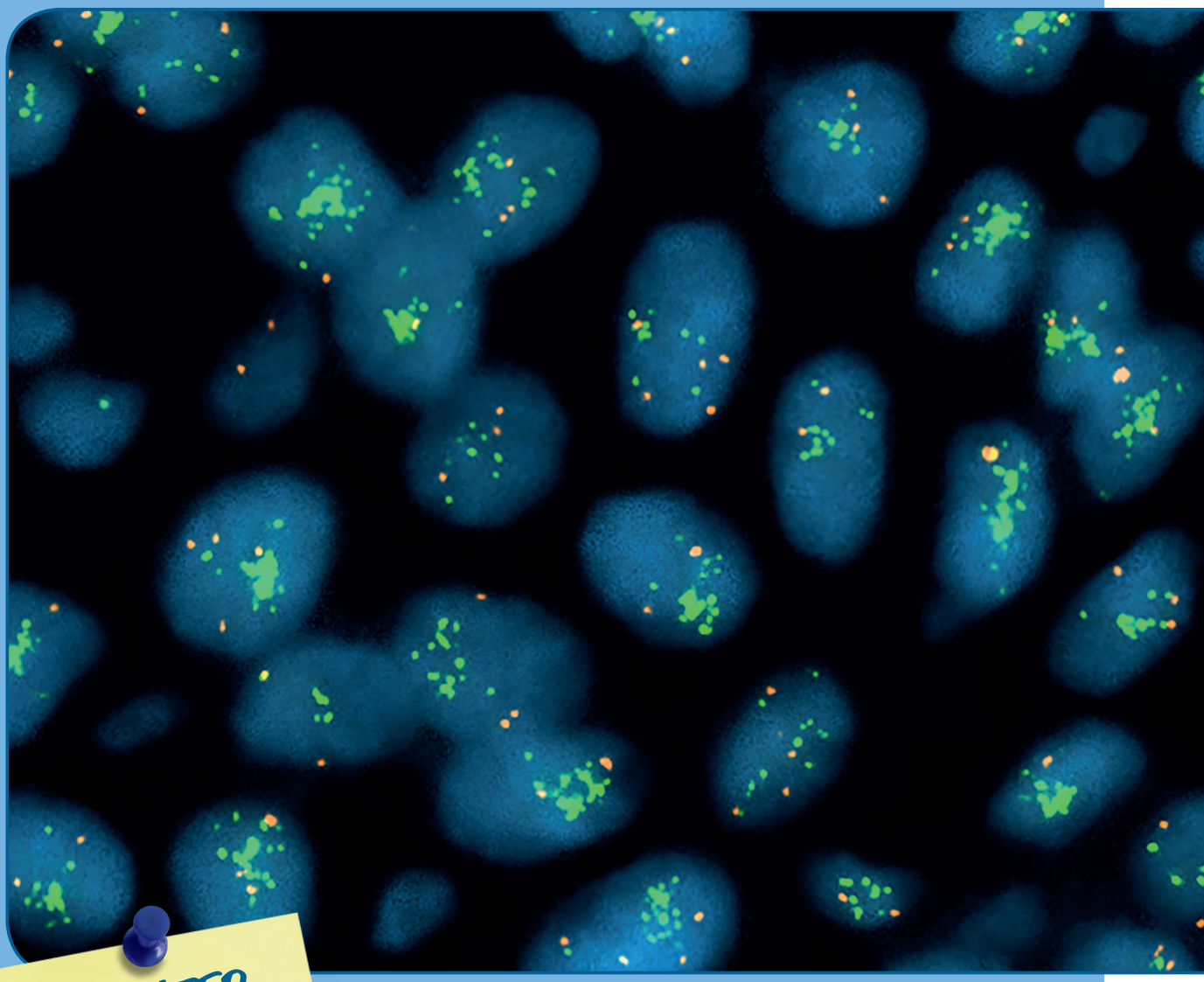
CE IVD

ZytoLight®

Products for FISH analysis

Breast Cancer Interpretation Guide



UPDATED

According to the
NEW ASCO/CAP
Guidelines 2018

ZYTOVISION

Molecular diagnostics simplified

Breast Cancer Interpretation Guide

ERBB2 (a.k.a. HER2) testing must be requested on every primary invasive breast cancer. Additionally, it is recommended to perform ERBB2 testing on metastatic sites, classified as stage IV, if tissue sample is available. This is to guide decision to pursue ERBB2-targeted therapy.
This Interpretation Guide is based on ASCO (American Society of Clinical Oncology) and CAP (College of American Pathologists) recommendations for ERBB2 testing in breast cancer (Wolff AC, et al. 2018)*.
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 ERBB2 FISH evaluation in regards to clinical decision making.

What's New?

- Revised definition of IHC 2+ (equivocal) cases as invasive breast cancer with „weak to moderate complete membrane staining observed in > 10% of tumor cells".
- If the initial ERBB2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new ERBB2 test on a surgical specimen is no longer stated as mandatory.
- Less common FISH patterns should be reviewed by IHC using the same tissue sample:
  1) ERBB2/CEN 17 ratio of ≥ 2.0 with an average ERBB2 gene copy number of < 4 signals/nucleus (FISH group 2)
  2) ERBB2/CEN 17 ratio of < 2.0 with an average ERBB2 gene copy number of ≥ 6 signals/nucleus (FISH group 3)
  3) ERBB2/CEN 17 ratio of < 2.0 with an average ERBB2 gene copy number of ≥ 4 signals/nucleus (FISH group 4)

IHC results:
a) IHC result is 3+: ERBB2 positive
b) IHC result is 2+: Recount at least 20 cells by FISH in the IHC 2+ staining area by an additional observer. New FISH category or ERBB2 diagnosis is negative (FISH group 2+4)/positive (FISH group 3)
c) IHC result is 0 or 1+: ERBB2 negative

- The concomitant IHC review for ISH group 2 to 4 is recommended to be performed in the same institution to ensure parallel interpretation and quality of the two assays.
