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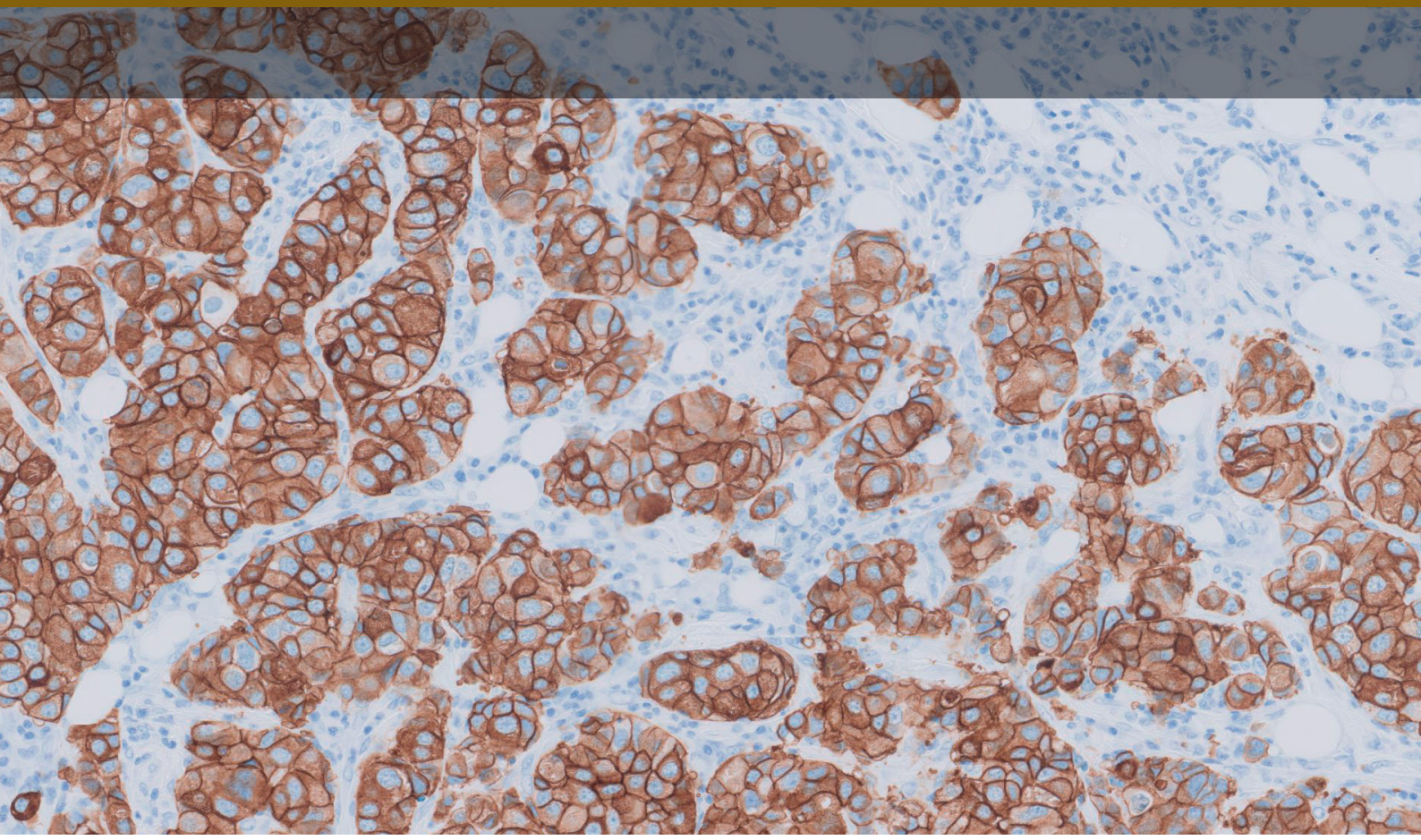
HercepTest™ mAb pharmDx (Dako Omnis) Interpretation Manual – Breast Cancer

