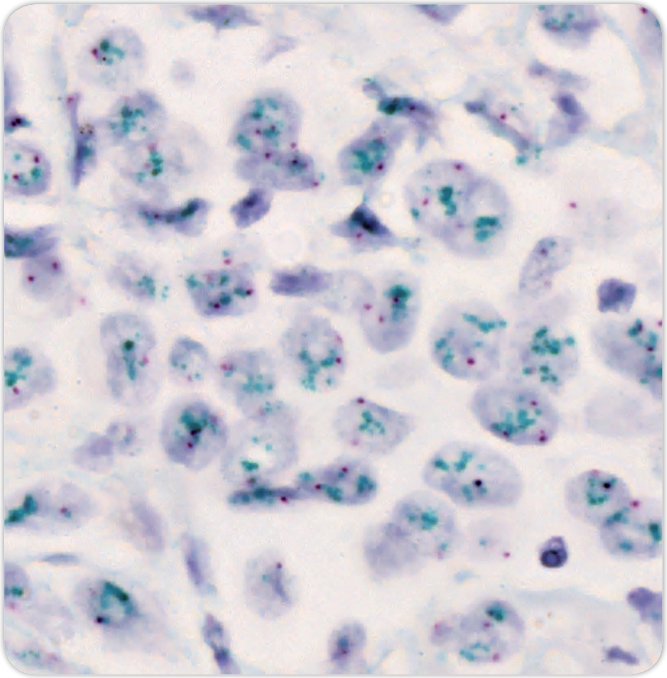
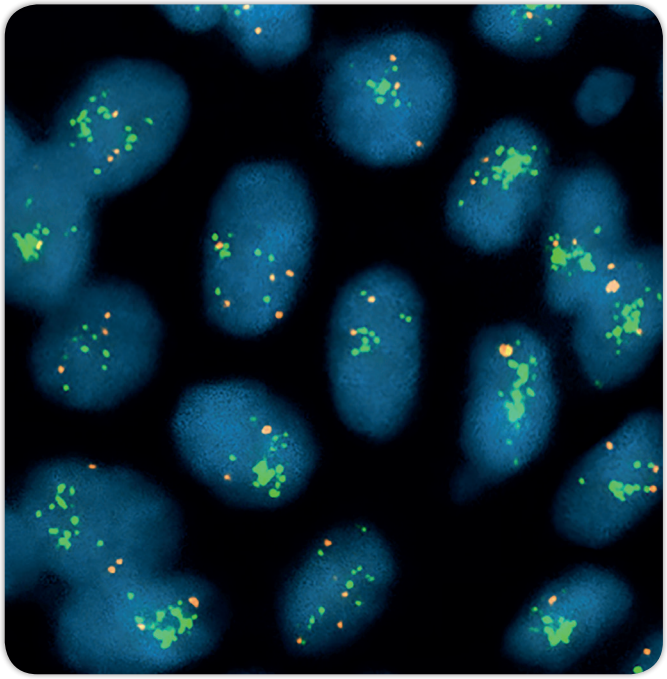
ZYTONEWS

Detection of ERBB2 Gene Amplification

Results of the Nordic Immunohistochemical Quality Control (NordiQC) - ERBB2 Assessment Run H13 2018



ZytoDot® 2C SPEC ERBB2/CEN 17 Probe



ZytoLight® SPEC ERBB2/CEN 17 Dual Color Probe

NordiQC ERBB2 ISH · www.nordiqc.org

Dear Readers,

With this new issue of our **ZYTONEWS** we would like to summarize the results of the ERBB2 assessment run H13 2018 of the international organization **Nordic Immunohistochemical Quality Control (NordiQC)**. NordiQC is an independent, non-profit scheme for external quality assurance (EQA) established in Denmark in 2003 that evaluates the inter-laboratory consistency of IHC and ERBB2 ISH, focusing mainly on the analytical part.

Enjoy reading, Yours ZYTONEWS TEAM

ZYTOVISION
Molecular diagnostics simplified

Aim of the ERBB2 Quality Testing Scheme

NordiQC is a scientific organization, whose aim it is to promote the quality of molecular testing methods and to expand their clinical use by arranging immunohistochemical (IHC) and *in situ* Hybridization (ISH) proficiency testing schemes.

