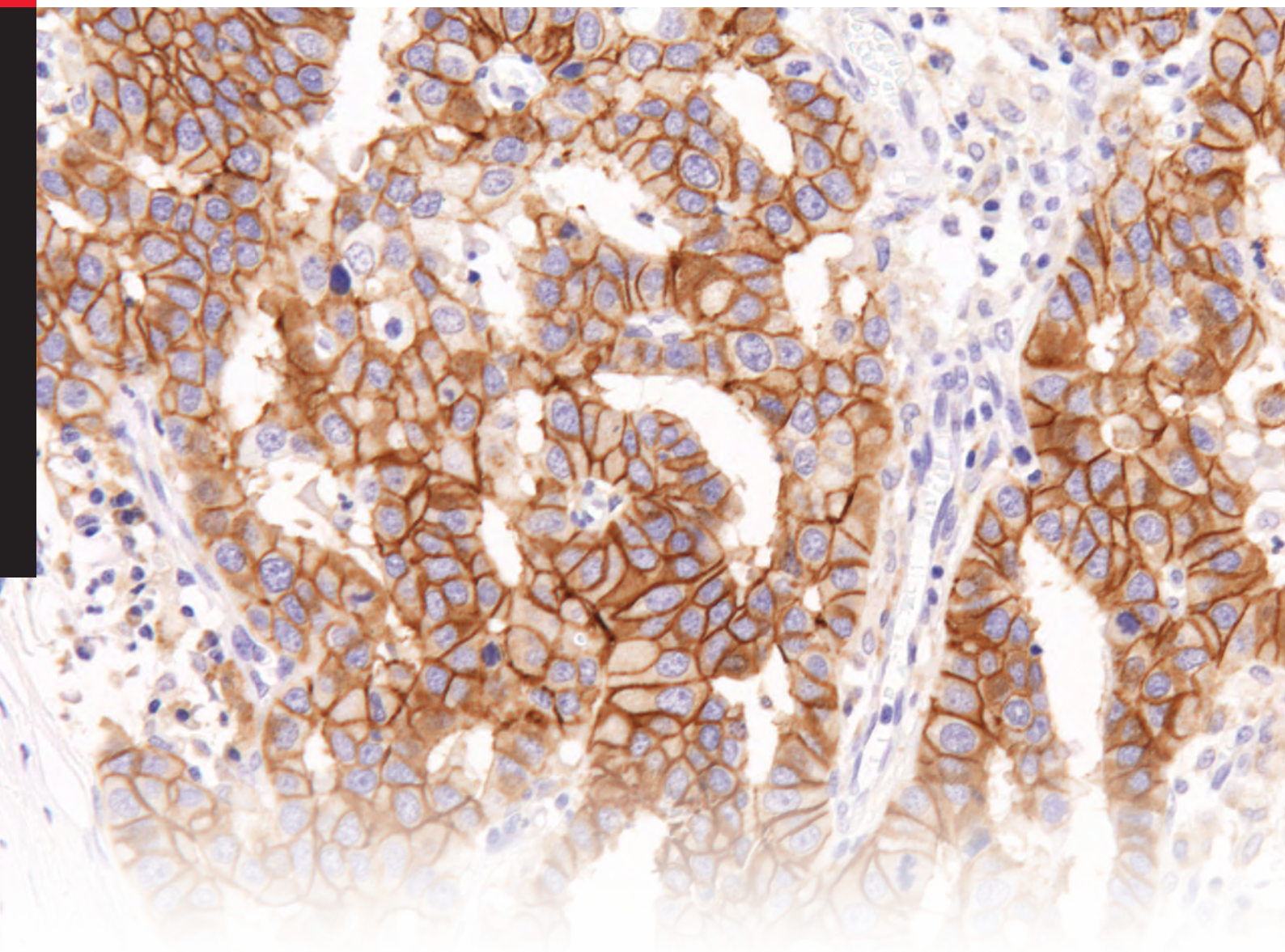
The Pathology Company

Leica BIOSYSTEMS



Bond Oracle™ HER2 IHC System

Interpretation Guide - Gastric Tissue

Bond Oracle™ HER2 IHC System

Interpretation Guide

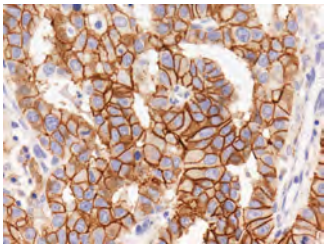
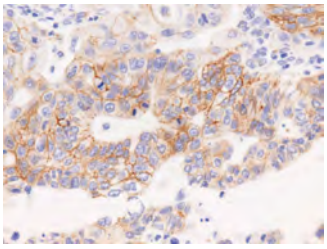
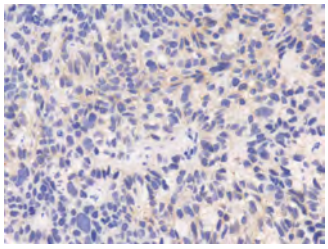
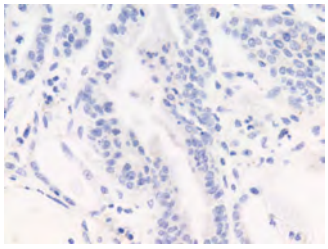
This document is provided as a guide to help scientists and pathologists achieve accurate, consistent and reproducible results.

The Interpretation Guide will familiarize you with the requirements for scoring adenocarcinomas of the stomach (including gastroesophageal junction) stained with the Bond Oracle HER2 IHC System and interpretation of the Oracle HER2 Control Slides.

The Interpretation Guide includes:

- Technical tips for ensuring high-quality HER2 staining and efficient throughput in your laboratory
- A review of the Bond Oracle HER2 IHC System Instructions For Use
- Guidance for interpretation of the Oracle HER2 Control Slides
- Examples of varying HER2 expression levels in gastric carcinomas

Consultation and continued review of the Bond Oracle HER2 IHC System Interpretation Guide provides a solid foundation for evaluating slides stained with the Bond Oracle HER2 IHC System.

This product is not for use in gastric cancer in the USA

Contents

Introduction

Bond Oracle HER2 IHC System

The Bond Oracle HER2 IHC System is a semi-quantitative immunohistochemical assay to determine HER2 (Human Epidermal Growth Factor Receptor 2) oncoprotein status in human breast cancer tissue and adenocarcinomas of the stomach (including gastroesophageal junction) processed for histological evaluation. The Bond Oracle HER2 IHC System is indicated as an aid in the assessment of patients for whom Herceptin® (trastuzumab) treatment is being considered (see Herceptin package insert). Decisions regarding Herceptin® treatment should be made within the context of the patient's clinical history.

The Bond Oracle HER2 IHC System contains components required to complete an immunohistochemical staining procedure for formalin-fixed, paraffin-embedded tissues. Following incubation with the ready-to-use HER2 Primary Antibody (clone CB11), this system employs ready-to-use Compact Polymer™ technology. The enzymatic conversion of the subsequently added chromogen results in the formation of a visible reaction product at the antigenic site. Tissue sections are counterstained, dehydrated, cleared and mounted. Results are interpreted using brightfield microscopy. Control slides with four, formalin-fixed, paraffin-embedded human breast cancer cell lines are provided to validate staining runs. The four cell lines demonstrate HER2 oncoprotein expression at 0, 1+, 2+ and 3+ intensities. The staining intensity of these cell lines has been correlated to both HER2 oncoprotein receptor load per cell and HER2 gene status.

For use on BOND fully automated, advanced staining system.

Product Code TA9145 is designed to stain 60 tests (150 slides):

60 test slides with HER2 Primary Antibody

60 test slides with HER2 Negative Control

15 HER2 Control Slides with HER2 Primary Antibody

15 positive in-house tissue controls with HER2 Primary Antibody

HER2 Overview

HER2, encoded by the c-erb-B2 gene, is one of four oncoproteins belonging to the Human Epidermal Growth Factor Receptor (HER1-4) family of tyrosine kinases and is overexpressed in 10–20% of invasive breast cancer cases[1,2,3]. Members of the HER family of receptors form ligand-mediated homo and heterodimers, where HER2 is the preferred partner for dimerization[4].

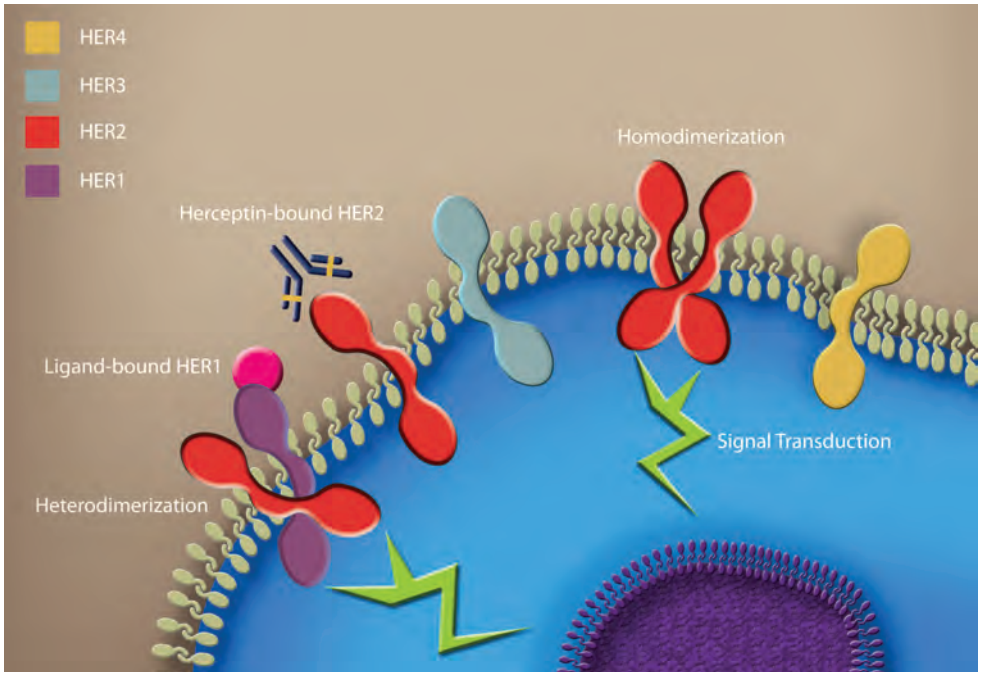
Dimerization of the HER family receptors initiates autophosphorylation cascades which in turn activate multiple cellular signalling pathways, including the Ras/Raf/MAPK and P13K/Akt cascades[5]. The resulting modification of gene transcription pathways has been shown to affect processes as diverse as cell division, angiogenesis, motility and adhesion[5].