For in vitro diagnostic use



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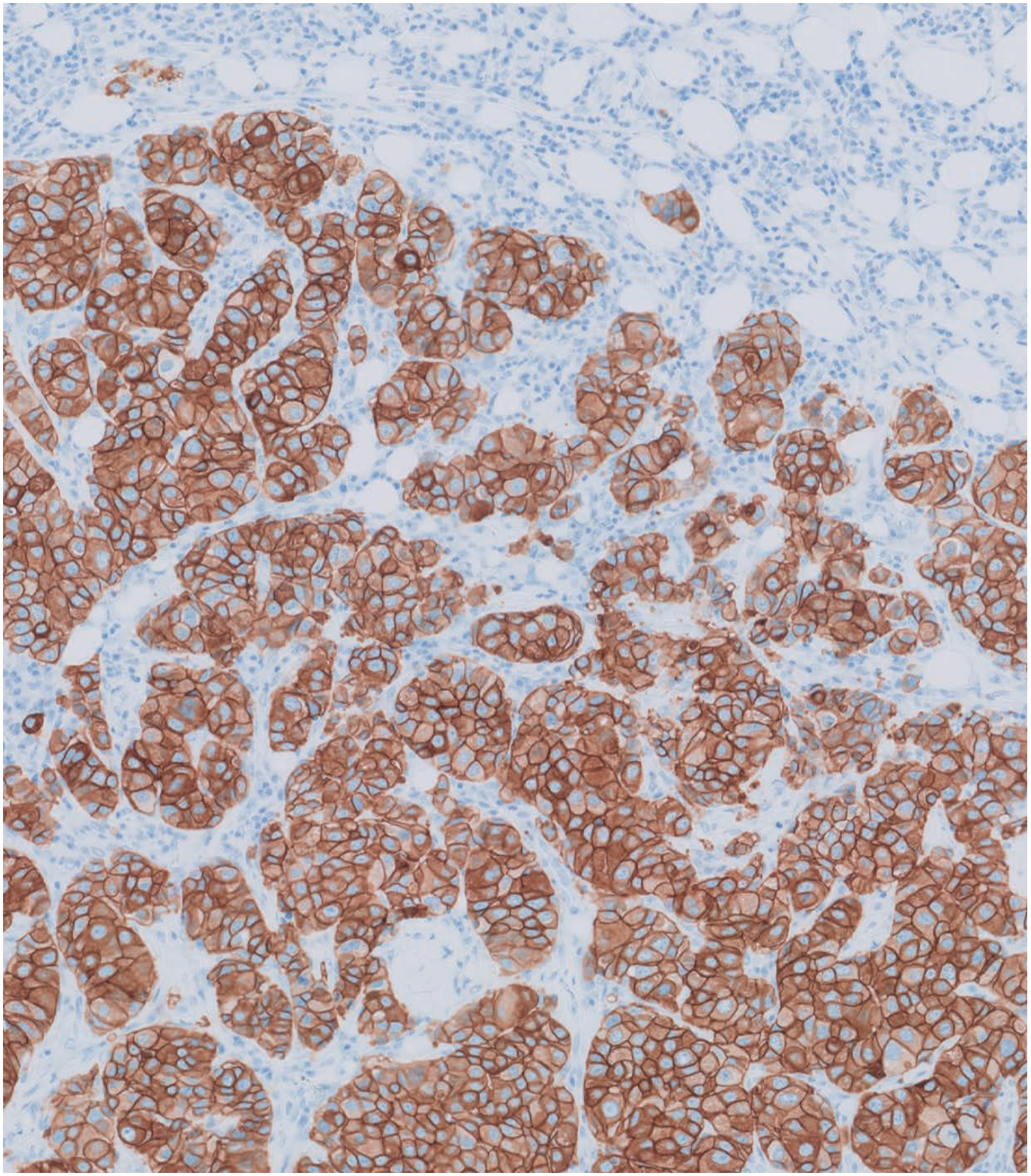
Intended Use

For in vitro diagnostic use.

HercepTest™ mAb pharmDx (Dako Omnis) is a semiquantitative immunohistochemical assay based on a primary monoclonal rabbit antibody (clone DG44) and an assay-specific visualization reagent. The assay determines HER2 protein overexpression in formalin-fixed, paraffin-embedded (FFPE) breast cancer tissues processed for histological evaluation. HercepTest™ mAb pharmDx (Dako Omnis) is indicated as an aid in the assessment of breast cancer patients for whom Herceptin® (trastuzumab) treatment is being considered (see Herceptin® package insert).

HercepTest™ mAb pharmDx (Dako Omnis) is for automated staining using Dako Omnis.

The licensed antibody is created by Epitomics Inc. (an Abcam company), using Abcam's proprietary rabbit monoclonal antibody technology covered under Patent No's 5,675,063 and 7,402,409.



Front page picture, breast carcinoma (FFPE) stained with HercepTest™ mAb pharmDx on Dako Omnis. Score 3+ (10x magnification).