NordiQC offers the **breast cancer ERBB2 ISH module** which comprises two annual runs with CISH and FISH tests for ERBB2 to be detected in formalin-fixed paraffin-embedded (FFPE) tissues. Because of the central role of ERBB2 testing in therapy selection for breast cancer, standardization of ERBB2 ISH assays and slide interpretation are of utmost clinical importance.

Assessment Method – ERBB2 ISH Scheme

- Each participating laboratory receives twice a year two unhybridized slides of an FFPE multi-tissue block comprising 5 breast carcinoma specimens for ERBB2 analysis by CISH and/or FISH using the standard protocol of the laboratory.
- All hybridized slides returned, the submitted protocols and the completed scoring sheets are assessed by a board of experienced pathologists and technicians in a blinded fashion. Each staining is evaluated by consensus as optimal, good, borderline, or poor.
- General staining results and results of the data analysis are published online. Individual assessment scores are communicated directly to the participating laboratories.

ERBB2 CISH and FISH Interpretation

Each participating laboratory had to submit a scoring sheet with their interpretation of the ERBB2/CEN 17 signal ratio. These evaluations were compared to the ERBB2 FISH results obtained by the NordiQC reference laboratories.

The **FISH reference laboratory used the Zyto*Light*® SPEC ERBB2/CEN 17 Dual Color Probe** for ERBB2 testing. For the interpretation of the ERBB2 status, the **ASCO/CAP 2013 guidelines** were applied:

Unamplified: ERBB2/CEN 17 ratio < 2.0 using a dual probe assay with an average of < 4 ERBB2 gene copies per nucleus (dual and single probe assay).

Equivocal: ERBB2/CEN 17 ratio < 2.0 using a dual probe assay with an average of ≥ 4 and < 6 ERBB2 gene copies per nucleus (dual and single probe assay).

Amplified: ERBB2/CEN 17 ratio ≥ 2.0 using a dual probe assay or an average of ≥ 4 ERBB2 gene copies per nucleus. Using a single probe assay an average of ≥ 6 ERBB2 gene copies per nucleus.

Results

Part I – Technical Assessment of CISH ERBB2 Results

Optimal demonstration and evaluation of the ERBB2 gene amplification status could be obtained in all four cores of the multi-tissue block by all the applied dual color and single color systems.
One core was excluded from the assessment due to technical problems.
When optimal protocol settings were applied, 71% and 100% of the submitted slides stained with the **ZytoDot® 2C SPEC ERBB2/CEN 17 Probe** and the **ZytoDot® SPEC ERBB2 Probe**, respectively, achieved a sufficient mark (optimal or good).

Tab. 1: Assessment of the ERBB2 CISH systems.

Dual color ERBB2 systems	No. of Labs	Optimal[1]	Good[2]	Borderline[3]	Poor[4]	Suff. OPS[5]
INFORM HER2 Dual ISH (Ventana/Roche)	93	32	28	24	9	69%
INFORM HER2 Dual ISH + IHC (Ventana/Roche)	17	14	3	0	0	100%
ZytoDot® 2C SPEC ERBB2/ CEN 17 Probe (ZytoVision)	**8**	**4**	**1**	**3**	**0**	**71%**
Single color ERBB2 systems						
INFORM HER2 DNA Probe (Ventana/Roche)	6	1	4	1	0	–
ZytoDot® SPEC ERBB2 Probe (ZytoVision)	**3**	**3**	**0**	**0**	**0**	**100%**

[1] ***Optimal staining:*** *Signals and ERBB2/CEN 17 signal ratios could be evaluated in all four tissues.*
[2] ***Good staining:*** *The ERBB2/CEN 17 signal ratios could be evaluated in all tissues, but the interpretation was slightly compromised e.g. due to weak or excessive counterstaining.*
[3] ***Borderline staining:*** *One of the tissues could not be evaluated properly e.g. due to weak signals, large negative areas with no signals or excessive background staining.*
[4] ***Poor staining:*** *Two or more tissues could not be evaluated properly e.g. due to weak signals, large negative areas with no signals or excessive background staining.*
[5] *Proportion of sufficient stains with optimal protocol settings only.*

