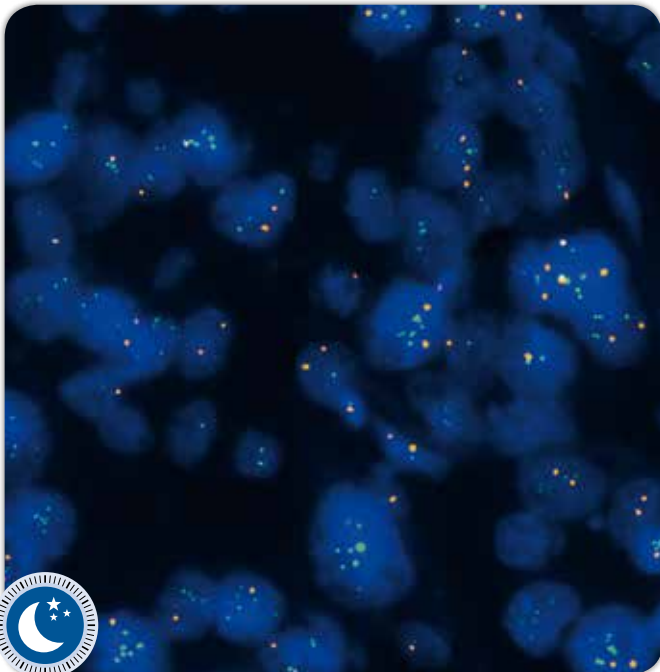


ZYTONNEWS

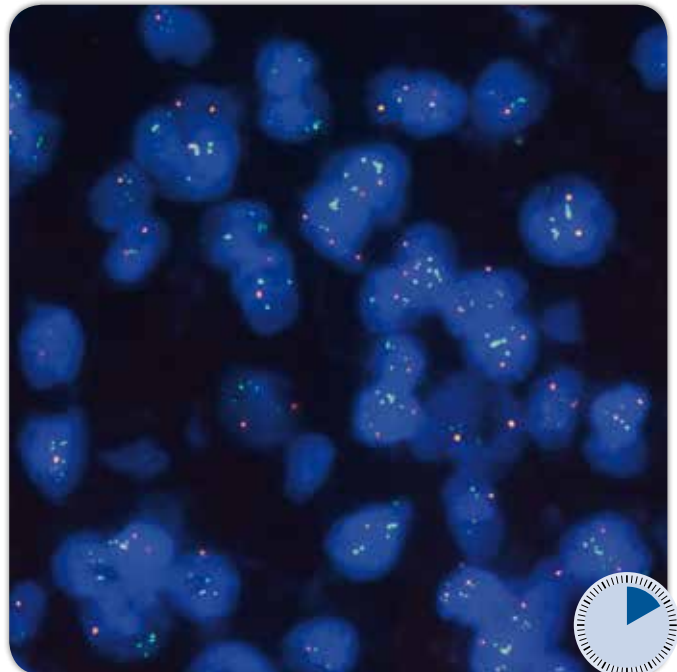
FlexISH[®] ERBB2/CEN 17 Dual Color Probe

The FlexISH[®] assay brings flexibility to cytogenetic HER2 testing



Hybridization time:
Overnight

FlexISH ERBB2/CEN 17 Dual Color Probe



Hybridization time:
120 min

FlexISH ERBB2/CEN 17 Dual Color Probe

Brockhoff G, et al. (2016) Histopathology 69: 635-46.

Dear Readers,
With this new issue of our **ZYTONNEWS** we would like to summarize the comparative study published by Brockhoff *et al.* (2016). By comparing different commercially available ERBB2 FISH assays, the use of the **FlexISH[®] ERBB2/CEN 17 Dual Color Probe** resulted in the highest flexibility in terms of time and laboratory management without negatively affecting the performance, specimen quality or diagnostic result!

Enjoy reading, Yours
ZYTONNEWS
TEAM

Aim of the Study

The assessment of the human epidermal growth factor receptor 2 (ERBB2) (a.k.a HER2) in breast cancer (BC) tissues has gained great clinical impact, as it helps to guide therapy decisions as an obligatory companion diagnostic method. Fluorescence *in situ* Hybridization (FISH) is the method of choice for quantitative ERBB2 gene testing in case of an equivocal IHC result. The performance of a FISH procedure on FFPE tissue sections traditionally takes ~2 days.

This article presents a comparative analysis of quantitative and qualitative counting results of commercially available ERBB2 FISH probes and corresponding FISH protocols recommended by the respective manufacturer in order to evaluate the usefulness and performance of the novel **FlexISH®** FISH assay designed to facilitate rapid and flexible (2h to overnight hybridization) FISH.