Introduction

This Interpretation Manual is provided as a tool to help pathologists in assessing HER2 protein expression in FFPE breast cancer specimens stained with HercepTest™ mAb pharmDx (Dako Omnis). It provides scoring guidelines and technical information from the HercepTest™ mAb pharmDx (Dako Omnis) Instructions for Use (IFU) to aid in diagnostic assessment.

To help familiarize you with the requirements for scoring breast cancer tissue specimens stained with HercepTest™ mAb pharmDx (Dako Omnis), example cases of various HER2 expression levels are provided as reference. These example cases and guidance for interpretation of breast cancer specimens stained with HercepTest™ mAb pharmDx (Dako Omnis) can help individual laboratories achieve reliable and reproducible assessments.

HercepTest™ mAb pharmDx (Dako Omnis) is a semiquantitative immunohistochemical assay with a scoring system reflecting the cell membrane staining intensity and staining pattern.

The assay is interpreted as negative for HER2 protein overexpression (0 and 1+ staining intensity), weakly positive (2+ staining intensity), and strongly positive (3+ staining intensity), respectively. HercepTest™ mAb pharmDx (Dako Omnis) is not intended to provide prognostic information to the patient and physician and has not been validated for that purpose.

For more details on staining and interpretation please refer to the current version of the IFU for HercepTest™ mAb pharmDx (Dako Omnis), Code GE001.

Assay Interpretation

The clinical interpretation of any positive staining or its absence must be evaluated within the context of clinical presentation, morphology and other histopathological criteria. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and proper controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist, who is familiar with the antibodies, reagents and methods used, to interpret the stained preparation.

The product is intended for in vitro diagnostic (IVD) use.

Reporting Results

To help understand what information should be reported to the treating physician, please refer to the Reporting Results section of this manual on page 68.

Staining Procedure

Please refer to HercepTest™ mAb pharmDx (Dako Omnis) IFU and Dako Omnis Basic User Guide for details on specimen preparation, staining procedure, and use of the Dako Omnis instrument.

Images

The included images from digital slide scans are of breast cancer tissue stained on Dako Omnis with HercepTest™ mAb pharmDx (Dako Omnis) unless otherwise noted.

Fully anonymized remnant human biological material was supplied by Trans-Hit, Cureline, and Conversant.

Special thanks to ProPath Services for supplying the images on page 15. The presented FISH images have not been edited or manipulated and are published in their original format as provided by ProPath Services, not in any way affiliated Agilent Technologies.

Note: Image magnification levels may appear different than indicated in respective annotations due to adjustment of image size.

HER2 Overview

HER2 Protein and HER2 Family

The gene encoding human epidermal growth factor receptor 2 (HER2) protein is located on chromosome 17 and is a member of the *EGF/erbB* growth factor receptor gene family, which also includes epidermal growth factor receptor (*EGFR*, or *HER1*), *HER3/erbB3* and *HER4/erbB4*.

All of these genes encode transmembrane growth factor receptors, which are tyrosine kinase type 1 receptors with growth stimulating potential.

Activation of HER family members generally occurs when the ligand and a dimer of the same monomer or other member of the HER family are bound together. HER2 has no known ligand. Once activation has occurred, tyrosine autophoshorylation of cytoplasmic signal proteins transmits signals to the nucleus, thus regulating aspects of cell growth, division, differentiation, and migration. Overexpression of HER2 receptors results in receptors transmitting excessive signals for cell proliferation to the nucleus. In a fraction of patients with breast cancer, the HER2 protein is overexpressed as part of the process of malignant transformation and tumor progression.

