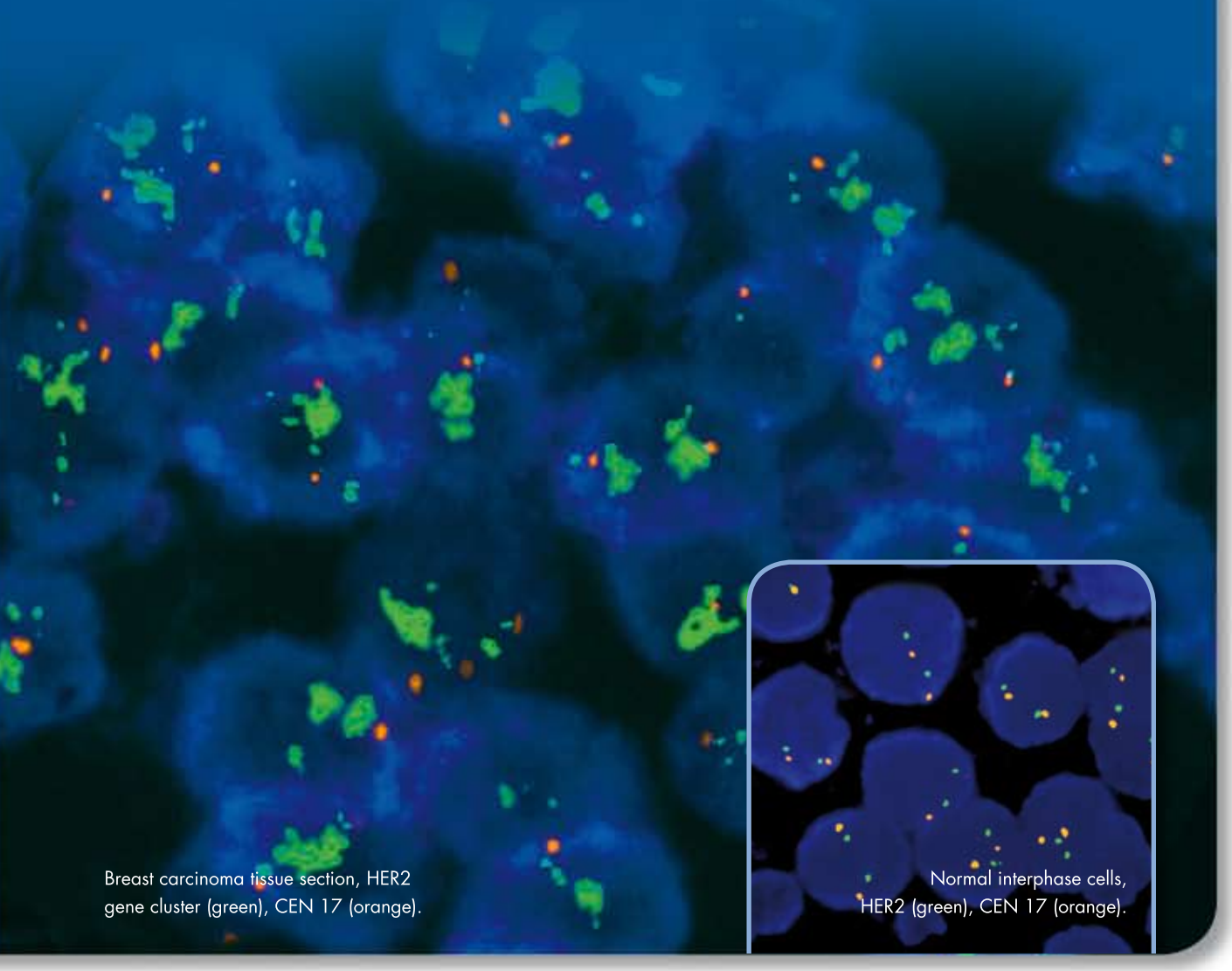
ZytoVision

Molecular diagnostics simplified

ZytoLight® SPEC HER2 / CEN 17 Dual Color Probe

Breast Cancer Interpretation Guide



Breast carcinoma tissue section, HER2 gene cluster (green), CEN 17 (orange).

Normal interphase cells, HER2 (green), CEN 17 (orange).

Basis of Interpretation Guidelines

This Interpretation Guide is based on ASCO (American Society of Clinical Oncology) and CAP (College of American Pathologists) recommendations for *HER2* testing in breast cancer (Wolff A C, *et al.* 2007).
It does not claim to be complete in reference to clinical usage and appraisal of results. Moreover it should be regarded as a practical help of HER2 FISH testing to determine and evaluate the obtained FISH signals.