Overexpression of HER2 leads to excessive activation of these pathways and may contribute to more aggressive growth associated with these tumor cells[6]. HER2 overexpression is associated with poor prognosis, including reduced disease-free survival and resistance to certain chemotherapeutic agents[7].

HER2 in Gastric Cancer

Studies have shown overexpression of HER2 in gastro-esophageal adenocarcinomas[8] and subsequent preclinical trials show that traztuzumab has significant anti-tumor activity in gastric cancer[9]

The ToGA trial (phase III trial) showed added benefit of combining Herceptin® with standard Chemotherapy in gastric cancer[10] which has led to the introduction of HER2 IHC testing of gastric cancer in routine histology laboratories.



References

1 Pawlowski V, Revillion F, Hebbar M. et al. Prognostic value of the Type I growth factor receptors in a large series of human primary breast cancers quantified with a real-time reverse transcription-polymerase chain reaction assay. ClinCancer Res. 2000 Nov(6): 4217-4225.

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7 Perez EA, Suman VJ, Davidson NE, et al, HER2 testing by local, central, and reference laboratories in specimens from the North Central Cancer Treatment Group N9831 intergroup adjuvant trial. J Clin Oncol. 2006 Jul 1;24(19): 3032-8.

8 Tanner M, et al. Amplification of HER2-2 in gastric carcinoma: association with Topoisomerase Iiα gene amplification, intestinal type, poor prognosis and sensitivity to trastuzumab. Ann. Oncol 2005;16: 273-8

9 Fujimoto-Ouchi K et al. Antitumor activity of trastuzumab in combination with chemotherapy in human xenograft models. Cancer Chemother. Pharmacol. 2007:;59: 795-805

10 Van Cutsem et al. Efficasy results from the ToGA trial: a phase III study of trastuzumab in combination with chemotherapy (CT) in first-line human epidermal growth factor receptor 2 (HER2)-positive advanced gastric cancer (GC) J. Clin. Oncol; 186: Abstract LBA4 509

Technical Considerations and Recommendations

Specimen Handling

Procedural deviations related to sample handling and processing can compromise HER2 assay performance. Variables that may alter assay performance are:

- Specimens drying prior to fixation
- Type of fixative
- Temperature, age, storage and pH of fixative
- Length of fixation, specimen size, ratio of size to fixative volume
- Length of time in alcohol after primary fixation
- Processing time, temperature, pressure and chemicals used
- Storage of paraffin blocks
- Storage of cut sections

Fixation, Processing and Embedding

It is recommended that tissues are prepared in formalin-based fixatives and are routinely processed and paraffin-embedded. For example, resection specimens should be blocked into a thickness of 3–4 mm and fixed for 18–24 hours in 10% neutral-buffered formalin. The tissues should then be dehydrated in a series of alcohols and cleared through xylene, followed by impregnation with molten paraffin wax, held at no more than 60 °C.

Tissue Section Preparation

Appropriate tissue preparation is integral to the continued performance of the Bond Oracle HER2 IHC System.

Embedded tissue specimens should be sectioned at a thickness between 3–5 µm. Overheating of tissues during embedding or sections during drying can be detrimental to immunostaining and therefore should be avoided.

The slides required for tumor verification (H&E) and HER2 oncoprotein evaluation (Bond Oracle HER2 IHC System) should be prepared at the same time. To preserve antigenicity, tissue sections mounted on slides (BOND Plus Slides – product code S21.2113) should be stained within 4–6 weeks of sectioning when held at room temperature (20–25 °C). Following sectioning, slides should be incubated for 12–18 hours (overnight) at 37 °C. Sections that require additional adherence may be incubated at 60 °C for a further hour.

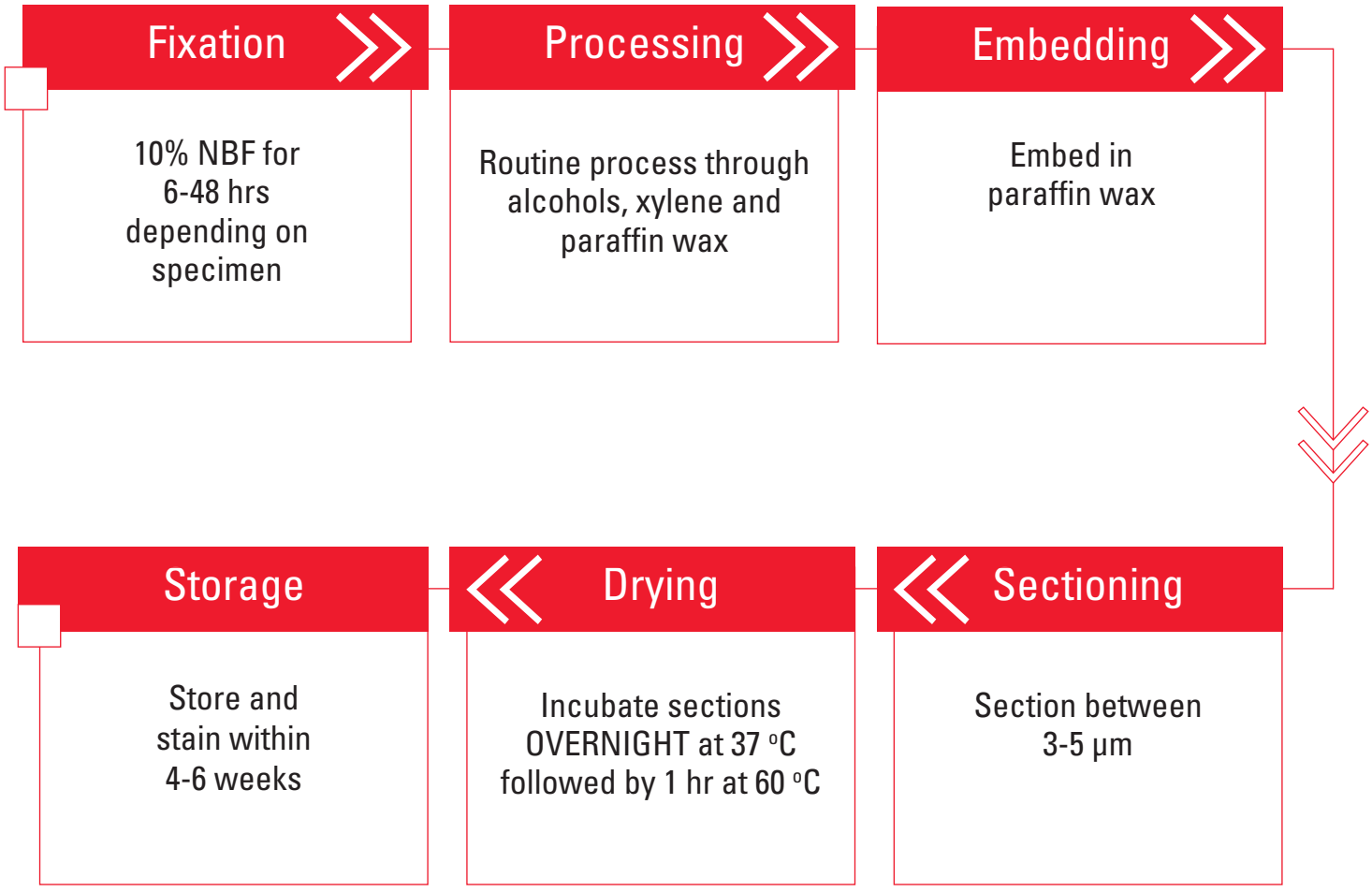
Protocol Defaults

The Bond Oracle HER2 IHC System and the BOND fully automated advanced staining system provide regulated, consistent epitope retrieval and controlled reagent incubations enabling reproducible results.

The following default settings are used with the Bond Oracle HER2 IHC System:

- Onboard dewaxing – *Dewax
- Regulated epitope retrieval – *HIER 25 min with ER1 (97)
- Controlled reagent incubations – *IHC Protocol H

For full details of the Bond Oracle HER2 IHC System protocol please consult the Instructions for Use document



The recommendations are compliant with current guidelines. Deviations from the recommended protocol should be vailidated by the laboratory.

