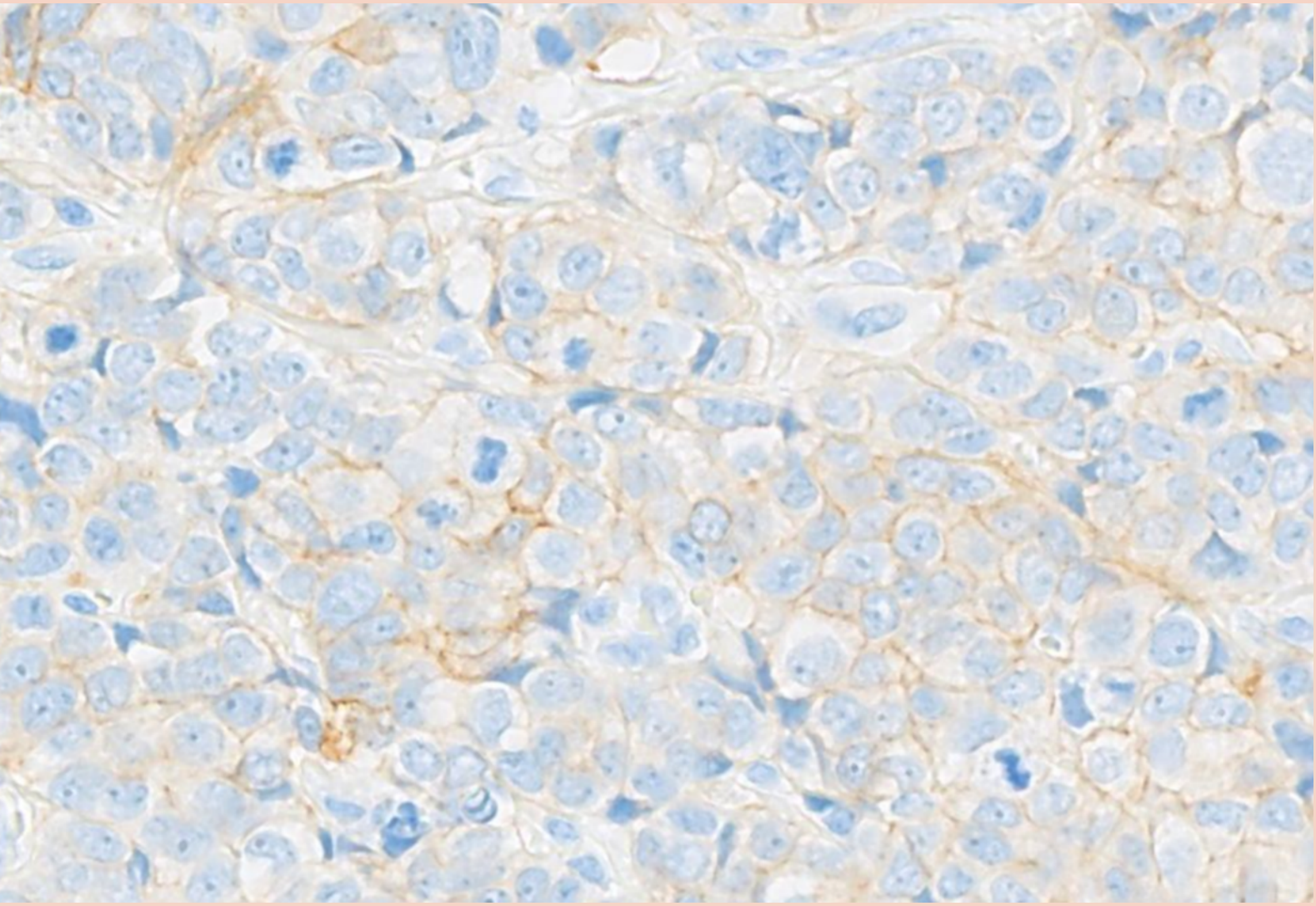
PATHWAY® anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody

FDA approved to identify HER2-low expression in mBC



US

Predictive

PATHWAY® anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody

Identifying patients with low expression of HER2 who may benefit from ENHERTU®

INTENDED USE

The PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody (PATHWAY anti-HER2 (4B5) antibody) is a rabbit monoclonal antibody intended for laboratory use for the semi-quantitative detection of HER2 antigen by IHC in sections of formalin-fixed, paraffin-embedded normal and neoplastic tissue using the *ultra*View Universal DAB Detection Kit on a BenchMark Ultra IHC/ISH instrument.