Comparative Analysis of the following Probes and Protocols

Manufacturer	Probe	Hybridization	Protocol
ZytoVision	FlexISH® ERBB2/CEN 17 Dual Color Probe	overnight	~ 19 h
ZytoVision	FlexISH® ERBB2/CEN 17 Dual Color Probe	2 h	~ 4.5 h
ZytoVision	ZytoLight® SPEC ERBB2/CEN 17 Dual Color Probe	overnight	~ 19 h
Abbott	PathVysion	overnight	~ 19 h

Analysed Breast Cancer Specimens

- 30 breast cancer specimens with chromosome 17 polysomy
- 60 non-polysomic/non-ERBB2-amplified breast cancer specimens
- 64 breast cancer specimens with obvious ERBB2 amplification
- 19 benign breast tissue specimens

All specimens were acquired from the tissue archive of the Institute of Pathology, University of Regensburg (Germany).

Evaluation Criteria

FISH Signal Counting

Signals were counted in 25 nuclei per specimen and the ERBB2/CEN 17 ratio was calculated.

Durability of hybridized FlexISH® specimens

A number of specimens were imaged 1 day after completion of the hybridization procedure, and were re-evaluated and photographed again up to 9 months later.

Data Analysis

Bland-Altman plots were generated to determine agreement between two assays. Cumulative data were compiled into comparative box plots.