A new BOND Universal Covertile™ should be used with each slide+

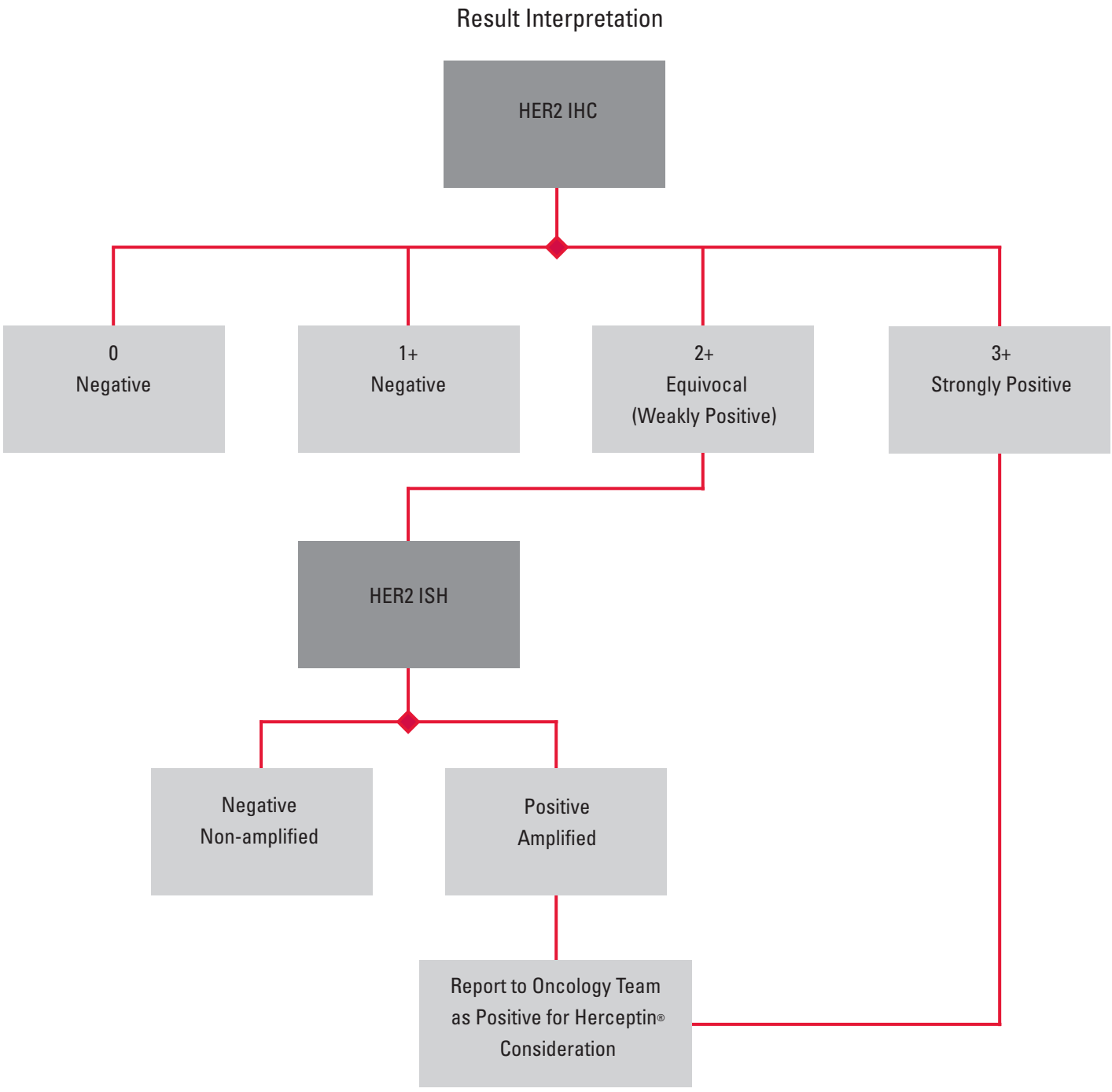
Using a new BOND Universal Covertile (product codes S21.2001, S21.4583 or S21.4611) every time will help ensure the consistency of this semi-quantitative test. The Covertile system allows gentle application and even flow of reagent across the sections to provide unmatched tissue care.

+The use of BOND Universal Covertiles which have been previously utilized for either immunohistochemical or in situ hybridization staining have not been validated with this test.

HER2 Testing

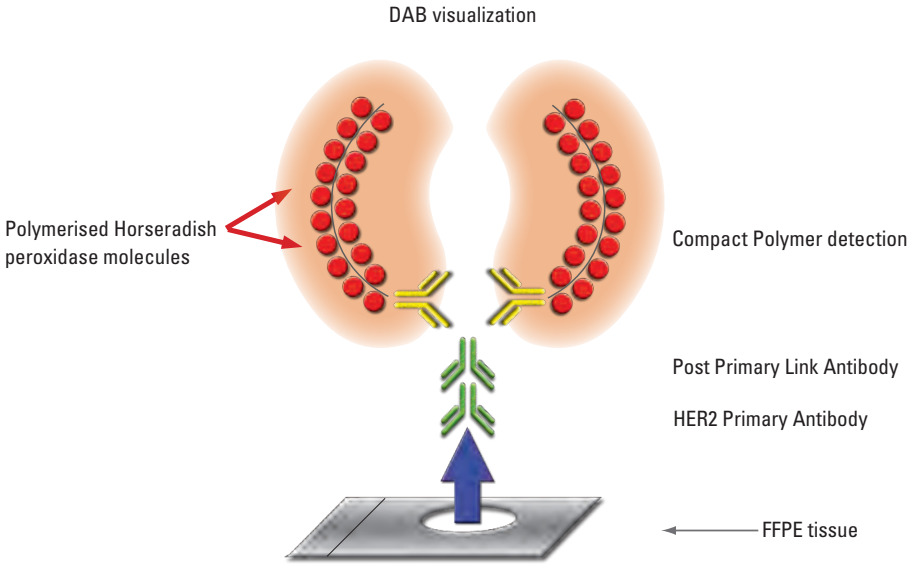
HER2 Testing Algorithm

First line assessment of patient samples using the Bond Oracle HER2 IHC System is used to determine HER2 oncoprotein levels at expression levels of 0, 1+, 2+ and 3+ immunohistochemical (IHC) staining intensities. Cases exhibiting weakly positive (2+) staining may be considered equivocal and reflexed to in situ hybridization (ISH) testing.



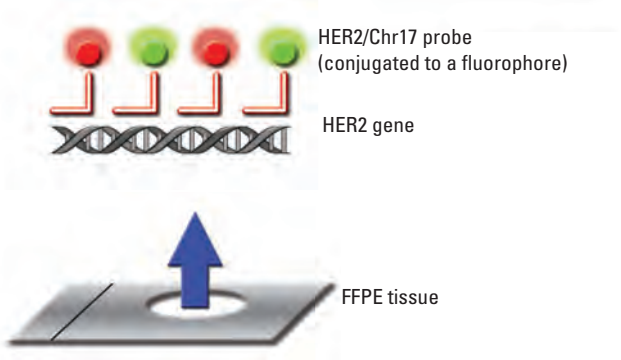
HER2 Oncoprotein Expression Determined by Immunohistochemistry

HER2 testing using IHC targets the HER2 oncoprotein located on the cell membrane. The Bond Oracle HER2 IHC System utilizes a target-specific primary antibody to label the HER2 protein. This antibody is then visualized using a multi-step Compact Polymer detection system.



HER2 Gene Status Determined by In Situ Hybridization

Cases found to be equivocal (2+, weakly positive) using the Bond Oracle HER2 IHC System may be further evaluated for HER2 gene status by in situ hybridization (ISH). ISH techniques utilize HER2 and Chromosome 17 signal enumeration probes, detected fluorescently to assess gene amplification status.



Bond Oracle HER2 IHC System

A complete solution including all reagents and control slides

Peroxide Block

A peroxide block is used in IHC techniques to block endogenous peroxidase within the tissue section. This is important as endogenous peroxidase may cause non specific background by association with the horseradish peroxidase (HRP) of the polymer components of the detection system.

HER2 Negative Control

The Bond Oracle HER2 IHC System contains a ready-to-use mouse IgG at an equivalent concentration to the HER2 Primary Antibody.

It is important to use a negative control antibody on each patient case to confirm the lack of detection system cross-reactivity to specifically targeted cells/cellular components.

HER2 Primary Antibody

The Bond Oracle HER2 IHC System contains the anti-HER2 antibody, clone CB11 affinity purified, mouse-monoclonal, in a fully optimized, ready-to-use format. Clone CB11, originally developed by Corbett et al, manufactured exclusively by Leica Biosystems Newcastle Ltd, is directed against the internal domain of the HER2 oncoprotein.

Performance monitoring of the Bond Oracle HER2 IHC System has shown a high degree of concordance to the Dako HercepTest and Ventana Medical Systems Inc. PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody, using recommended commercial interpretation guidelines.

Compact Polymer technology

The Compact Polymer™ detection system utilized by the Bond Oracle HER2 IHC System is part of a family of novel, controlled polymerization technologies that have been specifically developed to prepare polymeric HRP-linked antibody conjugates. The problem of nonspecific endogenous biotin staining, which may be seen with streptavidin/biotin detection systems, does not occur.

DAB visualization

Chromogen and Substrate Buffer combine in a reaction catalyzed by the polymerized enzymes to produce a brown precipitate which is viewed by brightfield microscopy.

Hematoxylin

Hematoxylin nuclear counterstain for IHC assessment of HER2 expression should be light; excessive counterstain can obscure staining results and make interpretation difficult.

HER2 Control Slide

A cornerstone of the Bond Oracle HER2 IHC System, the Oracle HER2 Control Slides, contain four formalin-fixed, paraffin-embedded human breast cancer cell lines expressing the HER2 oncoprotein at staining intensities of 0, 1+, 2+ and 3+. The cells are routinely processed using Peloris processing technology to ensure consistent manufacturing from batch to batch.

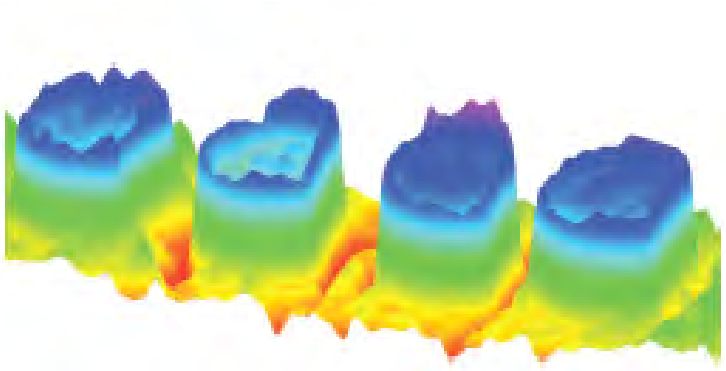
The HER2 control cell lines are designed as procedural controls, confirming procedural accuracy of the Bond Oracle HER2 IHC System. They validate:

- Reagent optimization and assay performance
- Correct protocol implementation
- BOND Instrument performance

Assay Validation using the Oracle HER2 Control Slide

The Oracle HER2 Control Slides, provide a comprehensive control method for assessing the consistency of assay performance using four formalin-fixed, paraffin embedded human breast cancer cell lines expressing the HER2 oncoprotein at staining intensities of 0, 1+, 2+ and 3+. The addition of a 2+ cell line provides additional confidence, by more closely monitoring the potential for assay variation.