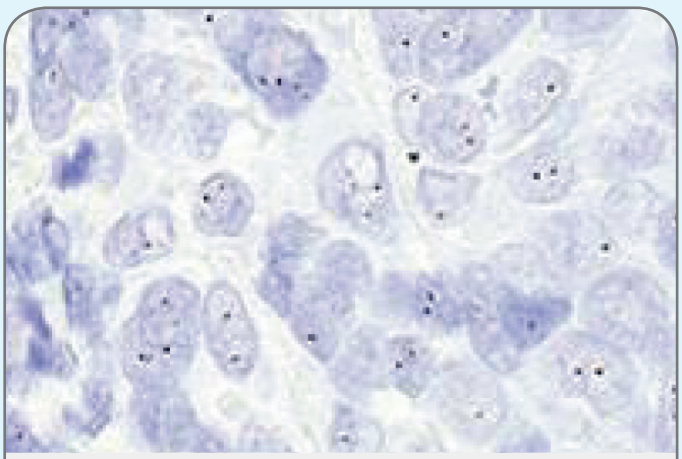


Fig. 1: Example image of an optimal CISH result using the ZytoDot® SPEC ERBB2 Probe on a breast carcinoma without ERBB2 gene amplification.

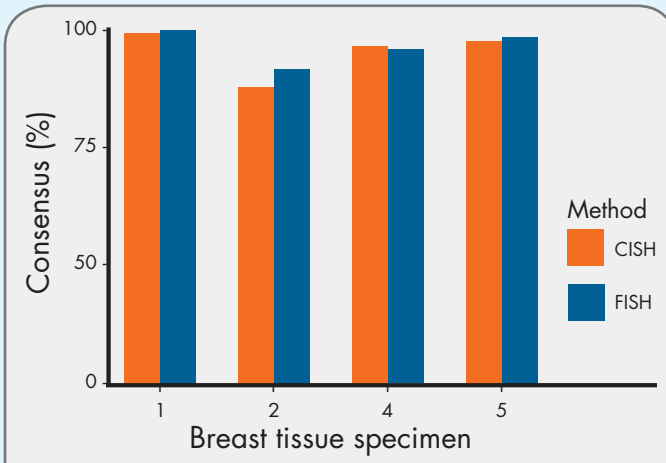


Fig. 2: Consensus rates of CISH and FISH results for each tissue core compared to the NordiQC reference data.
Core 3 was excluded due to suboptimal tissue quality.

Part II – Assessment of FISH ERBB2 Results

For the laboratories performing FISH, the consensus rate was 85% (47 of 55).
All participating laboratories using the **ZytoLight® SPEC ERBB2/CEN 17 Dual Color Probe** showed **100% concordance** with the NordiQC reference laboratory.

Tab. 2: Applied FISH assays and consensus rates compared to NordiQC reference data.

FISH Assay	No. of Laboratories	Consensus Rate
PathVysion HER-2 DNA Probe Kit II (Abbott)	17	88% (15/17)
ZytoLight® SPEC ERBB2/CEN 17 Dual Color Probe (ZytoVision)	**11**	**100% (11/11)**
HER2 IQFISH pharmDx™ (Dako/Agilent)	11	73% (8/11)
HER2 FISH System (Leica)	4	100% (4/4)
Other	12	75% (9/12)

Part III – Consensus Rates between CISH/FISH and NordiQC

In general, for both CISH and FISH, high consensus rates were observed between participating laboratories and the NordiQC reference data regarding the ERBB2 amplification status in all cores (Fig. 2). Laboratories performing FISH achieved a slightly higher (85%) consensus rate for the interpretation of ERBB2 amplification status compared to laboratories performing CISH (82%).

Conclusion

- The **ZytoLight® SPEC ERBB2/CEN 17 Dual Color Probe** was used for ERBB2 FISH testing in one of the NordiQC reference laboratories.
- Optimal ERBB2 CISH results could be obtained by using the dual color assay **ZytoDot® 2C SPEC ERBB2/CEN 17 Probe** and the single color assay **ZytoDot® SPEC ERBB2 Probe**.
- All participating FISH laboratories using the **ZytoLight® SPEC ERBB2/CEN 17 Dual Color Probe** reached a 100% consensus rate.
- Both methods (CISH and FISH) led to comparable results.

Product Information

ZytoDot® Products for CISH analysis

Prod. No.	Product	Label	Tests* (Volume)
C-3001-400	Zyto*Dot* SPEC ERBB2 Probe CE IVD	DIG	40 (400 µl)
C-3003-40	Zyto*Dot* SPEC ERBB2 Probe Kit CE IVD	DIG	40
	Incl. Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; Probe, 0.4 ml; Wash Buffer SSC, 560 ml; PBS/Tween, good for 2000 ml; Blocking Solution, 4 ml; Mouse-anti-DIG, 4 ml; Anti-Mouse-HRP-Polymer, 4 ml; DAB Solution A, 0.3 ml; DAB Solution B, 10 ml; Mayer's Hematoxylin Solution, 20 ml; Mounting Solution (alcoholic), 4 ml		

** 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.*

Background

The Zyto*Dot*® SPEC ERBB2 Probe is designed for the detection of ERBB2 gene amplification, frequently observed in solid malignant neoplasms, in formalin-fixed, paraffin-embedded tissue sections or cell samples.