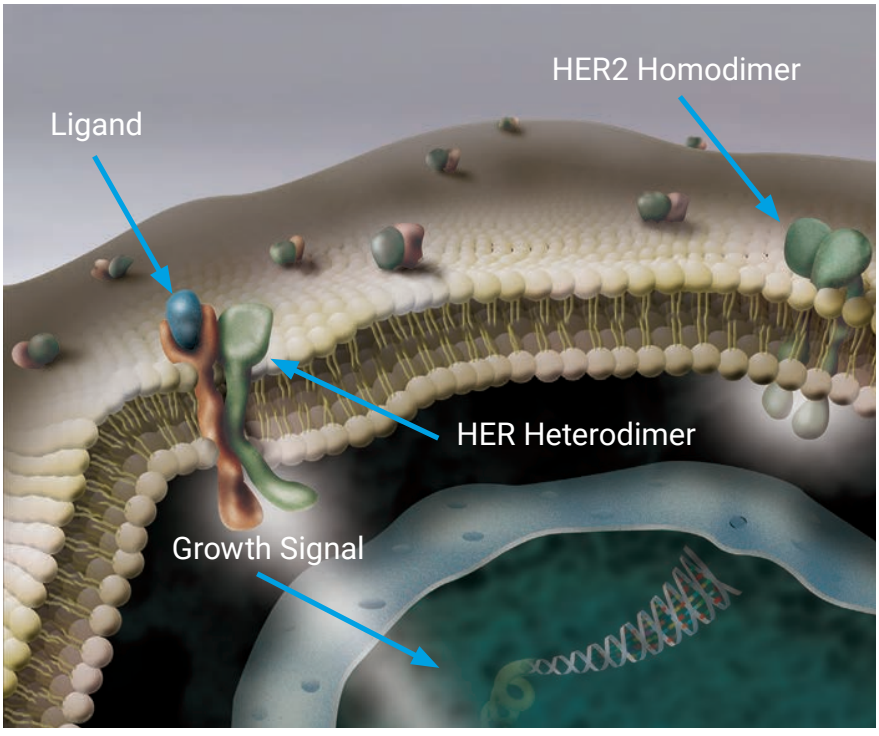


Figure 1: Representation of HER Family.

HER2 Testing with IHC and FISH

Immunohistochemistry (IHC) measures the level of HER2 receptor overexpression, while fluorescence in situ hybridization (FISH) quantifies the level of *HER2* gene amplification. Together they are the most commonly used methods of determining HER2 status in routine diagnostic settings.

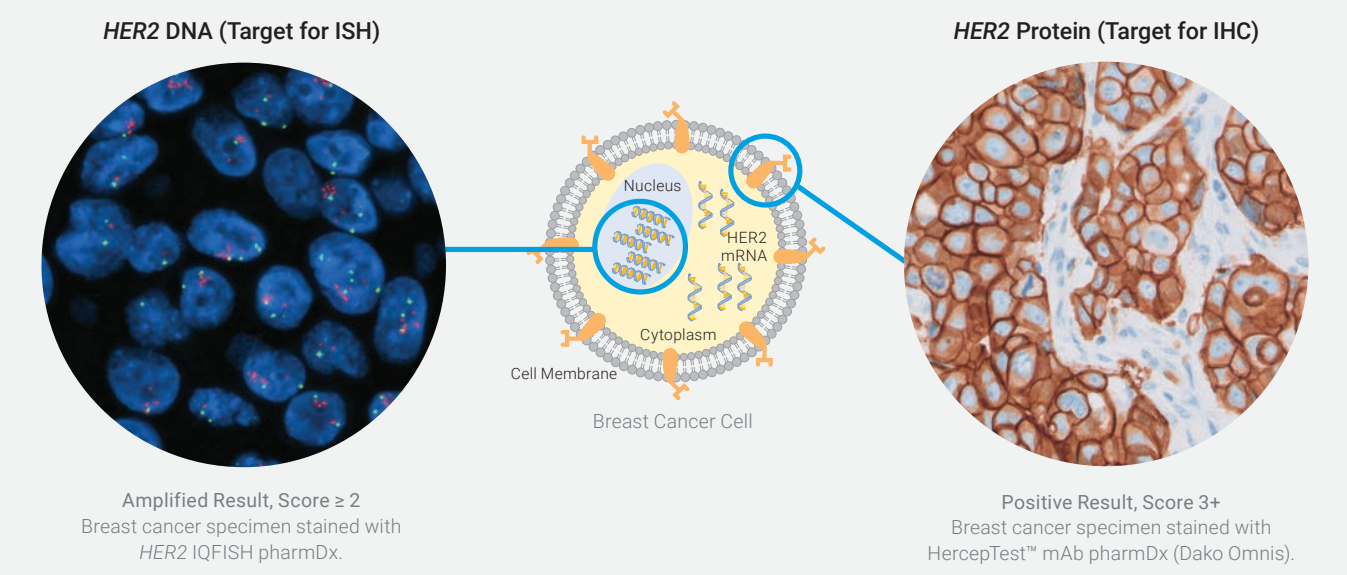


Figure 2: IHC and ISH targets for HER2 testing.