Each Oracle HER2 Control Slide is non-destructively QC tested using a patented white light interferometry system[1]. This unique process means accurate section thickness is maintained and control slides stain consistently. This level of control is critical to achieving accurate HER2 assay validation and continuous batch performance.



3D image of Oracle HER2 control cell line spots on a glass slide as generated by white light interferometry [1]
This technique provides an accurate method for consistent Oracle HER2 Control Slides that stain reproducibly.

Cell Line Characterization Data

The Oracle HER2 control cell lines have been fully characterized for immunohistochemical profile, HER2 gene status and HER2 receptor load.

Cell Line	Bond Oracle HER2 IHC System Profile	HER2 Receptor Load per Cell*	HER2 Gene Amplification Status+	
			HER2 Copy Number	HER2:Chr17 Gene Ratio
SK-BR-3	3+	$4.3x10^5$	13.35	3.55
MDA-MB-453	2+	$1.4x10^5$	5.73	2.05
MDA-MB-175	1+	$6.3x10^4$	3.33	1.20
MDA-MB-231	0	$9.3x10^3$	3.15	1.13

*HER2 receptor load analysis as assessed by flow cytometry.

+HER2 gene amplification status as assessed by dual probe (HER2 and Chromosome 17) FISH (Vysis PathVysion).

References:
1. Barker C, et al. Non-destructive quality control of HER2 control cell line sections: the use of interferometry for measuring section thickness and implications for HER2 interpretation on breast tissue. Accepted for publication 27 Feb 2009; AIMM

Interpretation of Oracle HER2 Control Slide Staining

For each staining assessment, slides should be examined in the order presented below to determine the validity of the staining run and to enable semi-quantitative assessment of the staining intensity of the sample tissue.

1. HER2 Control Slide
2. In-house positive control
3. In-house negative control
4. Patient tissue – HER2 Negative Control
5. Patient tissue – HER2 Primary Antibody

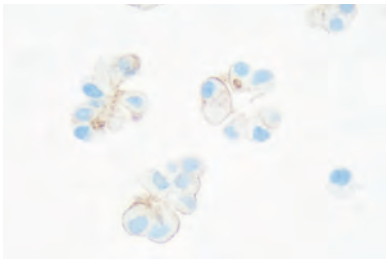
Levels of HER2 expression and their associated staining patterns for the HER2 control cell lines are represented below.

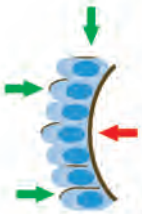
0

MDA-MB-231

No staining in the MDA-MB-231 cell line (0)

1+

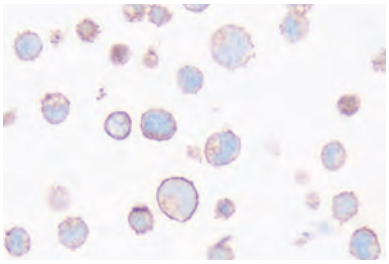




MDA-MB-175

Presence of faint/barely perceptible, brown, incomplete cell membrane staining in the MDA-MB-175 cell line (1+)

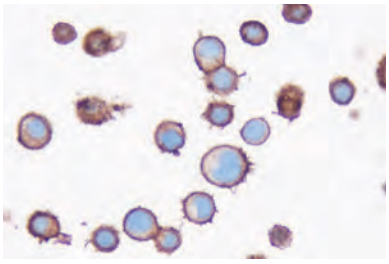
2+



MDA-MB-453

Presence of weak to moderate, brown, complete cell membrane staining in the MDA-MB-453 cell line (2+)

3+



SK-BR-3

Presence of strong brown, complete cell membrane staining in the SK-BR-3 cell line (3+)

Important note: A feature of the MDA-MB-175 cell line (1+) is a distinct growth pattern in which the cells form clusters.

These clusters give rise to a continuous luminal brush border region across the cell cluster. This brush border staining is stronger than that of the rest of the cell membrane.

It is the faint/barely perceptible incomplete cell membrane staining that is the correct HER2 oncoprotein (1+) staining pattern.

If the cell lines perform outside of these criteria the accompanying slides should be considered invalid.

Control cell lines are provided for qualifying the procedure and reagents not as an interpretation reference.

Interpretation of In-house Tissue Controls Staining

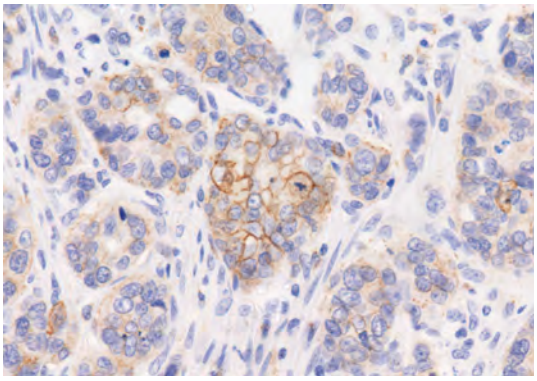
In-house tissue controls should be:

- **Included in each staining run**
- **Biopsy or surgical samples of known HER2 status, fixed, processed and embedded in the same manner as patient samples**

In-house Positive Tissue Control

Indicative of correctly prepared tissues and valid staining techniques. An ideal positive control section should demonstrate weak positive staining so as to define subtle changes in primary antibody sensitivity.

Known positive control tissue components should only be utilized for monitoring the correct performance of processed tissues together with test reagents, NOT as an aid in formulating a specific interpretation of patient samples. If the positive control tissue fails to demonstrate appropriate positive staining, results obtained with patient specimens should be considered invalid.

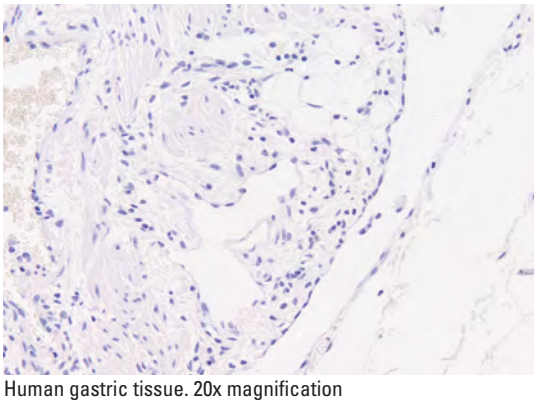


Human gastric tissue. 40x magnification

The in-house positive control tissue illustrated is an equivocal (2+) gastric adenocarcinoma.

In-house Negative Tissue Control

Verifies the specificity of the primary antibody and provides an indication of any nonspecific background staining. The variety of different cell types present in most tissue sections offers internal negative control sites (this should be verified by the user).



Human gastric tissue. 20x magnification

The in-house negative control component utilized in this case gastric adenocarcinoma cells are unstained demonstrating no cross reactivity with these normal cell components.

A multi-tissue control block containing tumors representing all four HER2 grades may also be effectively utilized as appropriate in-house control material.

If in-house tissue controls perform outside of the expected criteria the accompanying slides should be considered invalid.

Interpretation of Oracle HER2 Staining - Gastric

For the determination of HER2 oncoprotein expression, only membrane staining pattern and intensity should be evaluated using the scale presented below.

The use of 20–40x objective magnification should be used in the confirmation of the score. Cytoplasmic staining should be considered as non-specific staining and is not to be included in the assessment of membrane staining intensity. Only specimens from patients with stomach or gastroesophageal junction adenocarcinoma should be scored. In cases where both intestinal metaplasia and gastric adenocarcinoma are apparent, only the gastric adenocarcinoma component should be scored.

Surgical Specimens

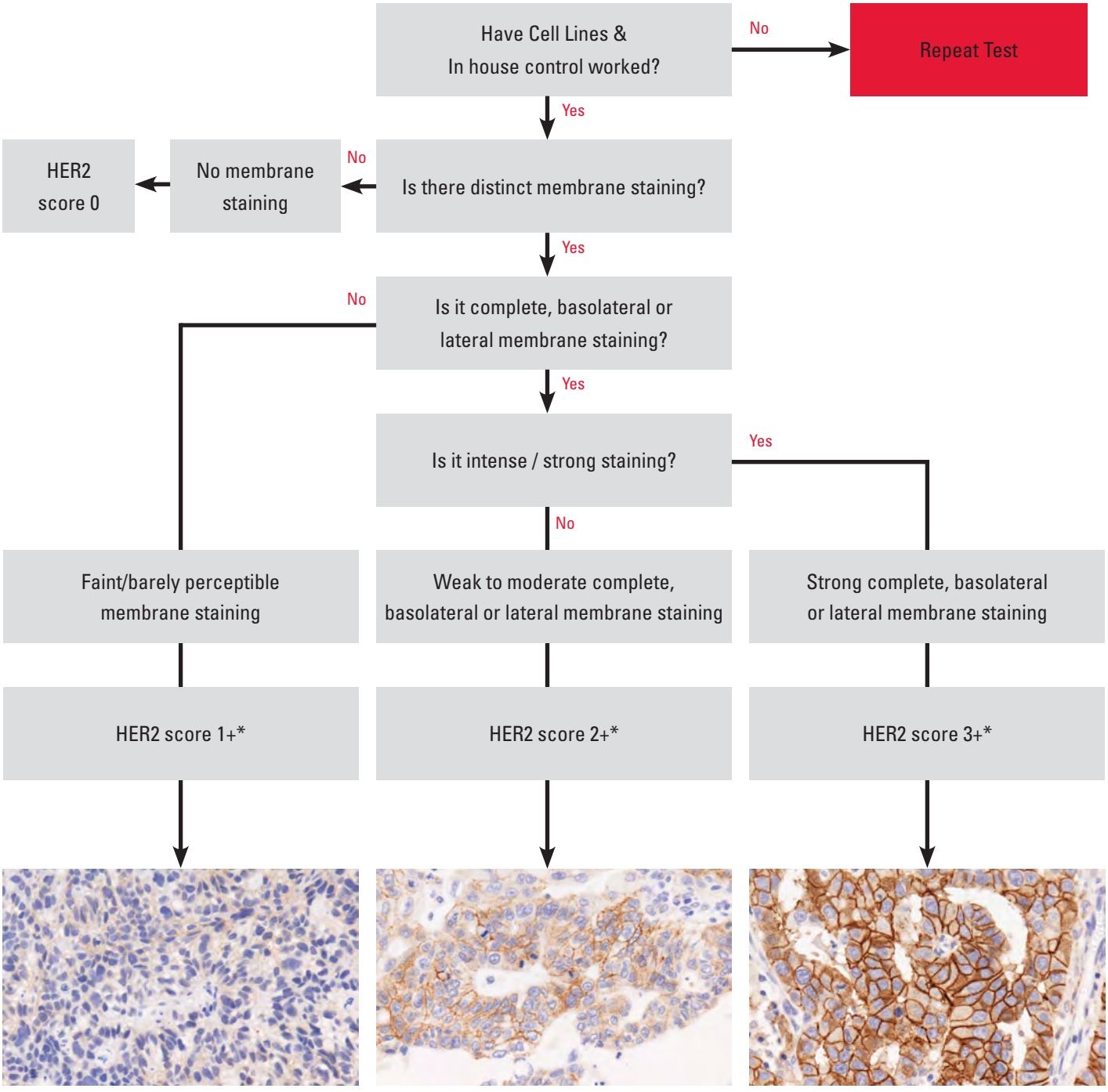
Immunohistochemical Staining Pattern	Score	Assessment
No staining is observed or membrane staining is observed **in less than 10% of the tumor cells.**	0	Negative
Faint/barely perceptible membrane staining is detected **in more than 10% of the tumor cells.** The cells are only stained in part of their membrane.	1+	Negative
Weak to moderate complete, basolateral or lateral membrane staining is observed **in equal to or more than 10% of the tumor cells.**	2+	Equivocal (Weakly Positive)
Strong complete, basolateral or lateral membrane staining **is observed in more than 10% of the tumor cells.**	3+	Strongly Positive