ZytoLight® SPEC HER2/CEN 17 Dual Color Probe Kit

The ZytoLight® SPEC HER2/CEN 17 Dual Color Kit contains all neccessary reagents to perform user-friendly and successful FISH experiments.

- Heat Pretreatment Solution Citric
- Pepsin Solution
- Wash Buffer SSC
- ZytoLight® SPEC HER2/CEN 17 Dual Color Probe
- 25x Wash Buffer A
- DAPI/Antifade-Solution

Filter Set Recommendations

- DAPI Single Bandpass Filter Set
- FITC/Rhodamine Dual Bandpass Filter Set
- FITC Single Bandpass Filter Set
- Rhodamine Single Bandpass Filter Set

Target	Fluorochrome	Excitation	Emission
HER2	● ZyGreen™	503 nm	528 nm
CEN 17	● ZyOrange™	547 nm	572 nm

ZytoLight® Method Description

This is a condensed protocol and should not replace the manual included in each product.

Dewax

Heat slides for 10 min at 70°C, incubate 2x 10 min in xylene and rehydrate through graded alcohols to dH_2O.
Note: It is important to change used xylene regularly to obtain completely dewaxed tissue.

Heat Pretreatment

Incubate slides for 15 min in 98°C Heat Pretreatment Solution Citric, then wash 2x in dH_2O.
Note: We recommend not to use more than six slides per staining jar.

Proteolysis

Apply Pepsin Solution for approx. 10 min, incubate 5 min in Wash Buffer SSC, then wash in dH_2O.
Note: Depending on multiple factors, e.g. nature and duration of fixing, thickness of sections, and nature of tissue/cells, different pepsin incubation times may be required.

Apply Probe

Dehydrate through graded alcohols and air dry slides. Apply 10 µl of probe on the tissue, cover with coverslip, and seal with rubber cement.
Note: 10 µl of probe per 22x22 mm are recommended. A gentle warming of the probe can make the pipetting process easier.

Denature & Hybridize

Co-denature probe and specimen DNA for 10 min at 75°C on a hot plate. Hybridize over night at 37°C.
Note: It may be necessary to optimize the denaturing temperature (73-77°C).

Wash

Remove coverslip by incubation in Wash Buffer A. Wash 2x 5 min in Wash Buffer A prewarmed to 37°C, dehydrate through graded alcohols and air dry samples protected from light.

Stain & Cover

Apply 30 µl DAPI/Antifade and cover with coverslip. Incubate 15 min protected from light.
Note: Using a pipette tip which has been cut off to increase the size of the opening to pipette the DAPI/Antifade solution, can make the pipetting process easier.

Evaluation

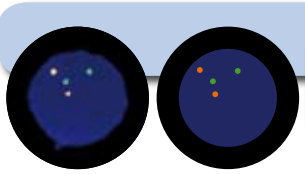
Evaluate slides using a fluorescence microscope equipped with a 100 watt mercury lamp and adequate filter sets.
Note: Slides can be stored in the dark at 2-8°C for up to approx. 2 weeks.

Prod. No.	Product	Label	Tests (Volume)*
Z-2015-50	ZytoLight SPEC HER2/CEN 17 Dual Color Probe CE IVD	●/●	5 (50 µl)
Z-2015-200	ZytoLight SPEC HER2/CEN 17 Dual Color Probe CE IVD	●/●	20 (200 µl)
Z-2020-5	ZytoLight SPEC HER2/CEN 17 Dual Color Probe Kit CE IVD	●/●	5
Z-2020-20	ZytoLight SPEC HER2/CEN 17 Dual Color Probe Kit CE IVD	●/●	20

** Using 10 µl probe solution per test. CE IVD only available in certain countries. All other countries research use only! Please contact your local dealer for more information.*

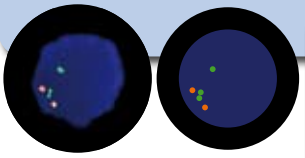
Count & Score

- Count at least 20 cells which are located in two different regions of the invasive component of the carcinoma.
- These regions must be confirmed by a pathologist.
- The area for counting should include clearly distinguishable and good distributed nuclei.
- Tissue artefacts like tissue at the boundary or retracted or squeezed tissue should be excluded from counting.