HercepTest™ mAb pharmDx (Dako Omnis) Overview

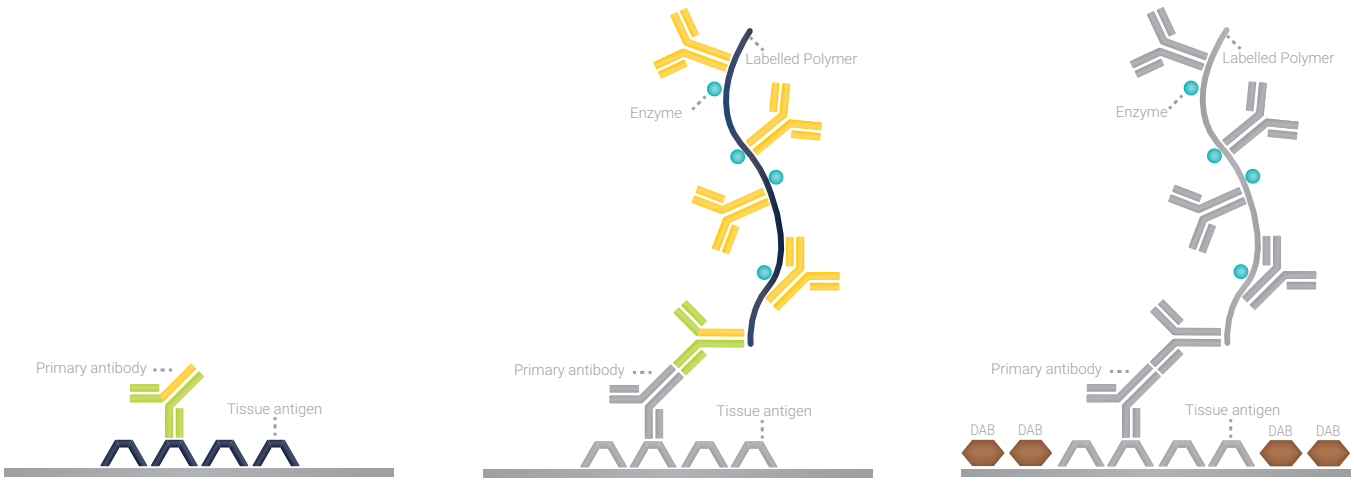
What is HercepTest™ mAb pharmDx (Dako Omnis)?

HercepTest™ mAb pharmDx (Dako Omnis) is a companion diagnostic assay indicated as an aid in the assessment of breast cancer patients for whom treatment with Herceptin® (trastuzumab) is being considered. HercepTest™ mAb pharmDx (Dako Omnis) is a semiquantitative immunohistochemical assay, which determines HER2 protein overexpression in breast cancer tissues processed for histological evaluation. The assay is intended for automated staining using the Dako Omnis instrument and is based on a primary monoclonal rabbit antibody (clone DG44) and an assay-specific, ready-to-use visualization reagent based on a biotin-free dextran technology.

Components of HercepTest™ mAb pharmDx (Dako Omnis)

The HercepTest™ mAb pharmDx (Dako Omnis) kit contains reagents required to complete a fully automated two-step immunohistochemical staining procedure for FFPE breast cancer specimens. The automated staining procedure includes deparaffinization of tissue sections and target retrieval using low pH target retrieval solution.

Following incubation with the ready-to-use primary antibody, this kit employs a ready-to-use visualization reagent consisting of secondary goat anti-rabbit immunoglobulin molecules and horseradish peroxidase molecules linked to a common dextran polymer backbone. The enzymatic conversion of the subsequently added DAB chromogen results in formation of a visible brown reaction product at the antigen site (Fig. 3). The specimen may then be counterstained and coverslipped. Results are interpreted using a bright field microscope.



Step 1: Application of Primary Antibody.

Step 2: Application of Visualization Reagent.

Step 3: Application of DAB+ Substrate Chromogen Solution.

Figure 3: HercepTest™ mAb pharmDx (Dako Omnis) staining procedure.

Kit Configuration

HercepTest™ mAb pharmDx (Dako Omnis) (Code GE001) contains reagents to perform 50 tests (50 tissue slides and 10 control slides incubated with Rabbit Anti-Human HER2 (Dako Omnis), and 10 slides incubated with an optional negative control reagent) (Fig. 4):

1. EnVision FLEX Peroxidase-Blocking Reagent (Dako Omnis)
2. Rabbit Anti-Human HER2 (Dako Omnis)
3. Visualization Reagent (Dako Omnis)
4. EnVision FLEX DAB+ Chromogen (Dako Omnis)
5. EnVision FLEX Substrate Buffer (Dako Omnis)
6. EnVision FLEX Target Retrieval Solution, Low pH (50x) (Dako Omnis)
7. Control Slides (Dako Omnis)

Figure 4: HercepTest™ mAb pharmDx (Dako Omnis) components.