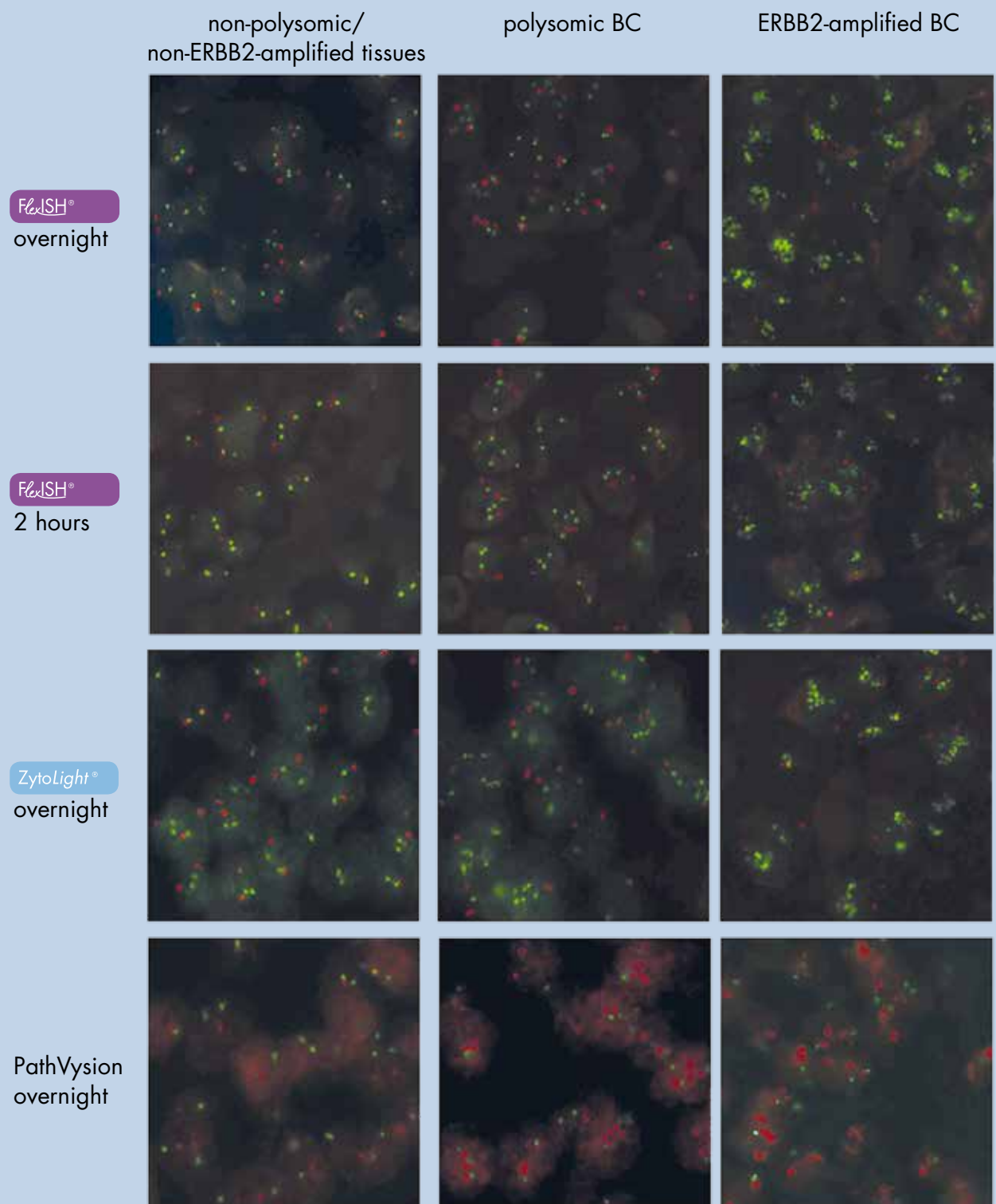
Results

Part I – Comparison of FISH signal quality

The comparison of the different protocols showed similar specimen quality in terms of signal intensity, clearness, and background. In particular, the hybridization signals could be easily identified and quantitatively counted regardless of the probe kit that was used.

FlexISH® specimens even tended to have the lowest background fluorescence, independently of the hybridization time.

Fig. 1: Example images of FISH results

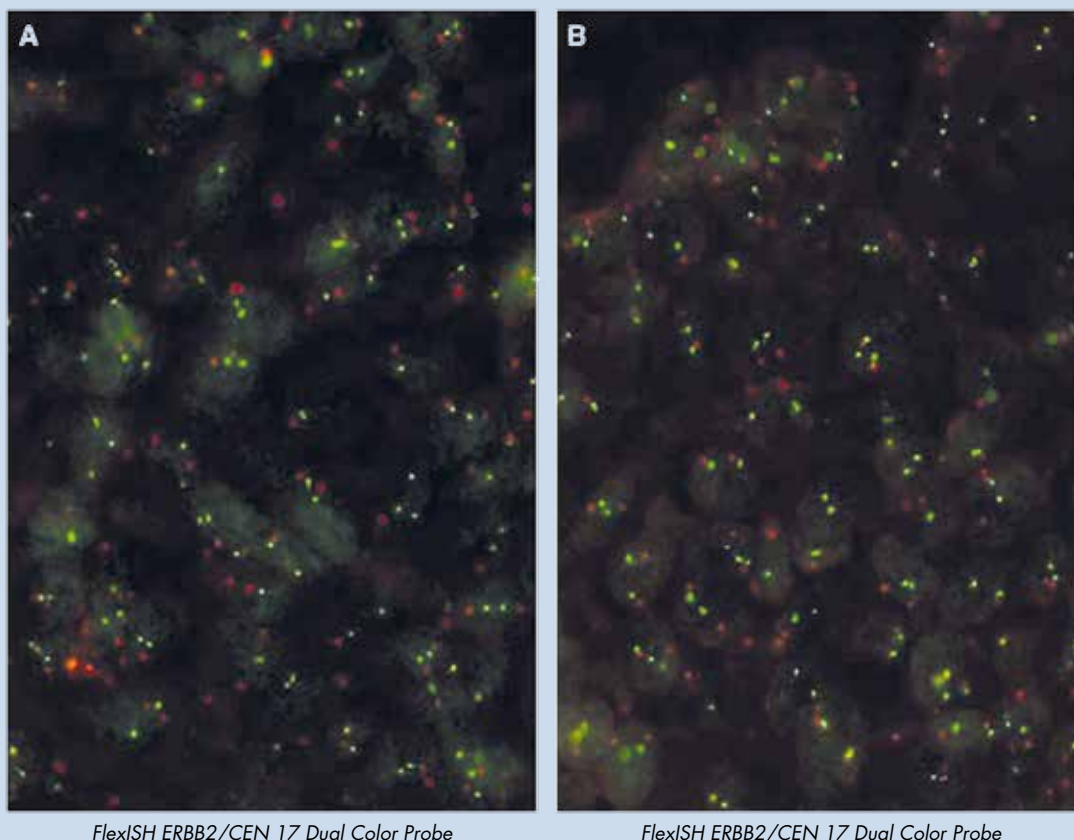


Results

Part II – Durability of hybridized FlexISH® specimens

Re-analysis of the long-term-stored FlexISH® specimens did not reveal any signal loss. Signal brightness and distinctness were well preserved, and were not inferior to the signal stability found in specimens hybridized with the other probes that were re-evaluated after long-term storage. The images in figure 2 verify the long-term durability of FlexISH® tissue specimens.

Fig. 2: Example of a FlexISH®-probed breast cancer tissue photographed 1 day after hybridization (A) and 9 months later (B).



Part III – Comparison of the counting results

The FISH signal quantification showed very good agreement between count values derived from the respective methods.

Tab. 1: Average ERBB2/CEN 17 ratio results of four different FISH procedures as indicated.