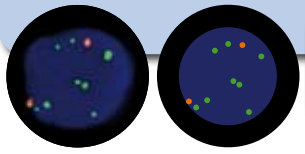
- Count: 2 green and 2 orange signals
- HER2/CEN 17 ratio = 1.0
- HER2 gene copy number = 2.0

Normal / Non-amplified



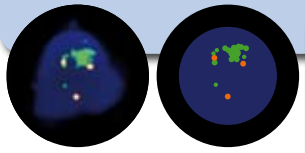
- Count: 2 green and 2 orange signals
  One orange signal is split but 2 signals of the same color separated by a distance of ≤ 1 signal diameter, are counted as one.
- HER2/CEN 17 = 1.0
- HER2 gene copy number = 2.0

Normal / Non-amplified



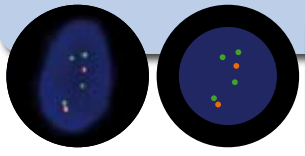
- Count: 7 green and 2 orange signals
- HER2/CEN 17 ratio = 3.5
- HER2 gene copy number = 7.0

Positive / Amplification



- Count: 12 green and 3 orange signals
  Number of green signals have to be estimated because of signal cluster formation.
- HER2/CEN 17 ratio = 4.0
- HER2 gene copy number = 12.0

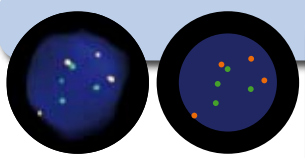
Positive / Amplification



- Count: 4 green and 2 orange signals
- HER2/CEN 17 ratio = 2.0
- HER2 gene copy number = 4.0

Equivocal

Sample is subjected to increased counting and/or repeated.



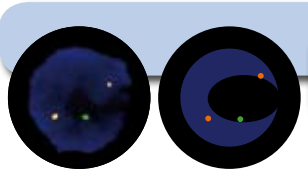
- Count: 4 green and 4 orange signals
- HER2/CEN 17 ratio = 1.0
- HER2 gene copy number = 4.0

Polysomy

Polysomic cases should be retested by strictly standardised IHC.

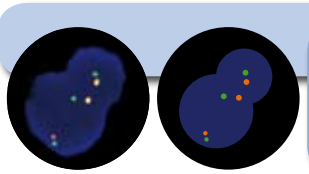
Reject & Repeat

- Do not count if controls are not as expected.
- If >25% of signals are weak the test cannot be scored.
- The test should be repeated if >10% of signals occur over cytoplasm.
- Reject the object if it shows strong autofluorescence.



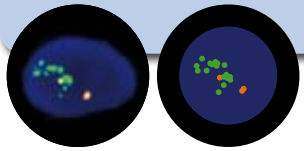
- Over-digestion can be recognized by dark areas visible inside of the nuclei.

Over-digested nuclei - Do not count!



- Nuclei are overlapping. Not all areas of the single nuclei are visible. Exact determination of signal per nucleus is hindered.

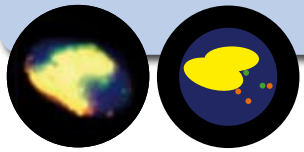
Overlapping nuclei - Do not count!



- Green signals overlapping orange signals.

Signal cluster overlapping signal

Check signals in single bandpass filter or do not count!



- Strong autofluorescence hindered signal recognition.

Autofluorescence - Do not count!

Breast Cancer *HER2 Evaluation Sheet*

ZYTOVISION
Molecular diagnostics simplified

Run Log ID: _____ Date of Service: _____

Specimen ID: _____ LOT _____

ZytoLight® SPEC HER2/CEN 17 Dual Color Probe/-Kit

Specimen Information

Fixative Type: _____ Time to Fixation (if available): _____

Duration of Fixation (if available): _____

Adequate Number of Invasive Tumor Cells present: Yes ☐ No ☐ ➡ Reject and Repeat

Signal Counting

Count only Cells of Invasive Tumor Component!

Nucleus	HER2 score (green)	CEN 17 score (orange)
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
Total		

Increased Counting (if necessary)!

Nucleus	HER2 score (green)	CEN 17 score (orange)
Total		

Results

Interpretation of: ☐ HER2/CEN 17 Ratio

(Total score HER2): _____

(Total score CEN 17): _____

= ☐

HER2 status:

Negative ☐ Ratio <1.8

Equivocal ☐ Ratio 1.8 - 2.2

Positive/Amplified ☐ Ratio ≥ 2.2

or

☐ Average HER2 gene copy number

(Total score HER2): _____

(Number of Counted Nuclei): _____

= ☐

HER2 status:

Negative ☐ Copy number <4.0

Equivocal ☐ Copy number 4.0 - 6.0

Positive/Amplified ☐ Copy number ≥ 6.0

Date and signature, Technican: _____

Date and signature, Pathologist: _____

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