Due to the high degree of heterogeneity in gastric cancer the percentage staining threshold for surgical and biopsy specimens is different. The threshold for surgical specimens is 10% of positive stained tumor cells, while that for biopsies is a cluster of at least 5 cells.

Biopsy Specimens

Immunohistochemical Staining Pattern	Score	Assessment
No staining is observed in any tumor cell	0	Negative
Tumor cell cluster with a faint/barely perceptible membrane staining is observed **irrespective of percentage of cells stained.**	1+	Negative
Tumor cell cluster with weak to moderate complete, basolateral or lateral membrane staining is observed **irrespective of percentage of cells stained.**	2+	Equivocal (Weakly Positive)
Tumor cell cluster with a strong complete, basolateral or lateral membrane staining is observed **irrespective of percentage of cells stained.**	3+	Strongly Positive

Oracle HER2 Staining Workflow



40x magnification

40x magnification

40x magnification

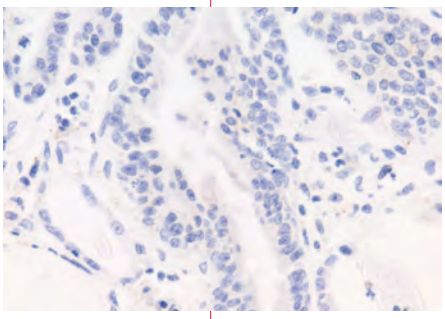
* Must at least be a cluster of 5 stained cells for biopsy specimens and at least 10% stained tumor cells for surgical specimens

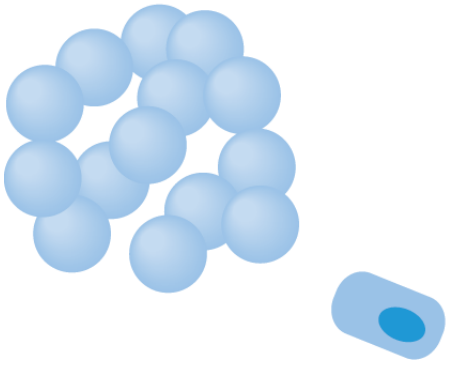
Bond Oracle HER2 IHC Staining Atlas

Bond Oracle HER2 IHC Profiling in Tumors

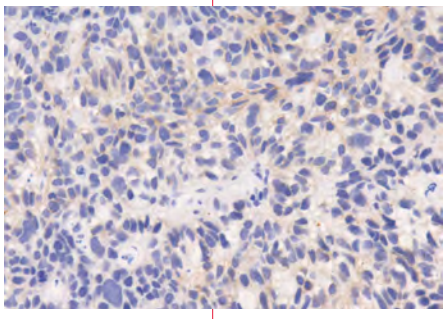
Levels of HER2 expression and associated staining patterns in tumor cells are represented below.

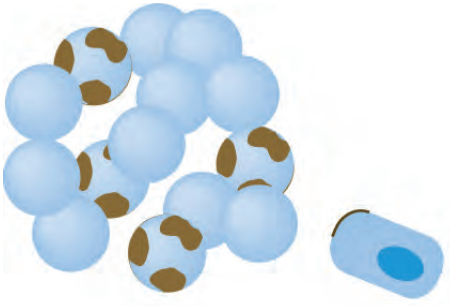
0



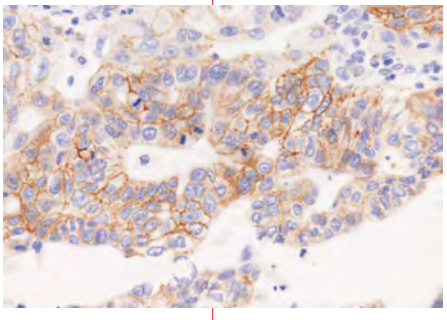


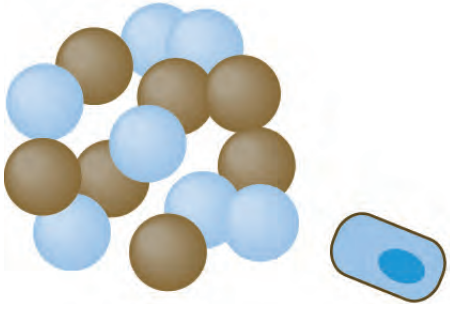
1+



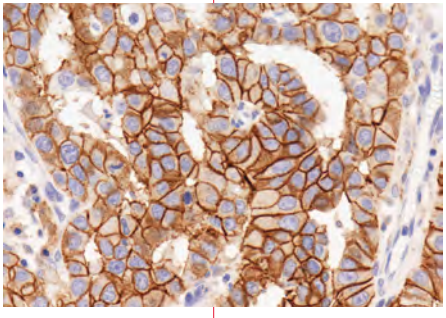


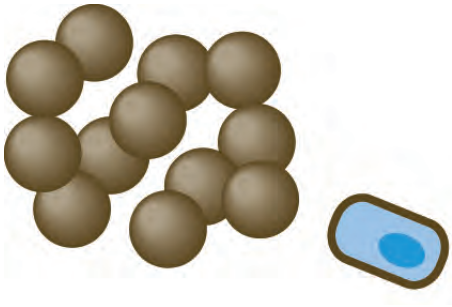
2+



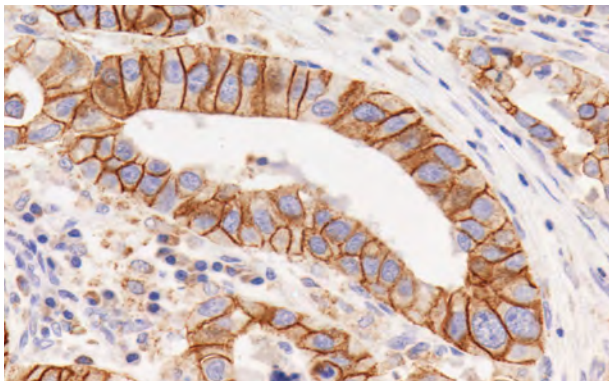


3+

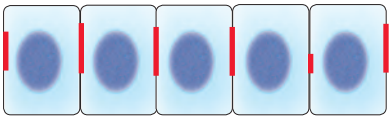




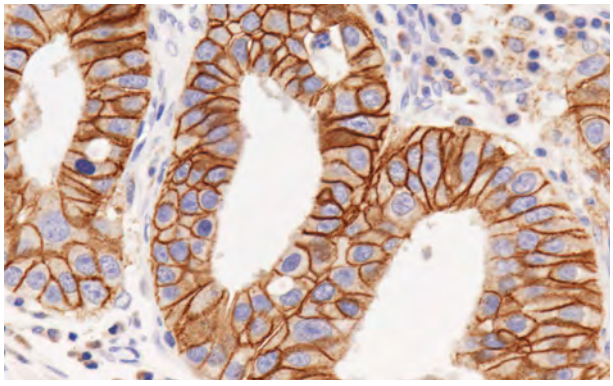
Tumor Profiling Interpretable Components



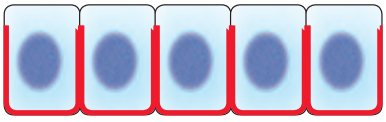
1. Lateral Membrane Staining



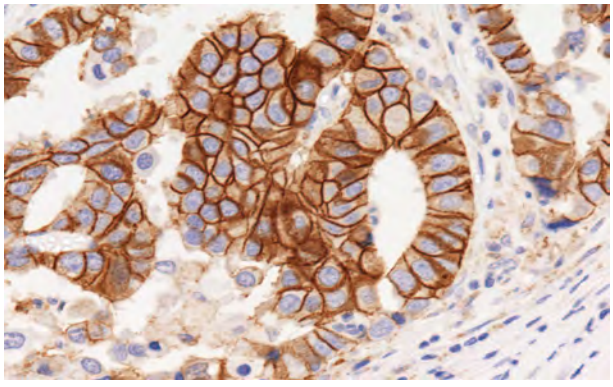
Lateral Membrane Staining – IHC x40
Oracle HER2 IHC stained section illustrating lateral staining in gastric cancer