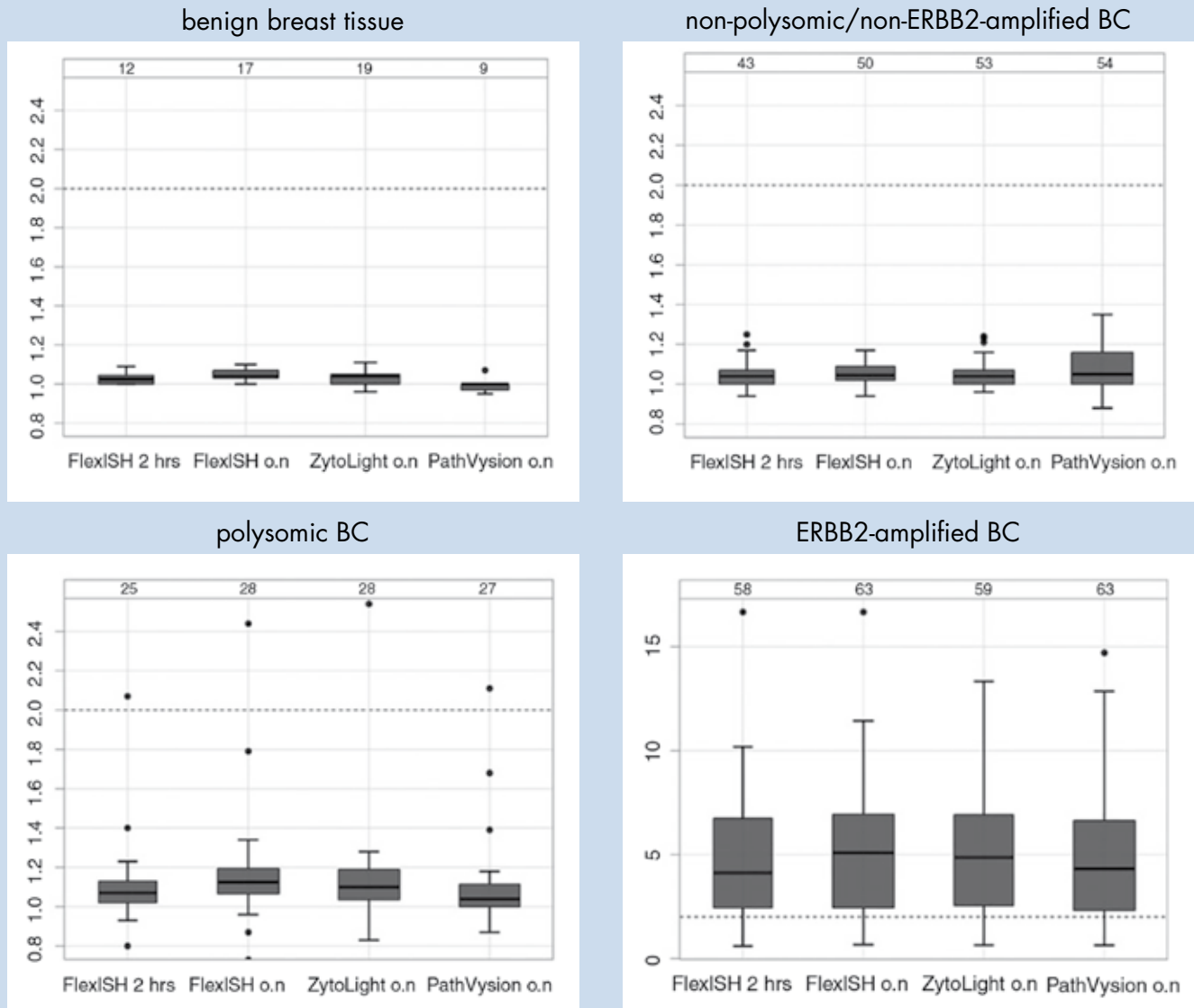
Specimen	FlexISH® overnight	FlexISH® 2 hours	ZytoLight® overnight	PathVysion overnight
Benign breast tissue	1.04 (0.03)	1.03 (0.03)	1.03 (0.04)	0.99 (0.04)
Non-polysomic/non-ERBB2-amplified BC	1.05 (0.05)	1.04 (0.07)	1.06 (0.07)	1.09 (0.10)
Polysomic BC	1.18 (0.30)	1.11 (0.23)	1.15 (0.29)	1.11 (0.25)
ERBB2-amplified BC	5.46 (3.34)	5.29 (3.43)	5.05 (2.97)	5.01 (3.24)

Numbers in brackets indicate the standard deviation of the mean.

Results

The box plots show good data correlation between datasets derived with the different hybridization approaches.

Fig. 3: Box plots of cumulative count values derived from the respective approaches. Mean ERBB2/CEN 17 ratio values are shown.