The ERBB2 gene (a.k.a. HER2 and NEU) is located in the chromosomal region 17q12 and encodes the cellular growth factor receptor p185.

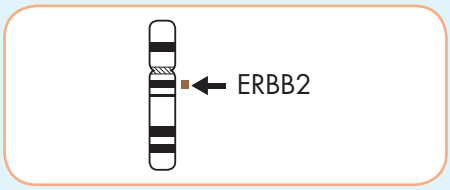
Amplification of the proto-oncogene ERBB2, observed in approximately 20% of all breast cancer samples, has been correlated with a poor prognosis of the disease. Similar results have been obtained for a variety of other malignant neoplasms e.g. ovarian cancer, stomach cancer, and carcinomas of the salivary gland.

References

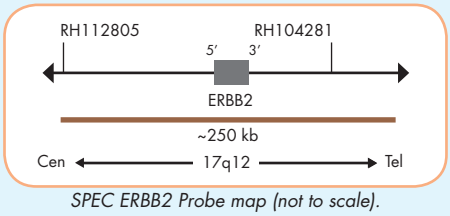
Baselga J, et al. (1999) Semin Oncol 26: 78-83.
Brockhoff G, et al. (2016) Histopathology 69: 635-46.
Brunello E, et al. (2012) Histopathology 60: 482-8.
Brunner K, et al. (2010) Anal Quant Cytol Histol 32: 78-89.
Coussens L, et al. (1985) Science 230: 1132-9.
Ettl T, et al. (2012) Br J Cancer 106: 719-26.
Hwang CC, et al. (2011) Histopathology 59: 984-92.
Hynes NE & Stern DF (1994) Biochim Biophys Acta 1198: 165-84.
Moelans CB, et al. (2011) Crit Rev Oncol Hematol 80: 380-92.
Park JB, et al. (1989) Cancer Res 49: 6605-9.
Popescu NC, et al. (1989) Genomics 4: 362-6.
Sassen A, et al. (2008) Breast Cancer Res 10: R2.
Slamon DJ, et al. (1987) Science 235: 177-82.
Voutsas IF, et al. (2013) Int J Radiat Biol 89: 319-25.
Wolff AC, et al. (2018) J Clin Oncol 14: 437-41.

Probe Description

The Zyto*Dot*® SPEC ERBB2 Probe is a Digoxigenin-labeled probe specific for the ERBB2 gene at 17q12, processed by the unique *ZytoVision® Repeat Subtraction Technique* resulting in advanced specificity and less background.



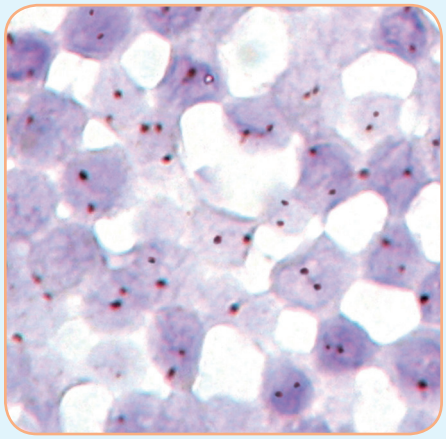
Ideogram of chromosome 17 indicating the hybridization locations.



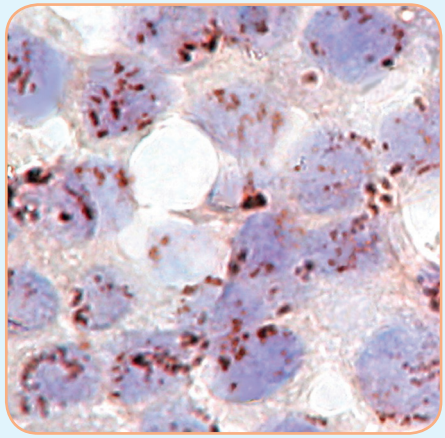
SPEC ERBB2 Probe map (not to scale).