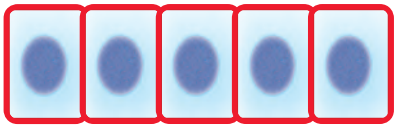
2. Basolateral Membrane Staining



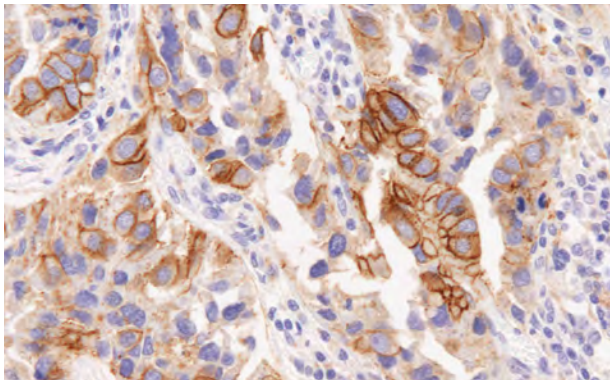
Basolateral Membrane Staining – IHC x40
Oracle HER2 IHC stained section illustrating basolateral staining in gastric cancer



3. Complete Membrane Staining



Complete Membrane Staining – IHC x40
Oracle HER2 IHC stained section illustrating complete membrane staining in gastric cancer



4. Heterogeneous Staining

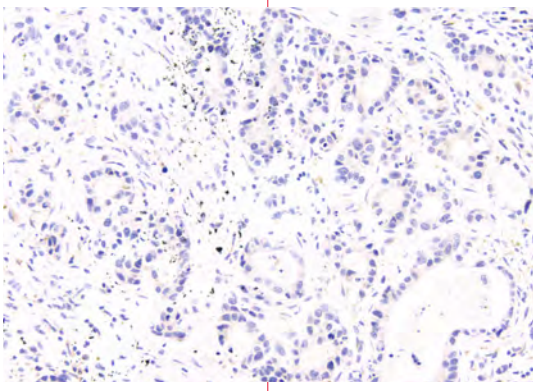
Heterogeneous Staining – IHC x40
Oracle HER2 IHC stained gastric cancer illustrating areas of heterogeneous staining, with areas of mixed 3+ and 2+ staining

Tumor Profiling - Reportable Staining

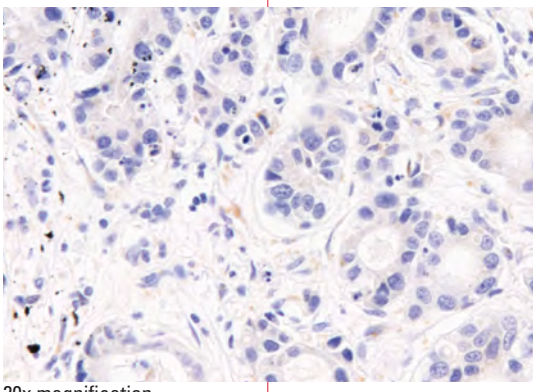
Gastric Adenocarcinoma (0)

Surgical Specimens	No staining is observed or membrane staining is observed **in less than 10% of the tumor cells.**
Biopsy Specimens	No staining is observed in any tumor cell

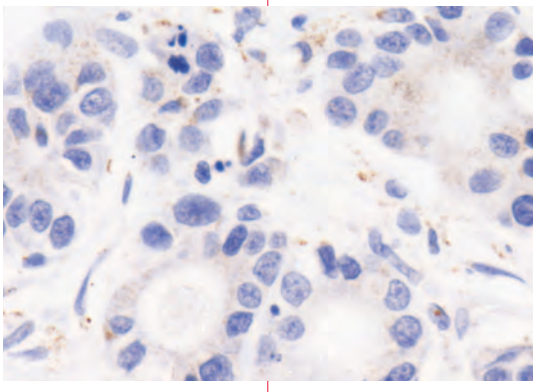
Case 1



10x magnification

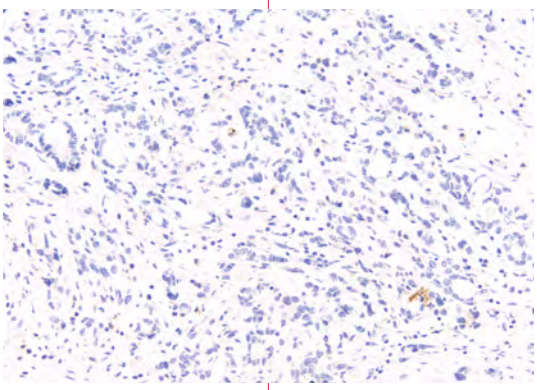


20x magnification

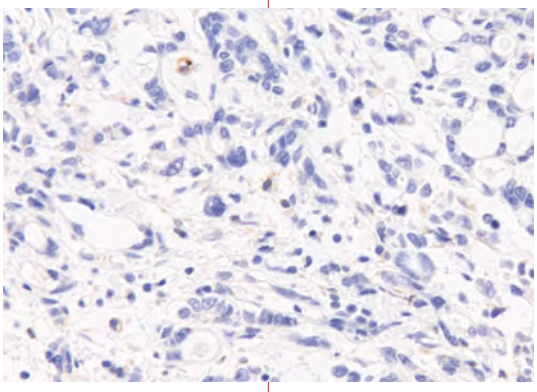


40x magnification

Case 2



10x magnification



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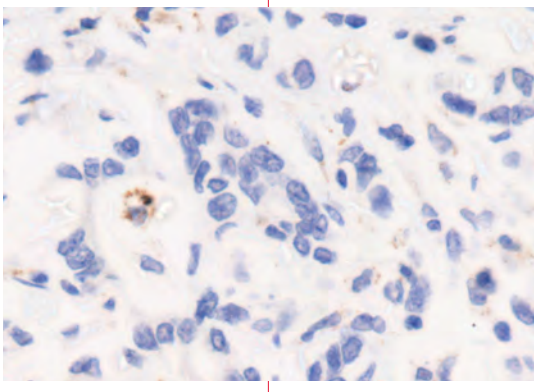


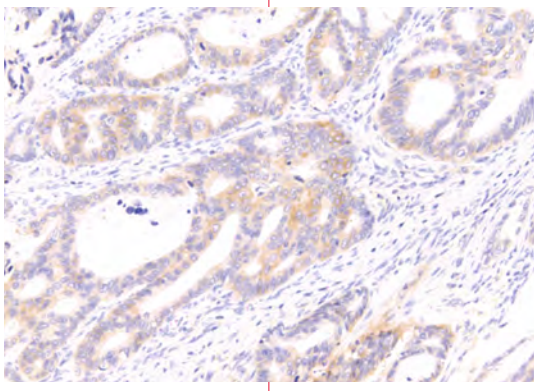
40x magnification

All images are surgical specimens unless otherwise stated

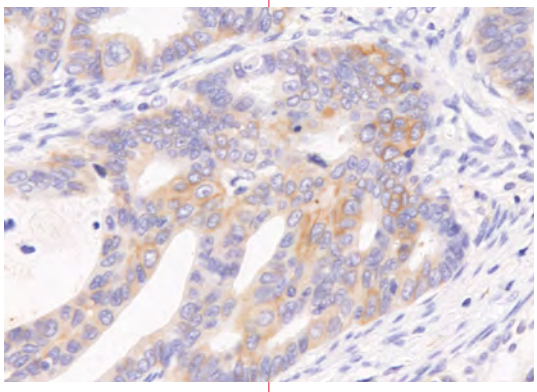
Gastric Adenocarcinoma (1+)

Surgical Specimens	Faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane.
Biopsy Specimens	Tumor cell cluster with a faint/barely perceptible membrane staining is observed irrespective of percentage of cells stained.