For reagents that are required but not included in the kit, please refer to the current version of the IFU provided with HercepTest™ mAb pharmDx (Dako Omnis), Code GE001.

Optional reagent not supplied:

– FLEX Universal Negative Control, Rabbit, Ready-to-Use (Dako Omnis) (Code GA600)

Substitutions for Kit Components

Certain kit components may be substituted with corresponding reagents from EnVision FLEX retail products for Dako Omnis. The retail products are available as different kits, represented by GV-codes:

Kit component	Product code	Substitution reagent vial code
EnVision FLEX Peroxidase-Blocking Reagent (Dako Omnis)	GV800/GV823/GV900	DM841
EnVision FLEX Target Retrieval Solution, Low pH (50x) (Dako Omnis)	GV805	DM849
EnVision FLEX DAB+ Chromogen (Dako Omnis)	GV800/GV823/GV825	DM847
EnVision FLEX Substrate Buffer (Dako Omnis)	GV800/GV823/GV825/ GV900/GV925	DM843

Primary antibody (HercepTest™ mAb pharmDx Rabbit Anti-Human HER2 (Dako Omnis)), visualization reagent (HercepTest™ mAb pharmDx Visualization Reagent (Dako Omnis)) and control slides (HercepTest™ mAb pharmDx Control Slides (Dako Omnis)) are formulated specifically for use with this test. For the test to perform as specified, no substitutions of these components can be made. The three components are lot-restricted, which means that the three components must have the same lot number.

Primary antibody, visualization reagent and control slides must have the same lot number.

Quality Control

Deviations from the recommended procedures including tissue fixation, processing and embedding in the user's laboratory may produce significant variability in results. Quality controls are described below and include lab-supplied positive and negative control tissues and a Control Slide.

Control Slides

Each of the supplied ready-to-use Control Slides contains four pelleted FFPE human breast cancer cell lines with different levels of HER2 protein expression, and hence staining intensity levels. The four cell lines display a range of HER2 staining intensity levels, i.e. no HER2 staining (negative), low, medium, and high HER2 staining intensity. The evaluation of the supplied Control Slide verifies reagent performance only and does not verify tissue preparation. One slide should be stained in the first run after loading, or reloading, the lot-restricted reagents (primary antibody and visualization reagent) onboard the Dako Omnis instrument.

The Control Slides have been heat treated for better adherence of sections to glass slides. Any additional heat treatment of the Control Slides prior to testing may compromise staining results.

Control Tissues (Lab-supplied)

Differences in pre-analytical procedures in the user's laboratory may produce significant variability in results. Include positive and negative control tissue on the same slide as the patient tissue. Controls should be biopsy/surgical resection specimens fixed, processed, and embedded in the same manner as the patient samples. Control tissues processed differently from the patient specimen verify reagent performance only and do not verify tissue preparation.

Positive control tissue is indicative of correctly prepared tissues, proper staining techniques, and reagent performance. The positive control tissue should give weak positive staining, to aid detection of subtle changes in the primary antibody (HercepTest™ mAb pharmDx Rabbit Anti-Human HER2 (Dako Omnis)) sensitivity. Use previously determined HER2 protein 2+ overexpressing invasive (infiltrating) human breast carcinoma tissue for the ideal positive control tissue.

Negative control tissue should be used for verification of the specificity of the primary antibody (HercepTest™ mAb pharmDx Rabbit Anti-Human HER2 (Dako Omnis)) and to provide an indication of background staining. The negative control should be a tissue known to be HER2 protein negative, refer to HercepTest™ mAb pharmDx (Dako Omnis) IFU, *Summary of HercepTest™ mAb pharmDx (Dako Omnis) normal tissue reactivity*, for potential negative control tissue. The variety of different cell types present in most tissue sections offers internal negative control structures, which should be verified by the user.

Nonspecific Negative Control Reagent (Optional)

Negative Control Reagent, FLEX Universal Negative Control, Rabbit, Ready-to-Use (Dako Omnis) (Code GA600) may be used in place of the primary antibody on a section of the patient specimen to evaluate nonspecific staining and allow better interpretation of specific staining at the antigen site.

HER2 ISH Reflex Testing

Reflex testing is recommended in case of equivocal/weakly positive IHC results.

Products such as Dako *HER2* IQFISH pharmDx (Code K5731) or *HER2* IQFISH pharmDx (Dako Omnis) (Code GM333) can be used. Both assays are direct fluorescence in situ hybridization (FISH) assays for quantitative determination of *HER2* gene amplification in FFPE breast cancer tissue specimens.