- The usage of a dual color ISH probe is recommended by the Expert Panel instead of using a single color ISH assay. Concomitant IHC review becomes part of the interpretation of single color ISH results.

Evaluation Procedure

1. Localize the invasive component of a breast cancer specimen on a corresponding H&E or IHC slide.
2. Screen the entire slide prior to FISH signal counting for areas of aggregate population of ERBB2 amplified cells.
3. The area for counting should include clearly distinguishable and good distributed nuclei.
4. Count at least 20 non-overlapping cells in two separate areas of a population of tumor cells in the invasive component of the carcinoma (at least 10 cells per area). If there is a second population of tumor cells with increased ERBB2 signals per cell comprising > 10% of tumor cells on the slide, perform a separate counting of at least 20 cells within this cell population.
5. Determine the ERBB2 status according to the ERBB2 Interpretation Guide.
6. A second person should count an additional 20 non-overlapping cells if ERBB2/CEN 17 ratio is 1.8 - 2.2 and ERBB2 copy number is < 6.0.
7. Report if ERBB2 status is indeterminate due to e.g. artifacts, analytic testing failure, etc. or if ERBB2 status is discordant with other histopathologic findings and repeat test with another specimen.

The validation of FISH probes is required for each type of tissue that is intended to be tested in clinical practice since different tissue types exhibit different cell types with different nuclei diameters which may result in different cut off values. 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.

* Wolff AC, et al. (2018) J Clin Oncol 36: 2105-2122

ERBB2 Interpretation Guide

Invasive component of breast cancer specimen

ERBB2 testing by validated IHC assay

- IHC 3+ positive
- IHC 2+ equivocal
- IHC 1+ negative
- IHC 0 negative

IHC 2+ equivocal:
- New specimen (if available): New IHC Test
- New specimen (if available): FISH Test

IHC 1+ negative:
- Same specimen (Reflex ERBB2 test): FISH Test

Interpretation of ERBB2/CEN 17 ratio
Total score ERBB2: ____
Total score CEN 17: ____ = ____