This IHC device is indicated for identifying patients who are eligible for Herceptin®, KADCYLA® (IHC 3+ or 2+/ISH+) or ENHERTU® (IHC 1+ or 2+/ISH-).

<table>
<tr><th></th><th>STAINING PATTERN</th><th>SCORE</th><th>RECOMMENDED REPORTING STATUS</th><th>CLINICAL APPLICATION</th></tr>
<tr><td rowspan="5">Breast Cancer</td><td>No membrane staining is observed
OR
Faint, partial staining of the membrane in 10% or LESS of the cancer cells*</td><td>0</td><td>HER2 Negative</td><td>None</td></tr>
<tr><td>Faint, partial staining of the membrane in greater than 10% of the cancer cells*</td><td>1+</td><td>HER2-low expression</td><td rowspan="2">ENHERTU®
(fam-trastuzumab deruxtecan-nxki)</td></tr>
<tr><td rowspan="2">Weak to moderate complete staining of the membrane in greater than 10% of the cancer cells</td><td>2+*
Reflex test: HER2 non-amplified</td><td>HER2-low expression</td></tr>
<tr><td>2+**
Reflex test: HER2 Amplified</td><td>HER2 Positive/ overexpression</td><td rowspan="2">Herceptin® (trastuzumab)
KACYLA® (trastuzumab emtansine)</td></tr>
<tr><td>Intense complete staining of the membrane in greater than 10% of the cancer cells</td><td>3+</td><td>HER2 Positive/ overexpression</td></tr>
</table>

*Recommend re-reading by a second pathologist for cases with "faint, partial staining of the membrane" and %TC near the threshold of 10%, when the range of %TC is between 5%-25%

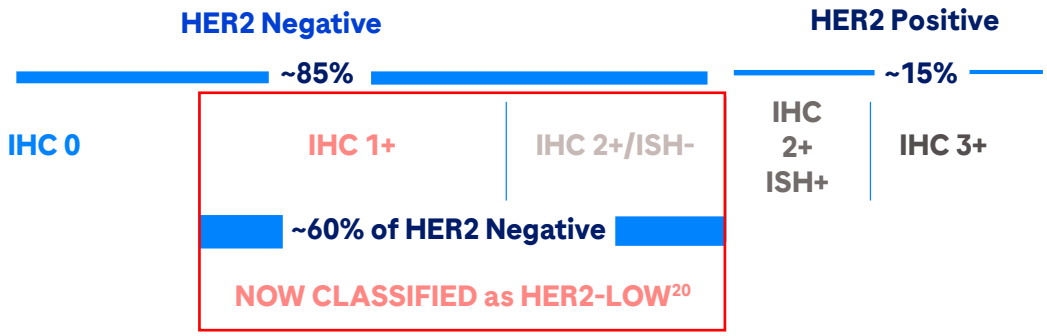
**Recommend reflex test to assess gene amplification per ASCO/CAP guidance

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls. This antibody is intended for in vitro diagnostic (IVD) use.

For more information on ENHERTU®, (fam-trastuzumab deruxtecan-nxki) please refer to the FDA-approved product labeling.