Dotted lines indicate reference values for tissues without genomic alterations. o.n.: overnight, hrs: hours

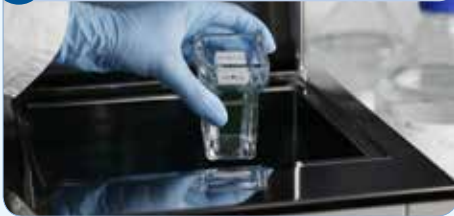
Conclusion

- Reliable ERBB2 findings can be delivered within 4.5 h by using the **FlexISH® ERBB2/CEN 17 Dual Color Probe Kit** without affecting the performance, specimen quality or diagnostic result.
- Excellent accordance with the results obtained with other commercially available FISH kits in terms of: signal counts, signal-to-noise ratio, brightness, and distinctness of ERBB2 and CEN 17 signals.
- **FlexISH®** probe kit can be individually adapted to the specific clinical requirements and the daily workload providing the highest flexibility in terms of time and laboratory management.

Protocol Workflow Overview

The protocol of the **FlexISH®-Tissue Implementation Kit** has been optimized for FFPE tissue (fixed in 10% neutrally buffered formalin) which has been cut in sections between 2-4 µm. Dewaxing with subsequent dehydration should be performed with regular changes of xylene and ethanol solutions!

1 Pretreatment



Incubate for 20 min in pre-warmed **Heat Pretreatment Solution Citric (PT1)** at 98°C.

2 Proteolysis



Apply ready-to-use **Pepsin Solution (ES1)** and incubate for approx. 15 min at 37°C in a humidity chamber.

3 Probe Application



Completely air dry section before pipetting 10 µl **FlexISH ERBB2/CEN 17 Dual Color Probe** each onto individual samples.

4 Cover with Coverslip



Cover the samples with a coverslip, avoiding trapped bubbles.

5 Seal with Rubber Cement



Seal the coverslip, e.g., with a layer of rubber cement.

6 Denaturation & Hybridization



Denature the slides at 75°C for 10 min and hybridize from 2 h to overnight at 37°C (in a humid environment).

7 Stringency Wash



Remove rubber cement and wash using 1x **FlexISH Wash Buffer (WB10)** for 10 min at 72°C.

8 Evaluation



Before evaluation, pipette 25 µl **DAPI/ DuraTect™-Solution (MT7)** onto the slides and cover the samples with a coverslip. Use appropriate filter sets for evaluation.

Product Information

FlexISH®

Products for flexible FISH

Prod. No.	Product	Label	Tests* (Volume)
Z-2166-50	FlexISH ERBB2/CEN 17 Dual Color Probe CE	●/●	5 (50 µl)
Z-2166-200	FlexISH ERBB2/CEN 17 Dual Color Probe CE	●/●	20 (200 µl)
Related Products			
Z-2182-5	FlexISH-Tissue Implementation Kit CE Incl. Heat Pretreatment Solution Citric, 150 ml; Pepsin Solution, 1 ml; 5x FlexISH Wash Buffer, 150 ml; DAPI/DuraTect-Solution, 0.2 ml		5
Z-2182-20	FlexISH-Tissue Implementation Kit CE Incl. Heat Pretreatment Solution Citric, 500 ml; Pepsin Solution, 4 ml; 5x FlexISH Wash Buffer, 500 ml; DAPI/DuraTect-Solution, 0.8 ml		20

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

Background

The FlexISH® 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), ERBB3 (HER3), and ERBB4 (HER4).

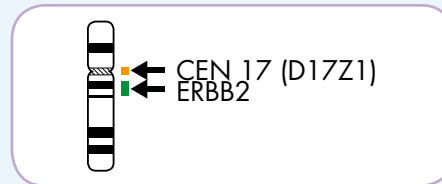
Amplification of the proto-oncogene ERBB2, observed in approximately 20% of all breast cancer samples, has been correlated with a poor prognosis of the disease. Similar results have been obtained for a variety of other malignant neoplasms, e.g., ovarian cancer, stomach cancer, and carcinomas of the salivary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

References

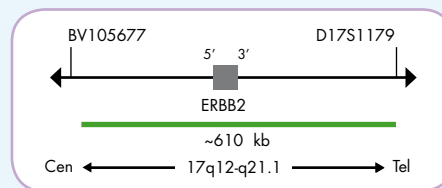
- Baselga J, et al. (1999) *Semin Oncol* 26: 78-83.
- Brockhoff G, et al. (2016) *Histopathology* [Epub ahead of print].
- Brunello E, et al. (2012) *Histopathology* 60: 482-8.
- Brunner K, et al. (2010) *Anal Quant Cytol Histol* 32: 78-89.
- Coussens L, et al. (1985) *Science* 230: 1132-9.
- Ehtl T, 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. (2013) *J Clin Oncol* 31: 3997-4013.

