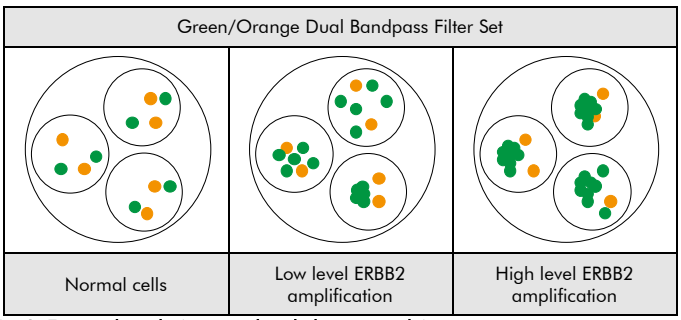

Fig. 2: Expected results in normal and aberrant nuclei

Other signal patterns than those described above may be observed in some abnormal samples. These 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 16 "Troubleshooting").
- 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.

13. 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.

14. Performance characteristics

14.1 Analytical performance

Analytical sensitivity:	100% (95% CI 98.5 – 100.0)
Analytical specificity:	100% (95% CI 97.0 – 100.0)

14.2 Clinical performance

Diagnostic sensitivity:	**Breast cancer**: 93% (95% CI 91.0 – 95.0) based on a bivariate model **Gastric cancer and gastroesophageal junction cancer:** 88% (95% CI 74.0 – 95.0) based on a bivariate model
Diagnostic specificity:	**Breast cancer:** 98% (95% CI 97.0 – 99.0) based on a bivariate model **Gastric cancer and gastroesophageal junction cancer**: 95% (95% CI 92.0 – 97.0) based on a bivariate model

15. Disposal

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

16. Troubleshooting

Any deviation from the operating instructions can lead to inferior staining results or to no staining at all. Please refer to www.zytovision.com for more information.

Weak signals or no signals at all

Possible cause	Action
Cell or tissue sample not fixed properly	Optimize fixing time and fixative or apply a post-fixation step as described in "assay procedure"
Proteolytic pretreatment not carried out properly	Optimize pepsin incubation time, increase or decrease if necessary
Probe evaporation	When using a hybridizer, the use of the wet stripes/water filled tanks is mandatory. When using a hybridization oven, the use of a humidity chamber is required. In addition, the coverslip should be sealed completely, e.g., with Fixogum, to prevent drying-out of the sample during hybridization
Inappropriate filter sets used	Use filter sets appropriate for the fluochromes of the probe. *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*

Cross hybridization signals; noisy background

Possible cause	Action
Incomplete dewaxing	Use fresh solutions; check duration of dewaxing
Proteolytic pretreatment too strong	Reduce pepsin incubation time
Slides cooled to room temperature before hybridization	Transfer the slides quickly to 37 °C

Morphology degraded

Possible cause	Action
Cell or tissue sample has not been fixed properly	Optimize fixing time and fixative or apply a post-fixation step as described in "assay procedure"
Proteolytic pretreatment not carried out properly	Optimize pepsin incubation time, decrease if necessary
Insufficient drying before probe application	Extend air-drying

Overlapping nuclei

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

Specimen floats off the slide

Possible cause	Action
Proteolytic pretreatment too strong	Reduce pepsin incubation time

Weak counterstain

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

17. Literature

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18. Revision

www.zytovision.com

Please refer to www.zytovision.com for the most recent instructions for use as well as for instructions for use in different languages.

Our experts are available to answer your questions.
Please contact helptech@zytovision.com
For the summary of safety and performance, please refer to www.zytovision.com.

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