Results

In normal cells, two distinct dot-shaped signals per nucleus will be observed. Nuclei with amplification of the ERBB2 gene locus or polysomy of chromosome 17 will show multiple dots or large signal clusters.



Normal nuclei each with two ERBB2 signals.



Breast carcinoma tissue section with ERBB2 amplification.

CE IVD

Product Information

ZytoDot® 2C Products for CISH analysis

FASTER • IMPROVED SIGNALS • LESS HANDS ON TIME • 2.0 version

Prod. No.	Product	Label	Tests* (Volume)
C-3032-100	ZytoDot 2C SPEC ERBB2/CEN 17 Probe CE IVD	DIG/DNP	10 (100 µl)
C-3032-400	ZytoDot 2C SPEC ERBB2/CEN 17 Probe CE IVD	DIG/DNP	40 (400 µl)
C-3022-10	ZytoDot 2C SPEC ERBB2/CEN 17 Probe Kit CE IVD Incl. Heat Pretreatment Solution EDTA, 150 ml; Pepsin Solution, 1 ml; Probe, 0.1 ml; Wash Buffer SSC, 210 ml; 20x Wash Buffer TBS, 50 ml; Anti-DIG/DNP-Mix, 1 ml; HRP/AP-Polymer-Mix,1 ml; AP-Red Solution A, 0.1 ml; AP-Red Solution B, 4 ml; HRP-Green Solution A, 0.2 ml; HRP-Green Solution B, 4 ml; Nuclear Blue Solution, 4 ml; Mounting Solution (alcoholic), 1 ml	DIG/DNP	10
C-3022-40	ZytoDot 2C SPEC ERBB2/CEN 17 Probe Kit CE IVD Incl. Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; Probe, 0.4 ml; Wash Buffer SSC, 560 ml; 20x Wash Buffer TBS, 2x 50 ml; Anti-DIG/DNP-Mix, 4 ml; HRP/AP-Polymer-Mix, 4 ml; AP-Red Solution A, 0.4 ml; AP-Red Solution B, 15 ml; HRP-Green Solution A, 0.8 ml; HRP-Green Solution B, 15 ml; Nuclear Blue Solution, 20 ml; Mounting Solution (alcoholic), 4 ml	DIG/DNP	40

** 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.*

Background

The ZytoDot® 2C SPEC ERBB2/CEN 17 Probe is designed for the simultaneous detection of ERBB2 and centromere 17 in formalin-fixed, paraffin-embedded tissue sections or cell samples.

The ERBB2 gene (a.k.a. HER2 and NEU) is located in the chromosomal region 17q12 and encodes the cellular growth factor receptor p185. 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.

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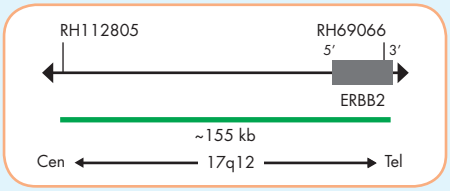
Slamon DJ, et al. (1987) Science 235: 177-82.
Voutsas IF, et al. (2013) Int J Radiat Biol 89: 319-25.
Wolff AC, et al. (2018) J Clin Oncol 14: 437-41.

Probe Description

The ZytoDot® 2C SPEC ERBB2/CEN 17 Probe is a mixture of a Digoxigenin-labeled probe specific for the ERBB2 gene at 17q12 and a Dinitrophenyl-labeled CEN 17 probe specific for the alpha satellite centromeric region of chromosome 17 (D17Z1).

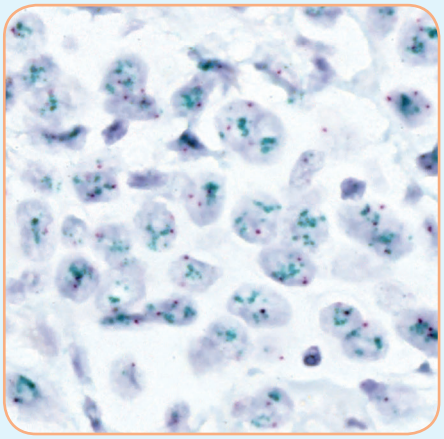
Ideogram of chromosome 17 indicating the hybridization locations.