Probe Description

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



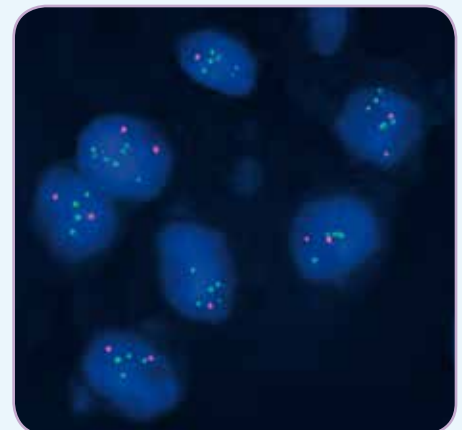
Ideogram of chromosome 17 indicating the hybridization locations.



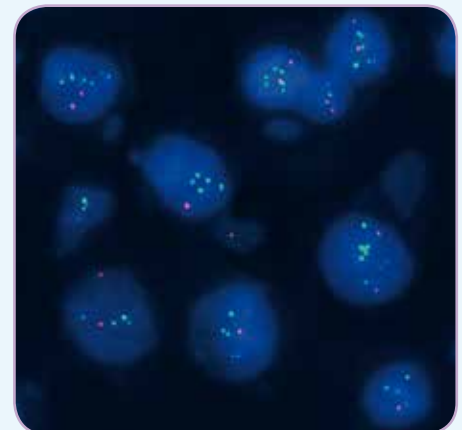
ERBB2 Probe map (not to scale).

Results

In a normal interphase nucleus, two green and two orange 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.



FlexISH ERBB2/CEN 17 Dual Color Probe hybridized for 2 hours on an endometrial carcinoma tissue section with ERBB2 (green) amplification.



FlexISH ERBB2/CEN 17 Dual Color Probe hybridized overnight on an endometrial carcinoma tissue section with ERBB2 (green) amplification.

Distributors

AFRICA

Algeria, Burkina Faso, Cameroun, Central African Republic, Côte d'Ivoire, Gabon, Guinea, Mauritania, Niger, Senegal, Chad, Tunisia
CliniSciences s.a.s
Fon: +33 9 77400909
Email: info@clinisciences.com
www.clinisciences.com

Egypt

Advanced Diagnostic Services (ADS) Ltd.
Fon: +44 7783 124383
Email: adserviceuk@aol.com
www.advands.com

Libya

Société Bioexport
Fon: +33 971 291329
Email: ofontes@wanadoo.fr
www.bio-export.com

Morocco

HexaBiogen, Groupe CliniSciences
Fon: +212 5 39933505
Email: maroc@hexabiogen.com
www.hexabiogen.com

South Africa

Cell Path Services (CPS)
Fon: +27 11 7043139
Email: cellpath@wol.co.za
www.cellpathservices.co.za

AMERICA

Argentina

BIOARS S.A.
Fon: +5411 4555 4601
Email: seccom@bioars.com.ar
www.bioars.com.ar

Brazil

Inopat Imp. E Exp. LTDA
Fon: +55 11 38650042
Email: inopat@inopat.com.br
www.inopat.com.br

Canada

ESBE Scientific
Fon: +905 4758232
Email: info@esbe.com
www.esbe.com

Chile

ProLab LTDA
Fon: +33 971 291329
Email: ofontes@wanadoo.fr
www.prolab.cl

Cuba

Société BioExport
Fon: +33 0 684064134
Email: contact@bio-export.com
www.bio-export.com

Ecuador

BIO SB, Inc.
Fon: +1 805 6922768
Email: info@biosb.com
www.biosb.com

Mexico

BIO SB, Inc.
Fon: +1 805 6922768
Email: info@biosb.com
www.biosb.com

Paraguay

BIO SB, Inc.
Fon: +1 805 6922768
Email: info@biosb.com
www.biosb.com

Tanirel SA

www.tanirel.com.uy

Uruguay

Tanirel SA
www.tanirel.com.uy

USA

Axxora LLC
Fon: +1 858 5508830
Email: axxora-usa@axxora.com
www.axxora.com

BIO SB, Inc.