PATHWAY® anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody

HER2-low is now an actionable classification in mBC



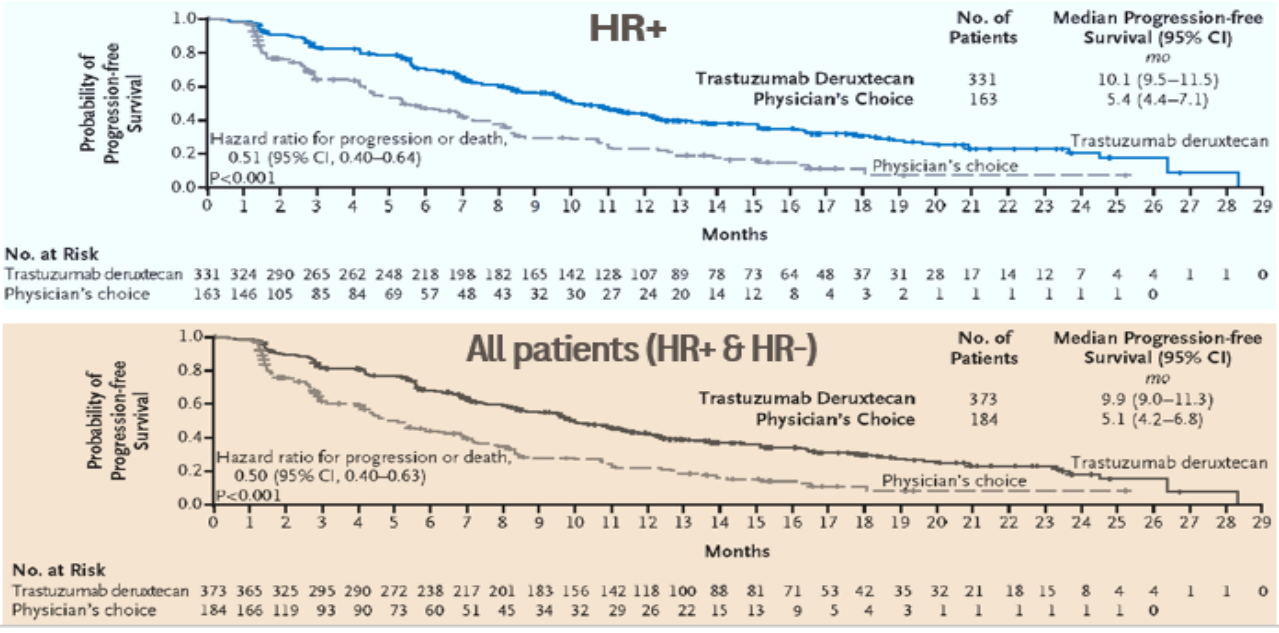
Approximately 40-50% percent of breast cancer patients have tumors that do not demonstrate amplification of the HER2 gene and do not over express the receptor; however, low levels of HER2 expression are detected.[20] HER2-low expressing cases (IHC score 1+ or 2+/ISH-) are typically considered HER2-negative and excluded from HER2-targeted treatment options.[8]

Recently, benefit has been observed with the anti-HER2 treatment fam-trastuzumab deruxtecan-nxki (ENHERTU®) in breast cancer patients with low levels of HER2 expression.[19-21]

- Fam-traastuzumab deruxtecan-nxki is an antibody-drug conjugate that contains a HER2 targeting monoclonal antibody (trastuzumab) base, a cleavable linker, and a cell membrane permeable exatecan derivative (a topoisomerase I inhibitor payload).[10]
- In vitro diagnostics for the determination of HER2 status in breast cancer patients are important to aid the clinician in determination of therapy with trastuzumab (Herceptin®), trastuzumab emtansine (KADCYLA®) or fam-trastuzumab deruxtecan-nxki (ENHERTU®).[11]
- The immunohistochemical detection of HER2 protein expression may be used as an aid in the assessment of breast cancer patients for whom the treatments Herceptin®, KADCYLA® or ENHERTU® are being considered.

HER2 clinical utility as evidenced by DESTINY-Breast04[20]

ENHERTU® (fam-trastuzumab deruxtecan-nxki) demonstrated a statistically significant and clinically meaningful progression-free survival benefit in HR+/HER2-low patients & all HER2-low patients (HR+ & HR-)[20]



For more information on ENHERTU, please refer to the FDA-approved product labeling.

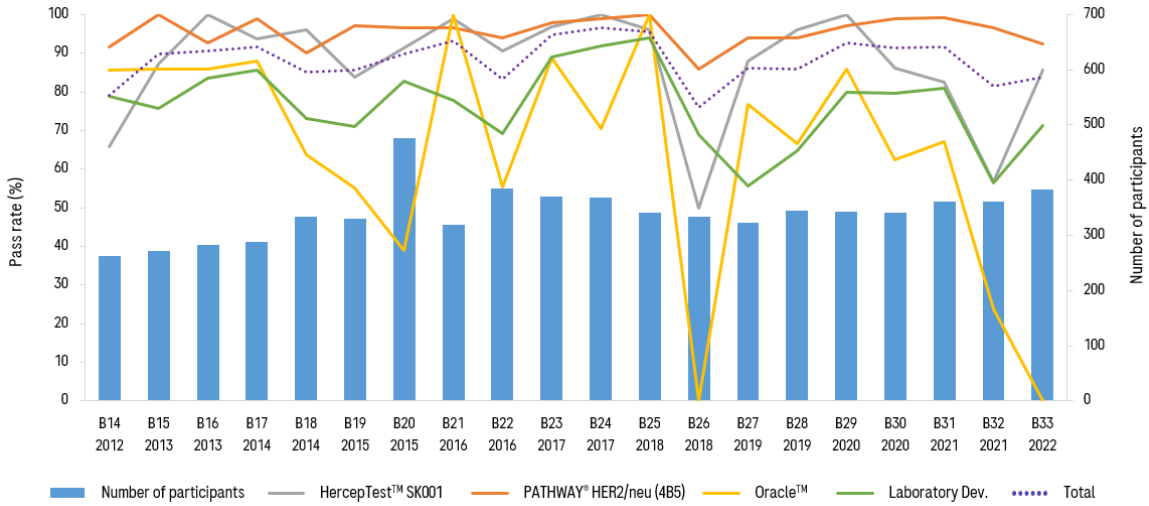
PATHWAY® anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody

Continuing to lead the way in HER2 testing

The use of pre-diluted PATHWAY HER2 (4B5),[37] in combination with the fully automated VENTANA BenchMark Ultra IHC/ISH slide staining instrument, standardizes all IHC Processes from baking through staining, and reduces the possibility of human error. It also minimizes inherent variability resulting from individual reagent dilution and other processes found in manual and semi-automated IHC methods.

The PATHWAY HER2 (4B5) Primary Antibody empowers you to:

- Achieve consistently high proficiency assessment scores with HER2 (4B5) antibody, compared to other clones[38]
- Employ the most widely adopted and reliable HER2-IHC primary antibody[38]
- Demonstrate high concordance with HER2 FISH[39,40]



NordiQC comparison data for HER2 clones[38]

PATHWAY® HER2 (4B5) CDx can identify HER2-low in mBC with Reproducibility[37]

An inter-laboratory reproducibility (ILR) study of PATHWAY HER2 (4B5) CDx for HER2-low (IHC 1 + or IHC 2+/ISH-) assessment demonstrated a **high concordance** across **5 days, 3 sites, and 6 readers** for HER2-low classification.[37]

>97%
Average Positive Agreement (APA)

Between-Site (N-7884/8102: 95% CI: 95.4, 98.8)
Between-Reader (N-398/409: 95% CI: 95.4, 98.8)
Between-Day (N=1580/1620; 95% CI: 95.9, 98.9)

>97%
Average Negative Agreement (ANA)

Between-Site (N-8240/8458; 95% CI: 95.7.98.8)
Between-Reader (N-414/425: 95% CI: 95.6, 98.8)
Between Day (N=1652/1692: 95% CI: 96.2, 98.9)

>97%
Overall Percent Agreement (OPA)

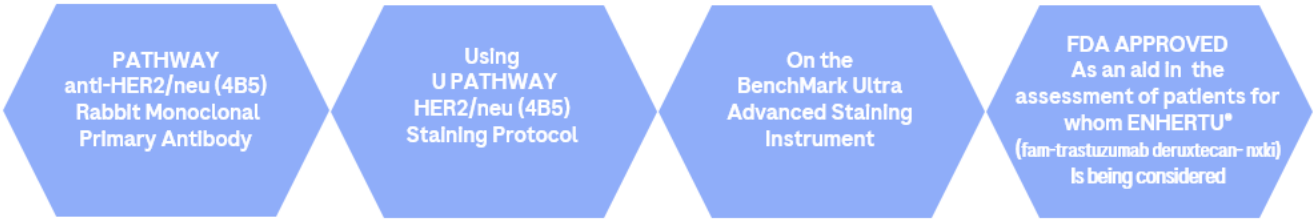
Between-Site (N-8062/8280: 95% CI: 95.5. 98.8)
Between-Reader (N-406/417: 95% CI: 95.5, 98.8)
Between-Day (N=1616/1656; 95% CI: 96.1, 98.9)

Laboratories are responsible for ensuring the reliability and accuracy of their results, by compliance with accreditation and proficiency testing requirements for HER2 testing assays.[13]

To calculate APA, ANA and OPA, HER2 IHC scores "1+" and "2" were categorize as "positive" as they were eligible for DESTINY-Breast04, and HER2 IHC scores "0," ">0, <1+," and "3+" were categorize as "negative"[37]

PATHWAY® HER2 (4B5) is FDA-approved to identify HER2-low expression[37]

Use U PATHWAY® HER2 (4B5) staining protocol for all HER2 assessment including potential HER2-low assessment on a Benchmark ULTRA instrument

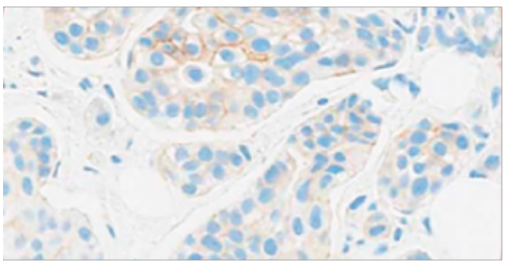


Deviations from the recommended cell conditioning or antibody incubation time may affect the HER2 score, particularly with HER2-low expression which may impact treatment decisions for patients[37]