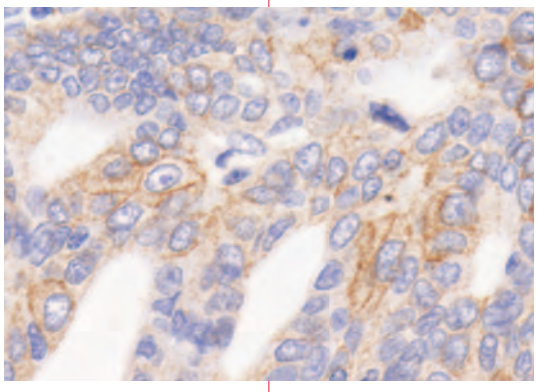
Case 3



10x magnification

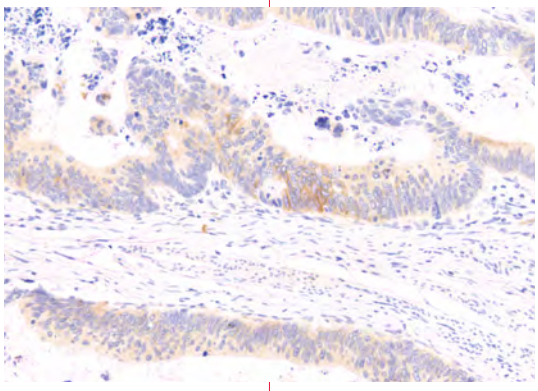


20x magnification

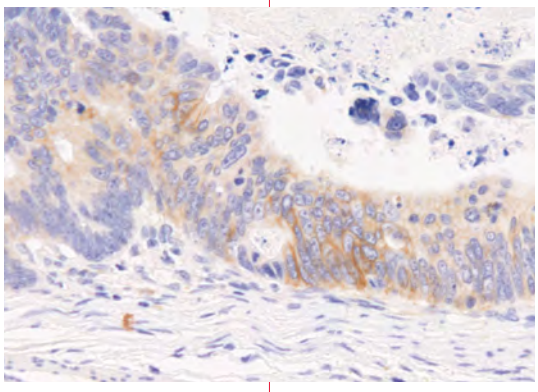


40x magnification

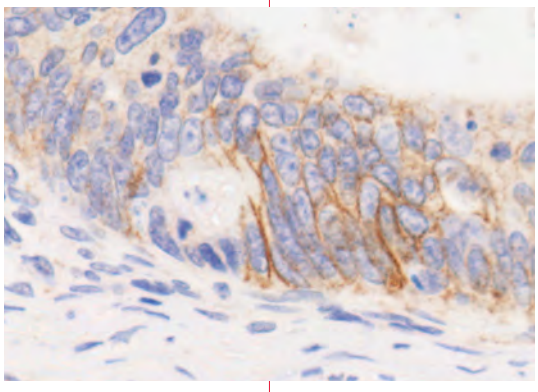
Case 4



10x magnification



20x magnification



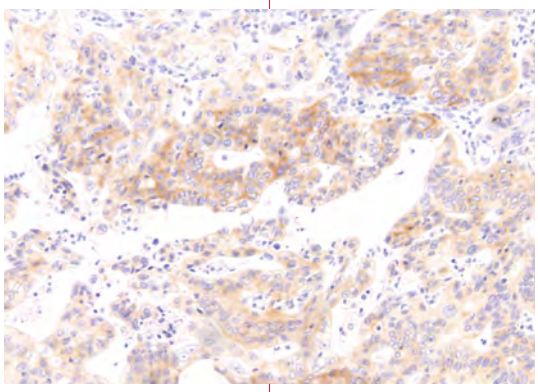
40x magnification

All images are surgical specimens unless otherwise stated

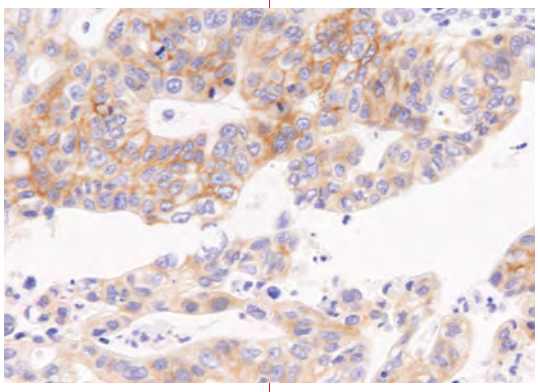
Gastric Adenocarcinoma (2+)

Surgical Specimens	Weak to moderate complete membrane basolateral or lateral staining is observed is equal to or more than 10% of the tumor cells
Biopsy Specimens	Tumor cell cluster with weak to moderate complete, basolateral or lateral membrane staining is observed **irrespective of percentage of cells stained**

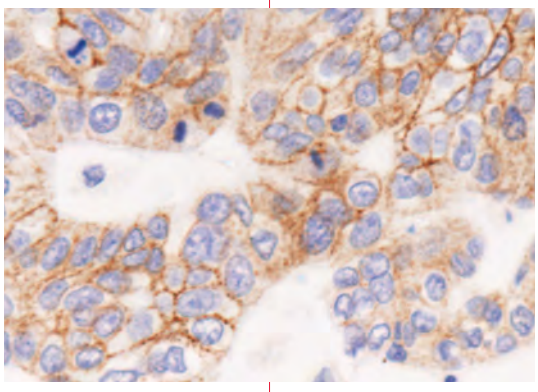
Case 5



10x magnification

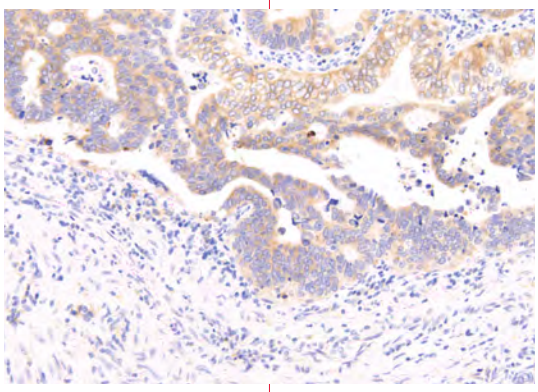


20x magnification

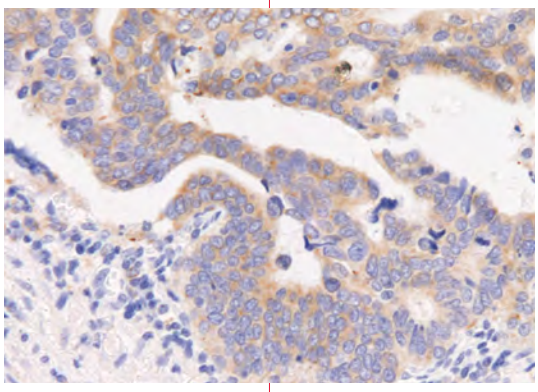


40x magnification

Case 6



10x magnification



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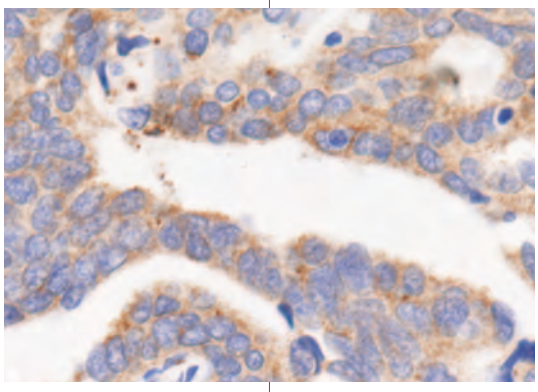


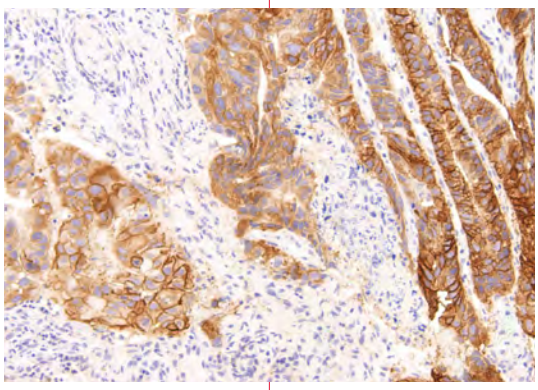
40x magnification

All images are surgical specimens unless otherwise stated

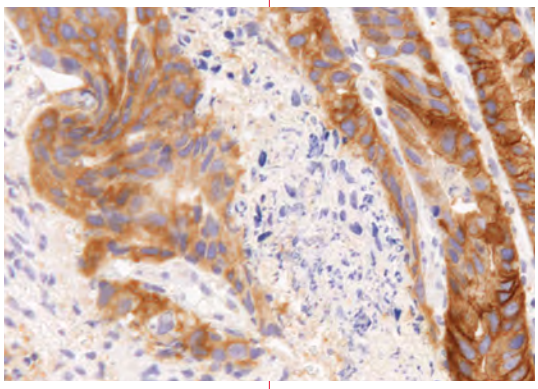
Gastric Adenocarcinoma (3+)

Surgical Specimens	Strong complete membrane staining is observed in more than 10% of the tumor cells.
Biopsy Specimens	Tumor cell cluster with a strong complete, basolateral or lateral membrane staining is observed **irrespective of percentage of cells stained.**

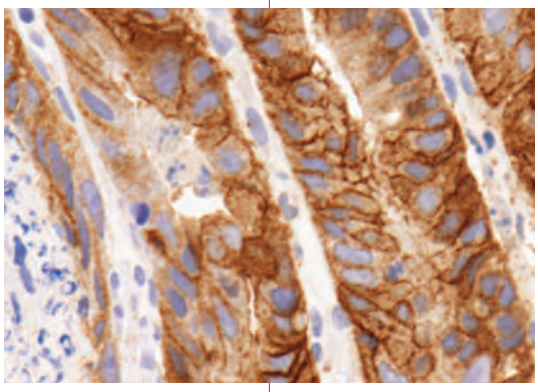
Case 7



10x magnification

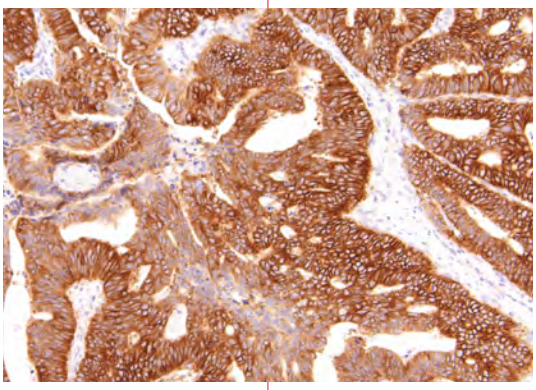


20x magnification



40x magnification

Case 8



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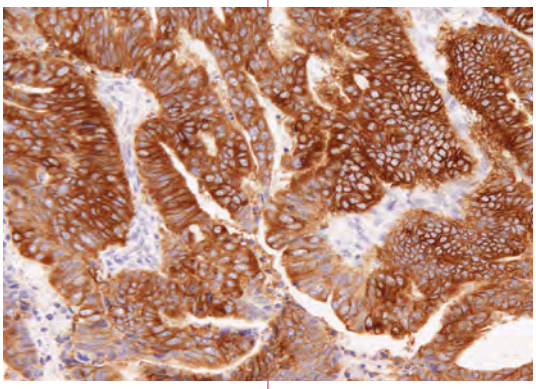
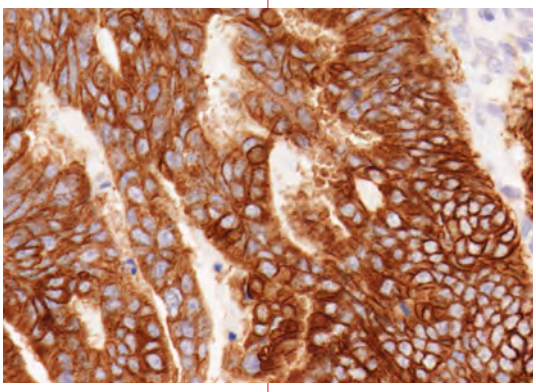