ERBB2/CEN 17 ratio ≥ 2.0

Interpretation of average ERBB2 copy number
Total score ERBB2: ____
Number of counted nuclei: ____ = ____

- FISH group 1) Average ERBB2 copy number ≥ 4.0 → FISH positive
- FISH group 2) Average ERBB2 copy number < 4.0 → IHC assessment

ERBB2/CEN 17 ratio < 2.0

Interpretation of average ERBB2 copy number
Total score ERBB2: ____
Number of counted nuclei: ____ = ____

- FISH group 3) Average ERBB2 copy number ≥ 6.0 → IHC assessment
- FISH group 4) Average ERBB2 copy number ≥ 4.0 and < 6.0 → IHC assessment
- FISH group 5) Average ERBB2 copy number < 4.0 → FISH negative

IHC assessment

Assess IHC using sections from the same tissue sample used for FISH

- IHC 0 or 1+ → ERBB2 negative*
- IHC 2+ → Observer blinded to previous results recounts FISH, counting at least 20 cells
- IHC 3+ → ERBB2 positive

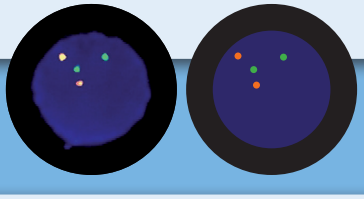
Observer blinded to previous results recounts FISH, counting at least 20 cells

- Same FISH result (FISH group 2+4) → ERBB2 negative*
- Same FISH result (FISH group 3) → ERBB2 positive
- Other FISH result/category → Final category should be adjudicated per internal procedures

* Evidence is limited on the efficacy of ERBB2-targeted therapy

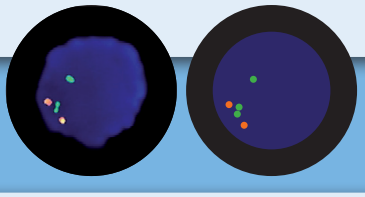
Signal Interpretation Guide

ERBB2 non-amplified cell



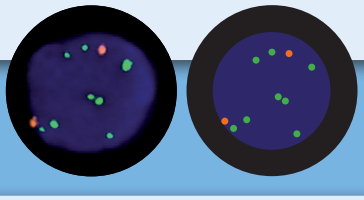
- Count: 2 green and 2 orange signals.

ERBB2 non-amplified cell



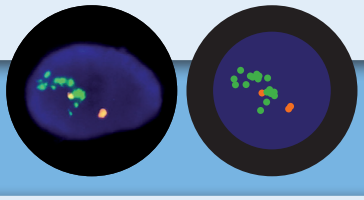
- Count: 2 green and 2 orange signals
  One green signal is split but 2 signals of the same color separated by a distance of ≤ 1 signal diameter, are counted as one.

Cell with low level amplification of ERBB2



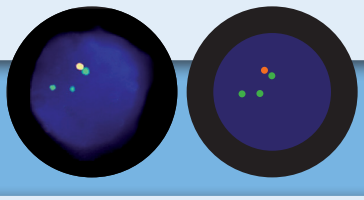
- Count: 7 green and 2 orange signals.

Cell with high level amplification of ERBB2



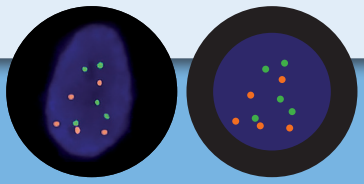
- Green signals overlapping orange signals.
  Signal cluster overlapping signal
  Check signals in single bandpass filter.

Cell with monosomy of chromosome 17



- Count: 3 green signals and 1 orange signal.

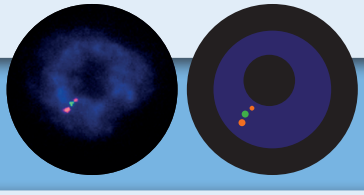
Cell with polysomy of chromosome 17



- Count: 5 green and 5 orange signals.