Dako *HER2* IQFISH pharmDx (Code K5731) and *HER2* IQFISH pharmDx (Dako Omnis) (Code GM333) are indicated in adjunction to HercepTest™ in the assessment of patients for whom Herceptin® treatment is being considered.

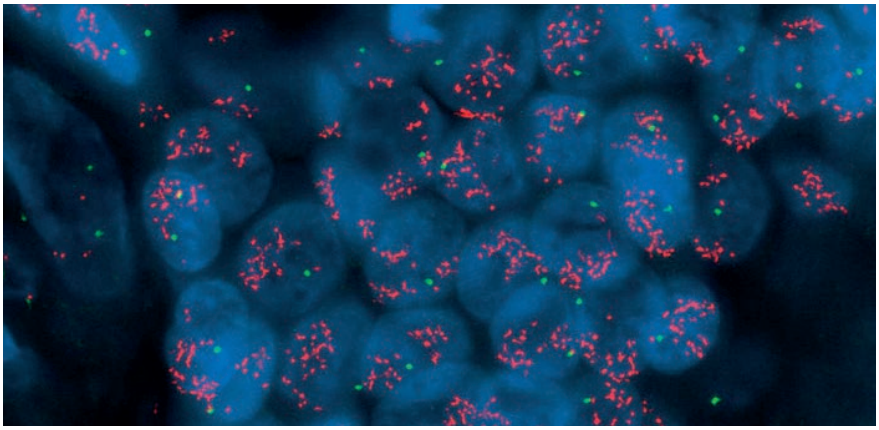


Figure 5: *HER2* IQFISH pharmDx stain of a *HER2* amplified breast tumor.

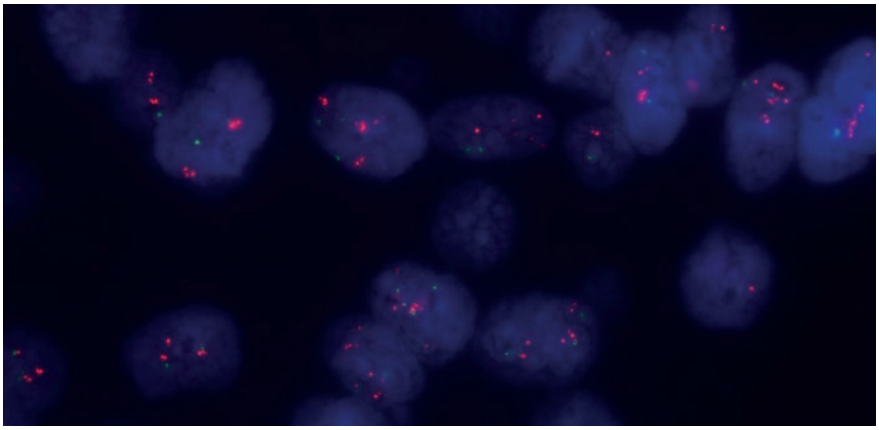


Figure 6: *HER2* IQFISH pharmDx (Dako Omnis) *HER2* amplified breast tumor.

Interpretation Guidelines

General Considerations

HercepTest™ mAb pharmDx (Dako Omnis) evaluation should be performed by a qualified pathologist using a bright field microscope. For details of the HercepTest™ mAb pharmDx (Dako Omnis) scoring guidelines refer to the section starting on page 22. Before examining the patient specimen for HER2 staining, it is important to examine the controls to assess staining quality. HER2 interpretation is best assessed by examining slides in the following order:

1. A hematoxylin and eosin (H&E) stain of the tissue specimen
2. Control Slide containing the four cell lines – ***only included in the first run after lot-restricted reagents (primary antibody and visualization reagent) have been loaded, or reloaded, onto Dako Omnis***
3. Positive control tissue
4. Negative control tissue
5. Patient tissue slide stained using the negative control reagent (**optional**)
6. Patient tissue slide stained using the primary antibody (HercepTest™ mAb pharmDx Rabbit Anti-Human HER2 (Dako Omnis))

H&E Staining

A hematoxylin and eosin (H&E) stain of the patient tissue is evaluated first to assess tissue histology and preservation quality. Tissue specimens should be undamaged, well preserved, and should confirm tumor indication. The presence of a tumor may not be obvious when looking at the sample stained with HercepTest™ mAb pharmDx (Dako Omnis). An H&E stain allows the pathologist to verify the presence of the invasive tumor.

Only specimens from patients with invasive breast carcinoma should be scored.