20x magnification



40x magnification

All images are surgical specimens unless otherwise stated

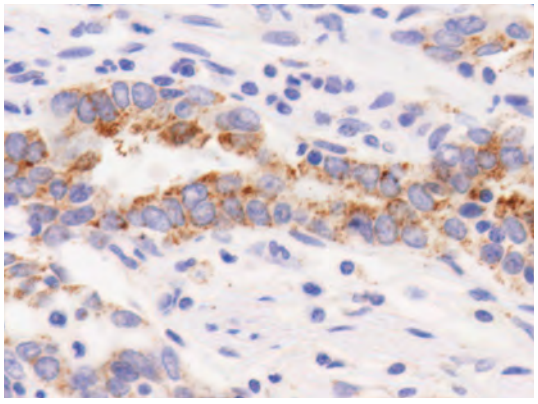
HER2 Expression in Normal Tissue

Normal Tissue Type	Staining Pattern	
	HER2 Primary Antibody	HER2 Negative Control
Adrenal	Negative	Negative
Brain, cerebellum	Negative	Negative
Brain, cerebrum	Negative	Negative
Breast	Negative	Negative
Bone marrow	Negative	Negative
Colon	Negative	Negative
Esophagus	Negative	Negative
Eye	Negative	Negative
Heart	Negative	Negative
Hypophysis	Moderate cytoplasmic staining observed in hypophyseal cells (1/3)	Negative
Kidney	Negative	Negative
Larynx	Negative	Negative
Liver	Negative	Negative
Lung	Negative	Negative
Mesothelium	Negative	Negative
Ovary	Negative	Negative
Pancreas	Negative	Negative
Parathyroid	Negative	Negative
Peripheral nerve	Negative	Negative
Prostate	Negative	Negative
Salivary gland	Negative	Negative
Skin	Negative	Negative
Small intestine	Negative	Negative
Spleen	Negative	Negative
Stomach	Weak cytoplasmic staining observed in gastric glands (2/3)	Negative
Striated muscle	Negative	Negative
Testis	Negative	Negative
Thymus	Negative	Negative
Thyroid	Negative	Negative
Tonsil	Negative	Negative
Uterine cervix	Negative	Negative
Uterus	Negative	Negative

Exclusions From Scoring

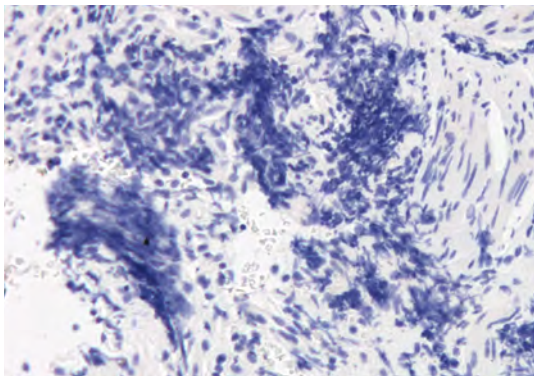
These areas should not be scored

Cytoplasmic Staining in the Absence of Membrane Staining



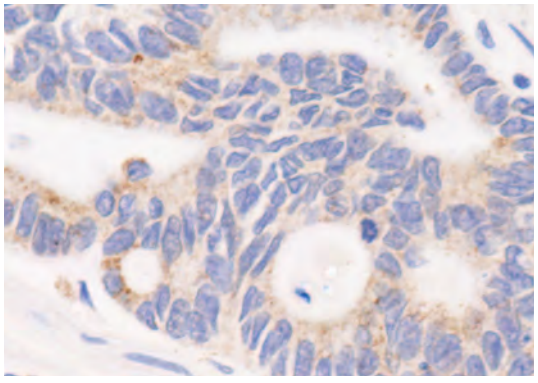
Human Gastric tissue x 40 magnification. Cytoplasmic staining in the absence of specific membrane staining of the gastric epithelia should NOT be scored positive for HER2.

Crush Artefact



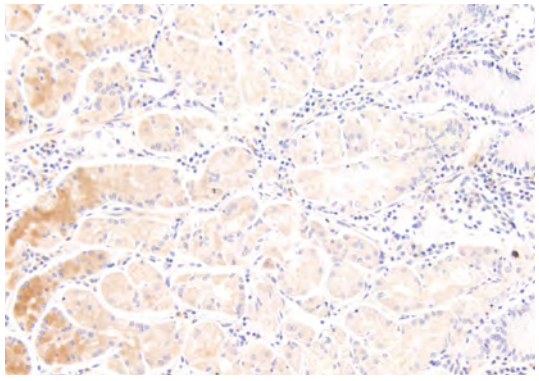
Human Gastric tissue x 20 magnification, illustration of crushed nuclei and damaged connective tissue. Note the intense deposition of hematoxylin in overlapping nuclei as a result of the tissue crush damage.

Luminal Staining



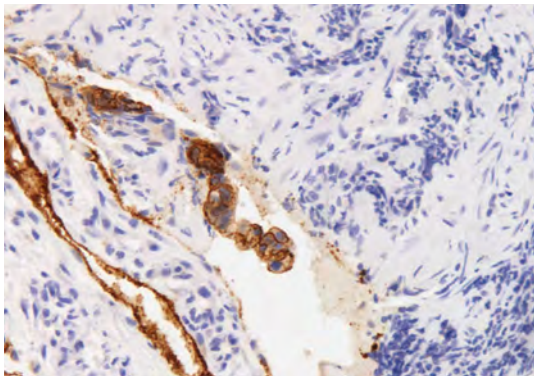
Human Gastric tissue x 40 magnification. Diffuse staining only at the luminal surface of the gastric epithelia should NOT be scored positive for HER2.

Peptic (Chief) Cell Staining



Human Gastric tissue x 10 magnification. Non-specific staining of Chief cells is cytoplasmic in nature and does not affect the ability to score adjacent tumor for specific HER2 staining.

Retraction Artefact



Human Gastric tissue x 20 magnification. Note detachment of tumor cells from the surrounding stromal surfaces which allows non-specific aggregation of DAB between retracted surfaces.

Bond Oracle™ HER2 IHC System – Training Checklist

Trainee Name:_____ Institution_____

Equipment for Training

- ❑ Bond Oracle HER2 IHC System
- ❑ BOND Ancillary reagents and consumables
  - ❑ BOND ER1
  - ❑ BOND Wash
  - ❑ BOND Dewax
  - ❑ BOND Universal Covertiles

- ❑ Bond Oracle HER2 IHC System Instructions for Use
- ❑ Bond Oracle HER2 IHC System Interpretation Guide
- ❑ Bond Oracle HER2 IHC System Scoring Guide
  These can be downloaded from website: www.LeicaBiosystems.com/TA9145-IFU
- ❑ Sufficient tissue of known HER2 status for procedural and interpretation training

Before Training

- ❑ Trainee has completed Bond Oracle HER2 IHC System e-Learning module - gastric

www.LeicaBiosystems.com/TA9145-elearning Certificate number: _____

- ❑ The BOND instrument software version: _____
- ❑ BOND instrument date of last PM: _____
- ❑ The BOND instrument is equipped with a new/clean FTP probe
- ❑ There is a cleaning schedule for the BOND instrument

Storage of Reagents

- ❑ Storage of Bond Oracle HER2 IHC System at 2-8 °C
- ❑ Check storage of bulk reagents
  - ❑ ER1 at 2-8 °C
  - ❑ BOND Wash at 2-8 °C
  - ❑ Dewax at 2-26 °C
- ❑ The lab uses de-ionised H_2O

Tissue Handling, Fixation and Processing

- ❑ Appropriate formalin fixation and processing schedule (see IFU)
- ❑ Sectioning at 3-5 µm
- ❑ Sufficient drying and adherence

Procedure

- ❑ Check Bulk reagents, hazardous and non-hazardous waste
- ❑ Register Bond Oracle HER2 IHC System components on BOND
- ❑ Set up label profiles
- ❑ Set up a case
- ❑ Slide set up and optimal layout for the most efficient usage of the system
  Reference: Table 2 Bond Oracle IHC System IFU

Quality Control

- ❑ The importance of positive and negative control components
- ❑ The importance of in-house tissue controls
- ❑ The importance of using control cell lines (highlighting 1+ and 2+ cell lines)
- ❑ The importance of always using new BOND Universal Covertiles

Interpretation - Use slides stained in training run (on a pre-stained set)

- ❑ Slide Screening Order Rationale – the order in which to screen slides (see IFU)
- ❑ Review of Tables 5 & 6: (IFU) "Interpretation of Staining - gastric"
- ❑ What to expect of the Bond Oracle HER2 IHC System Control Cell Slides
  - ❑ Special attention to brush border staining of 1+ control cell line
- ❑ Interpretation of test slides:
  1. Biopsy Vs. Surgical Specimen?
  2. What percentage of the tumor stains?
  3. What is the intensity?
  4. Artifacts exclusions from staining

See IFU, Interpretation Guide and Scoring Guide

Signed on behalf of Leica Biosystems (Trainer):

……(Signature)

……(Name and Title)

……(Date)

Signed by End User (Trainee):

……(Signature)

……(Name and Title)

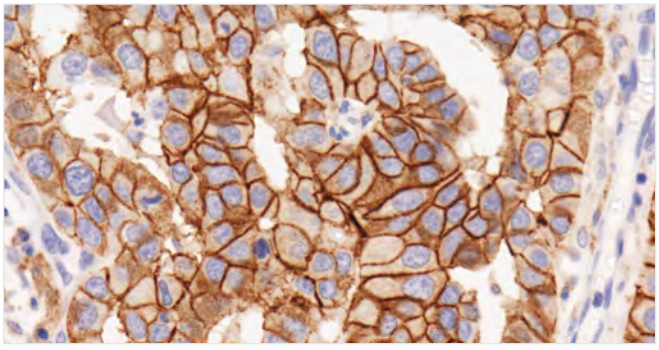
……(Date)

Please keep a copy of this checklist and the e-Learning certificate for each trainee for your training records

Notes

Notes

LeicaBiosystems.com



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- Breast Pathology
- Hematopathology
- Dermatopathology
- Head & Neck Pathology

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*Independent analysis commissioned by Leica Biosystems and conducted by Nordi QC according to the manufacturer's instructions for use on the corresponding staining platform.