Fon: +1 805 6922768
Email: info@biosb.com
www.biosb.com

Venezuela

Macrosearch C.A.
Fon: +58 212 9914531
Email: macrosearch@gmail.com

ASIA

Armenia

"Gysane" LLC
Fon: +374 91403610
www.davlab.am

Bahrain

ExpressMed Supplies
Fon: +973 77298888
Email: info@expressmedlabs.com
www.expressmed-supplies.com

Bangladesh

ABC Corporation
Fon: +880 17 10026938
Email: info@abc-bd.com
www.abc-bd.com

China

Anhui Huite Medical Technology
Fon: +86 551 62319209
Email: Lisa_chen@vip.126.com
www.huitesci.com

Ascend Biotechnology Company, Ltd.
Fon: +86 20 34685552
Email: sales@ascendbio.cn
www.ascendbio.cn

Fuzhou Maixin Biotech, Co., Ltd
Fon: +86 800 8581156
mail: info@maxim.com.cn
www.maxim.com.cn

GeneDiagnostic Inc.
Fon: +86 571 86042915
Email: info@genediagnostic.com
www.genediagnostic.com

GeneFormation Technology Inc.
Fon: +86 10 64484022 & 64484033
Email: info@geneformation.com
www.geneformation.com

Hangzhou Xixing Medical
Technology Co., Ltd
Fon: +86 18106505953
Email: info@xxmedpharm.com
www.xxmedpharm.com

Wuhan Bioinborn Scientific Inc.
Fon: +86 027 84621823
Email: info@bioinborn.com
www.bioinborn.com

Georgia

Lab Service Georgia
Fon: +995 591273090
Email: armazi1@yahoo.com

Hong Kong

Fuzhou Maixin Biotech Co., Ltd.
Fon: +86 800 8581156
Email: info@maxim.com.cn
www.maxim.com.cn

GeneFormation Technology Inc.
Fon: +86 10 64484022 & 64484033
Email: info@geneformation.com
www.geneformation.com

Onwon Trading Ltd.

Fon: +852 27577569
Email: info@onwon.com.hk
shopping.netsuite.com/onwon

India

Diagnostic Biosystems (India)
Fon: +91 11 25703615
Email: dbsindia@vsnl.net
www.dbiosyindia.net

Indonesia

Pt. Biozatih Indonesia
Fon: +62 21 47885303
Email: sales@biozatih.net
www.biozatih.net

Iran

Tashkhis Baft Aragene Co. Ltd.
Fon: +98 21 889720067
www.tbai-inc.com

Iraq

LSB Medical
Fon: +964 771 1111841
Email: info@lsbmedical.com
www.lsbmedical.com

Israel

Biotech Ltd.
Fon: +972 9 7667454
Email: biotech@biotech.co.il
www.biotech.co.il

Japan

Cosmo Bio Co., Ltd
Fon: +81 3 56329610
Email: mail@cosmobio.co.jp
www.cosmobio.co.jp

Filgen, Inc.

Fon: +81 52 6244388
E-mail: biosupport@filgen.jp
www.filgen.jp

Wako Pure Chemical Ind., Ltd.

Fon: 0120 052099
Email: labchem-tec@wako-chem.co.jp
www.wako-chem.co.jp

Jordan

Genetics Company
(Biotechnology Products and Services)
Fon: +962 6 5536402
Email: sales@genetics-jo.com
www.genetics-jo.com

Paramount International
Fon: +962 79 5970957
Email: ztahr11@hotmail.com

Lebanon

LSB Medical
Fon: +961 3 699947
Email: info@lsbmedical.com
www.lsbmedical.com

Malaysia

Nano Life Quest Sdn. Bhd.
Fon: +603 80632688
Email: info@nanolifequest.com
www.nanolifequest.com

Morocco

HexaBiogen
Fon: +212 524 358475
Email: maroc@hexabiogen.com
www.hexabiogen.com

Pakistan

Qualitron Corporation
Fon: +92 21 34539991 - 4
Email: qualtra@super.net.pk
www.qualitron.com.pk

Mongolia

Setunari LLC
Fon: +976 70177016
www.setunari.com

Russia

OPTEC LLC*
Fon: +7 495 9335151
Email: office@optecgroup.com
www.optecgroup.com

Singapore

Precision Technologies Pte Ltd
Fon: +65 62734573
Email: scitech@pretech.com.sg
www.pretech.com.sg

South Korea

ZytoVision Korea Co Ltd
Fon: +82 32 2340306
Email: help@zytovisionkorea.com

Tunisia

HexaBiogen
Fon: +216 50 762907
www.hexabiogen.com

Turkey

SitoGen Biomedikal ve Laboratuvar Sist.
San. Tic. Ltd. Şti.
Fon: +90 216 4893344
Email: sitogen@sitogen.com.tr
www.sitogen.com.tr

Vietnam

Namviet supplied technology Company
Ltd.
Fon: +84 - 4 66864744
Email: nguyentuan.ets@gmail.com