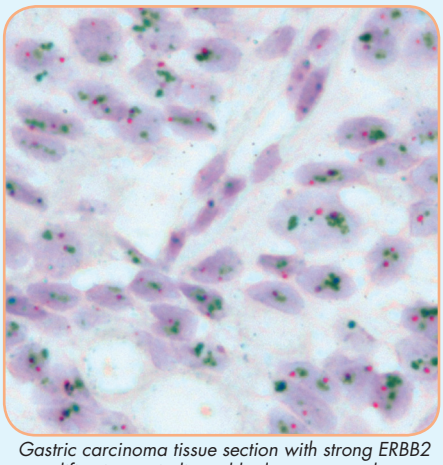
SPEC ERBB2 Probe map (not to scale).

Results

Using the ZytoDot® 2C SPEC ERBB2/CEN 17 Probe Kit, two green (ERBB2) and two red (CEN 17) signals are expected in a normal interphase nucleus. In a cell with amplification of the ERBB2 gene locus, multiple copies of the green signal or green signal clusters will be observed.



Breast cancer tissue section with ERBB2 amplification as indicated by multiple green signals in each nucleus.



Gastric carcinoma tissue section with strong ERBB2 amplification as indicated by large green clusters.

CE IVD

Product Information

ZytoLight® Products for FISH analysis

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-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

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Background

The ZytoLight® SPEC ERBB2/CEN 17 Dual Color Probe is designed for the detection of ERBB2 gene amplification frequently observed in solid malignant neoplasms e.g. breast cancer samples. 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, HER1), 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.

References

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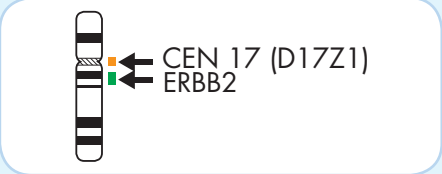
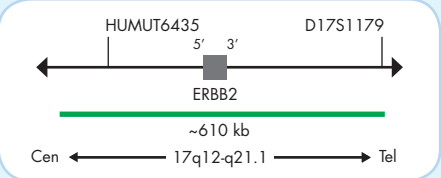
Slamon DJ, et al. (1987) Science 235: 177-82.
Voutsas IF, et al. (2013) Int J Radiat Biol 89: 319-25.
Wolff AC, et al. (2018) J Clin Oncol 14: 437-41.

Probe Description

The SPEC ERBB2/CEN 17 Dual Color Probe is a mixture of an orange fluorochrome direct labeled CEN 17 probe specific for the alpha satellite centromeric region of chromosome 17 (D17Z1) and a green fluorochrome direct labeled SPEC ERBB2 probe specific for the chromosomal region 17q12-q21.1 harboring the ERBB2 gene.



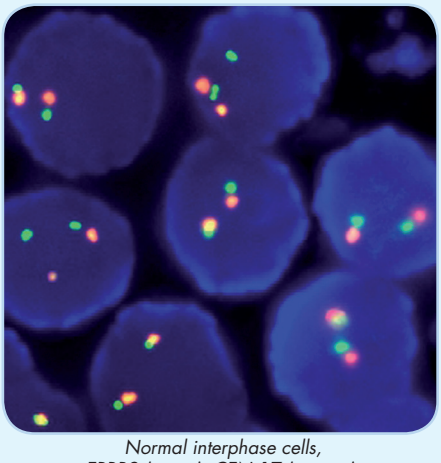
Ideogram of chromosome 17 indicating the hybridization locations.



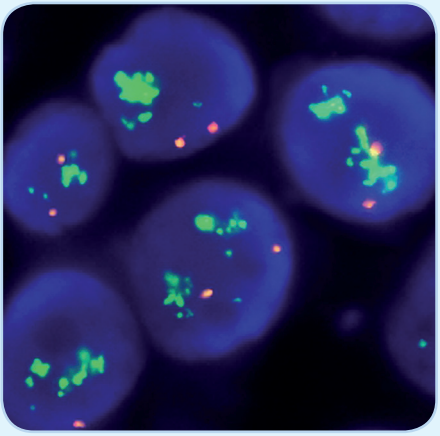
SPEC ERBB2 Probe map (not to scale).

Results

In a normal interphase nucleus, two orange and two green signals are expected. In a cell with amplification of the ERBB2 gene locus, multiple copies of the green signal or green signal clusters will be observed.



Normal interphase cells, ERBB2 (green), CEN 17 (orange).



Breast carcinoma tissue section, ERBB2 gene cluster (green), CEN 17 (orange).

CE IVD

ZYTOVISION

Molecular diagnostics simplified

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M-5015-004-02-2019

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