Procedure Type	PATHWAY anti-HER2 (4B5)
Staining Procedure	U PATHWAY 4B5
Cell Conditioning	Mild, 36 minutes
Antibody (Primary or Neg Ctrl Rbt IG)	12 minutes
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

Identify HER2-low expression by distinguishing subtle differences between HER2 IHC 1+ and IHC 0 with PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody[37]

Heterogeneous staining pattern[41]

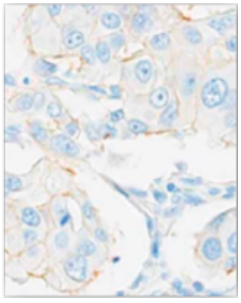


- Partial membrane staining should be scored as 0 or 1+ depending on the percentage of tumor cells (10% cutoff) [41]
- Examination of multiple areas of the slide is recommended to assess HER2 status[13,32]

Cytoplasmic blush[41]

- Only membrane staining in tumor cells, both partial and complete, is interpreted for HER2 IHC[41]
- Cytoplasmic staining, artifactual staining and other non-specific staining are excluded from the interpretation and subsequent score[41]

Retraction Artifact[41]



Up to 40X magnification may be required for HER2-low status evaluation[41]

Ordering Information

Product name	Catalog number	Ordering Code	Quantity
PATHWAY® anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody	790-2991	05278368001	50 tests
ultraView Universal DAB Detection Kit	760-500	05269806001	250 tests
PATHWAY® HER2 4 in 1 Control Slides	781-2991	05273510001	10 tests