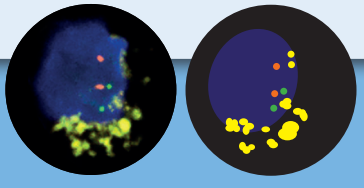
- Artifacts (crush or edge artifacts) that make interpretation difficult should be excluded from counting.
- Do not count if controls are not as expected.
- If >25% of signals are weak the test cannot be scored.
- The test should be repeated if >10% of signals occur over cytoplasm.

Over-digested cell



- Over-digestion can be recognized by dark areas visible inside of the nuclei.
  Over-digested nuclei - Do not count!

Cell with autofluorescence



- Strong autofluorescence hinders signal recognition.
  Autofluorescence - Do not count!

ZytoLight® Products for FISH analysis

ZytoLight® SPEC ERBB2/CEN 17 Dual Color Probe Kit

The ZytoLight® SPEC ERBB2/CEN 17 Dual Color Probe Kit contains all necessary reagents to perform user-friendly and successful FISH experiments.

- Heat Pretreatment Solution Citric
- Pepsin Solution
- Wash Buffer SSC
- ZytoLight® SPEC ERBB2/CEN 17 Dual Color Probe
- 25x Wash Buffer A
- DAPI/DuraTect™-Solution

Prod. No.	Product	Label	Tests* (Volume)
Z-2015-50	ZytoLight SPEC ERBB2/CEN 17 Dual Color Probe CE IVD	●/●	5 (50 µl)
Z-2015-200	ZytoLight SPEC ERBB2/CEN 17 Dual Color Probe CE IVD	●/●	20 (200 µl)
Z-2190-50	ZytoLight SPEC ERBB2/D17S122 Dual Color Probe CE IVD	●/●	5 (50 µl)
Z-2190-200	ZytoLight SPEC ERBB2/D17S122 Dual Color Probe CE IVD	●/●	20 (200 µl)
Z-2020-5	ZytoLight SPEC ERBB2/CEN 17 Dual Color Probe Kit CE IVD Incl. Heat Pretreatment Solution Citric, 150 ml; Pepsin Solution, 1 ml; Wash Buffer SSC, 210 ml; Probe, 0.05 ml; 25x Wash Buffer A, 50 ml; DAPI/DuraTect-Solution, 0.2 ml	●/●	5
Z-2020-20	ZytoLight SPEC ERBB2/CEN 17 Dual Color Probe Kit CE IVD Incl. Heat Pretreatment Solution Citric, 500 ml; Pepsin Solution, 4 ml; Wash Buffer SSC, 560 ml; Probe, 0.2 ml; 25x Wash Buffer A, 100 ml; DAPI/DuraTect-Solution, 0.8 ml	●/●	20
Related Products			
Z-2028-5	ZytoLight FISH-Tissue Implementation Kit Incl. Heat Pretreatment Solution Citric, 150 ml; Pepsin Solution, 1 ml; Wash Buffer SSC, 210 ml; 25x Wash Buffer A, 50 ml; DAPI/DuraTect-Solution, 0.2 ml		5

** 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.*

ZytoLight® Fluorochromes

Two factors that mainly influence FISH analyses:
- **Fluorochromes of the FISH probes**
- **Appropriate filter sets**

Fluorochrome	Excitation	Emission	Equivalent to
● ZyGreen™	503 nm	528 nm	FITC
● ZyOrange™	547 nm	572 nm	Rhodamine

Recommended Filter Sets

All filter sets have a superior signal-to-noise ratio and need to be assembled in fluorescence filter holders specific for the respective microscope. Please contact info@zytovision.com for more information.

Prod. No	Product	Detected Fluorochrome
E-4030-1	DAPI Single Bandpass Filter Set v2	DAPI
E-4012-1	ZyGreen™ Single Bandpass Filter Set v2	●
E-4013-1	ZyOrange™ Single Bandpass Filter Set v2	●
E-4016-1	ZyGreen™/ZyOrange™ Dual Bandpass Filter Set v2	●/●
E-4010-1	DAPI/ZyGreen™/ZyOrange™ Triple Bandpass Filter Set	DAPI/●/●

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