AUSTRALIA

Australia

Abacus ALS Australia
Fon: +61 7 3386 7999
Email: info@abacus-als.com
www.abacus-als.com

New Zealand

Abacus ALS New Zealand
Fon: 0800 222170
Email: info@abacus-als.co.nz
www.abacus-als.com

EUROPE

Austria

Sanova Pharma GesmbH
Fon: +43 1 801042569
Email: medicalsystems@sanova.at
www.medicalsystems.sanova.at

Belarus

Paynet LLC
Email: manager.paynet@gmail.com

Belgium

VWR International bvba
Fon: +32 16 385011
Email: info.be@vwr.com
https://be.vwr.com

Czech Republic

Pragostem s.r.o.
Fon: +420 72 5930929
Email: info@pragostem.cz
www.pragostem.cz

Denmark

AH diagnostics as
Fon: +45 87459010
Email: info@ahdiagnostics.dk
www.ahdiagnostics.dk

Eire

Generon Ltd
Fon: +44 1753 866511
Email: info@generon.co.uk
www.generon.co.uk

Finland

AH diagnostics Oy
Fon: +358 9 3509100
Email: ahdiagnostics@ahdiagnostics.fi
www.ahdiagnostics.fi

France

CliniSciences s.a.s
Fon: +33 9 77400909
Email: info@clinisciences.com
www.clinisciences.com

Germany

ZYTOMED Systems GmbH
Fon: +49 30 804984990
Email: info@zytomed-systems.de
www.zytomed-systems.de

Greece

MENARINI Diagnostics SA
Fon: +30 21 09944950
Email: mendiagr@otenet.gr
www.menariniagnostics.gr

Hungary

Izinta Trading Co. Ltd.
Fon: +36 1 3922654
Email: info@izinta.hu
www.izinta.hu

Italy

Bio-Optica Milano S.p.A.
Fon: +39 2 2127131
Email: info@bio-optica.it
www.bio-optica.it

Latvia

SIA Hydrox
Fon: +371 67551517
Email: info@hydrox.lv
www.hydrox.lv

Lithuania

Medfarmos laboratorijos Ltd
Fon: +37 05 2700101
Email: medfarmoslaboratorijos@yahoo.com

SIA Hydrox

Fon: +371 67551517
Web: www.hydrox.lv

Luxembourg

Klinipath BVBA
Fon: +32 14 702100
Email: info@klinipath.be

Netherlands

Klinipath / Immunologic BV - a VWR
Company
Fon: +31 316 266466
Email: info@klinipath.nl
www.klinipath.nl

Norway

AH diagnostics as
Fon: +47 23 233260
Email: ahdiagnostics@ahdiagnostics.no
www.ahdiagnostics.no

Poland

ELEKTROMED Grzegorz Palkowski
Fon: +48 12 28891 40
Email: biuro@elektromed.pl
www.elektromed.com.pl

Portugal

Menarini Diagnósticos, Lda
Fon: +351 210930000
Email: mdportugal@menariniadiag.pt
www.menariniadiag.pt

Romania

SC GenomicALL Solutions SRL
Fon: +40 752081534
Email: office@genomicall.ro
www.genomicall.ro

Slovakia

Pragostem s.r.o.
Fon: +420 72 5930929
Email: info@pragostem.cz
www.pragostem.cz

Slovenia

Sanova Pharma GesmbH
Fon: +43 1 801042569
Email: medicalsystems@sanova.at
www.medicalsystems.sanova.at

Spain

Anacrom Diagnosticos
Fon: +34 95 4155282
Email: anacrom@anacrom.com
www.anacrom.com

Menarini Diagnosticos S.A.

Fon: +34 93 5071000
Email: informacion@menariniadiag.es
www.menariniadiag.es

Sweden

AH diagnostics ab
Fon: +45 8 6800845
Email: ahdiagnostics@ahdiagnostics.se
www.ahdiagnostics.se

Switzerland

LabForce AG
Fon: +41 61 7959620
Email: info@labforce.ch
www.labforce.ch

United Kingdom

Generon Ltd
Fon: +44 1753 866511
Email: info@generon.co.uk
www.generon.co.uk

REST OF THE WORLD

ZytoVision GmbH
Fon: +49 471 4832300
Email: export@zytovision.com
www.zytovision.com

* ZytoVision products are distributed by OPTEC LCC also in the following countries: Armenia, Azerbaijan, Georgia, Kazakhstan, Kyrgyzstan, Moldova, Tajikistan, Turkmenistan, Ukraine, and Uzbekistan.



ZytoVision GmbH · Fischkai 1 · 27572 Bremerhaven · Germany · www.zytovision.com

ZYTOVISION
Molecular diagnostics simplified