Automation: optimized for use on BenchMark Ultra IHC/ISH instruments

References

1. Akiyama T, Sudo C, Ogawara H, Toyoshima K, Yamamoto T. The product of the human c erbB 2 Gene: A 185-kilodalton glycoprotein with tyrosine kinase activity. Science. 1986;232:1644-1646.
2. Kraus MH, Popescu NC, Amsbaugh C, King RC. Overexpression of EGF receptor-related proto-oncogene erbB-2 in human mammary tumor cell lines by different molecular mechanisms. EMBO J. 1987;6:605-610.
3. Schrohl AS, Pedersen HC, Jensen SS, Nielsen SL, Brünner N. Human epidermal growth factor receptor 2 (HER2) immunoreactivity: specificity of three pharmacodiagnostic antibodies. Histopathology. 2011;59(5):975-983.
4. Jay JI, Brunhoeber PS, Smith MH, et al. Immunohistochemical analysis of the monoclonal antibody 4B5 in breast tissue expressing human epidermal growth factor receptor 4 (HER4). Histopathology. 2013;62(4):563-577.
5. Moasser MM. The Oncogene HER2: Its Signaling and Transforming Functions and Its Role in Human Cancer Pathogenesis. Oncogene. 2007;26(45):6469-6487.
6. Hsu JL, Hung MC. The Role of HER2, EGFR, and Other Receptor Tyrosine Kinases in Breast Cancer. Cancer Metastasis Rev. 2016;35(4):575-588.
7. Iqbal N, Iqbal N. Human Epidermal Growth Factor Receptor 2 (HER2) in Cancers: Overexpression and Therapeutic Implications. Mol Biol Int. 2014:2014:852748.
8. Tarantino P, Hamilton E, Tolaney SM, et al. HER2-low Breast Cancer: Pathological and Clinical Landscape. J Clin Oncol. 2020;38:1951-1962.
9. Osborne CK, Shou J, Massarweh S, et al: Crosstalk between estrogen receptor and growth factor receptor pathways as a cause for endocrine therapy resistance in breast cancer. Clin Cancer Res 11:8655-870s, 2005.
10. Eiger D, Agostinetto E, Saude-Conde R, de Azambuja E. The Exciting New Field of HER2-low Breast Cancer Treatment. Cancers (Basel). 2021;13.
11. Zhang H, Katerji H, Turner BM, Hicks DG. HER2-low Breast Cancers. Am J Clin Pathol. 2021.
12. The Global Cancer Observatory: Cancer Today (GLOBOCAN) 2020. International association of Cancer Registries. Nov 2020.
13. Wolff AC, Hammond MEH, Allison KH, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. Arch Pathol Lab Med. 2018;142:1364-1382.
14. Dickson RB and Lippman ME. Genes, Oncogenes, and Hormones. Boston: Kluwer Academic Publishers; 1992.
15. Keatings L, Sinclair J, Wright C, et al. cerbB 2 oncoprotein expression in mammary and extramammary Paget's disease: an immunohistochemical study. Histopathology. 1990;17:234-247.
16. Hudis CA. Trastuzumab--Mechanism of Action and Use in Clinical Practice. N Engl J Med. 2007;357(1):39-51.
17. Herceptin (Trastuzamab) [Package Insert). EMEA (European Medicines Agency) .
18. vonMinckwitz g, Huang CS Mano MS et al. Transtuzumab Emtansine for Residual Invasive HER2-positive Breast Cancer NEJM 2019; 380(7):617-628
19. Modi S, Park H, Murthy RK, et al. Antitumor Activity and Safety of Trastuzumab Deruxtecan in Patients with HER2-low-Expressing Advanced Breast Cancer: Results From a Phase lb Study. J Clin Oncol. 2020;38:1887-1896.
20. Modi S, et al. Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer. N Engl J Med. 2022. doi: 10.1056/NEJMoa2203690. Online ahead of print.
21. Denkert C, Seither F, Schneeweiss A, et al. Clinical and molecular characteristics of HER2-low-positive breast cancer: pooled analysis of individual patient data from four prospective, neoadjuvant clinical trials. Lancet Oncol. 2021,22:1151-1161.
22. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press, 2009.
23. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology, 2nd Edition. St. Louis, Missouri: The C.V. Mosby Company, 1980.
24. Department of Health, Education and Welfare, National Institute of Occupational Safety and Health, Rockville, MD. "Procedures for the decontamination of plumbing systems containing copper and/or lead azides." DHHS (NIOSH) Publ No. 78-127, Current 13. August 16, 1976.
25. Roche PC, Hsi ED.Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.
26. College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist, 2010.
27. CLSI. Quality Assurance for Immunocytochemistry: Approved Guideline. CLSI document MM4-A- (ISBN 1-56238-396-5). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 1999.
28. Herman GE, Elfont EA. The taming of immunohistochemistry: the new era of quality control. Biotech Histochem. 1991;66:194-199.
29. Omata M, Liew CT, Ashcavai M, Peters RL. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen. A possible source of error in immunohistochemistry. Am J Clin Pathol. 1980:73:626-32.
30. Nadji M, Morales AR. Immunoperoxidase: part 1. The technique and its pitfalls. Lab Med. 1983;14:767.
31. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.
32. Ahn S, Woo JW, Lee K, et al. HER2 status in breast cancer: changes in guidelines and complicating factors for interpretation. J Pathol Trans Med.2020:54:34-44
33. Thomson TA, Hayes MM, Spinelli JJ, et al. HER-2/neu in breast cancer: interobserver variability and performance of immunohistochemistry with 4 antibodies compared with florescent in situ hybridization. Mod Pathol. 2001;14:1079-86.
34. Kay EW, Walsh CJ, Cassidy M, Curran B, Leader M. C-erbB-2 immunostaining: problems with interpretation. J Clin Pathol. 1994;47:816-22.
35. Bilous M, Dowsett M, Hanna W, et al. Current Perspectives on HER2 Testing: A Review of National Testing Guidelines. Mod Pathol. 2003;16:173-182.
36. Vyberg M, Nielsen S, Røge R, et al. Immunohistochemical expression of HER2 in breast cancer: Socioeconomic impact of inaccurate tests. BMC Health Services Research. 2015;15:352.
37. PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody Package Insert.
38. NordiQC. (2021). HER2 IHC.https://www.nordiqc.org/epitope.php?id=11 Accessed 7/7/2022
39. Mayr D, et al. Comprehensive immunohistochemical analysis of Her-2/neu oncoprotein overexpression in breast cancer: HercepTest™ (Dako) for manual testing and Her-2/neuTest 4B5 (VENTANA) for VENTANA BenchMark automatic staining system with correlation to results of BenchMark automatic staining system with correlation to results of fluorescence in situ hybridization (FISH). Virchows Archiv. 2009;454(3):241-248.
40. Brügmann A, Lelkaitis G, Nielsen S, et al. Testing HER2 in breast cancer: a comparative study on BRISH, FISH, and IHC. Appl Immunohistochem Mol Morphol. 2011;19(3):203-211.
41. PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody Interpretation Guide

roche.com diagnostics.roche.com