Evaluating Controls

Control Slide

Evaluate the HercepTest™ mAb pharmDx Control Slide (Dako Omnis) to verify performance of the lot-restricted reagents (primary antibody and visualization reagent). The presence of a brown staining reaction at the cell membrane is indicative of positive reactivity. Verify that the staining intensity of the four control cell lines, from no HER2 expression (no staining intensity, negative) to the cell line with a high HER2 protein expression (high staining intensity) show a gradual increase in staining intensity among the four different control cell lines.

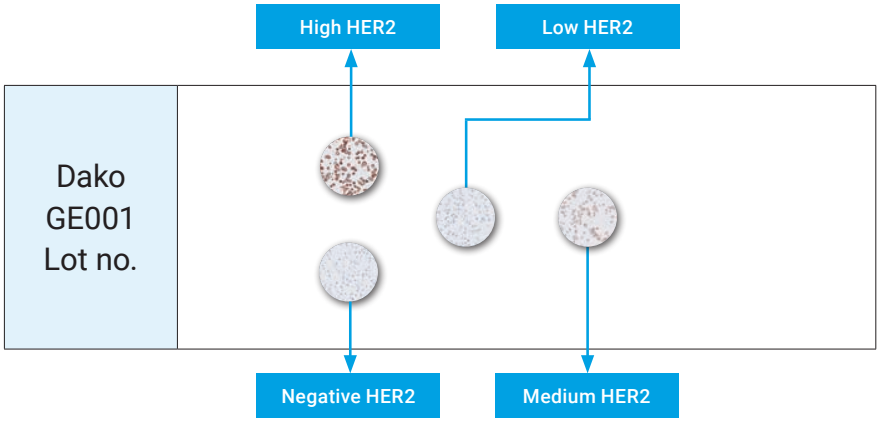


Figure 7: Ready-to-use Control Slide contains sections of four FFPE breast carcinoma cell lines (control cell lines) representing different levels of HER2 protein expression: Negative HER2 (MDA-231), Low HER2 (MDA-175), Medium HER2 (MDA-453), and High HER2 (SK-BR-3).

Evaluate the overall staining intensity using the following guidelines:

Table 1. Staining pattern for HercepTest™ mAb pharmDx Control Slides (Dako Omnis)

Staining intensity level	Level of HER2 protein expression
No staining	Negative. No HER2 protein expression
Weak to moderate brown partial cell membrane staining*	Low HER2 protein expression
Moderate to strong brown complete cell membrane staining	Medium HER2 protein expression
Strong brown complete cell membrane staining	High HER2 protein expression

** Certain exceptions to the staining pattern can be observed in the low expressing (Low) control cell line: 1) Dot-like immunostaining of the Golgi region of the cytoplasm can be observed. 2) Few cells with complete cell membrane staining may be present.*

If any of the control cell lines on the Control Slide perform outside of these criteria, the lot-restricted reagents used for the run should be discarded and the slides stained with these reagents must be considered invalid. Only slides stained after the load/reload of reagents that led to unapproved Control Slides should be discarded.

Do not use the Control Slide as an aid in interpretation of patient results.

Negative/No HER2 protein expression

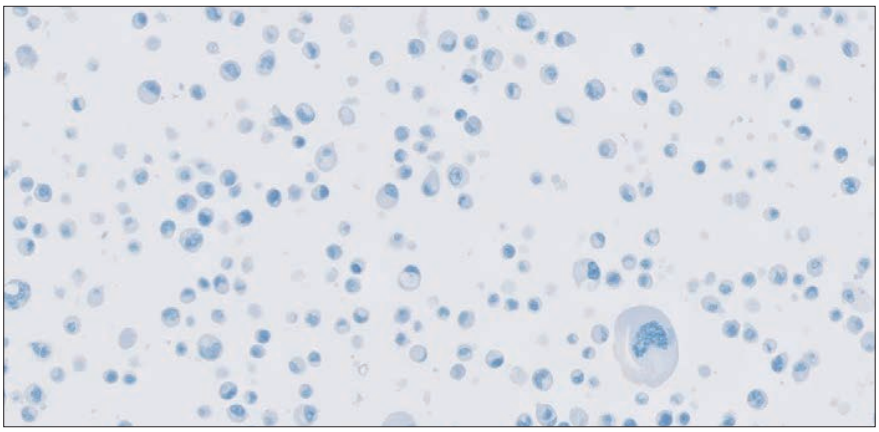


Figure 8: The negative control cell line displays no staining of the membrane (20x magnification).

Low HER2 protein expression

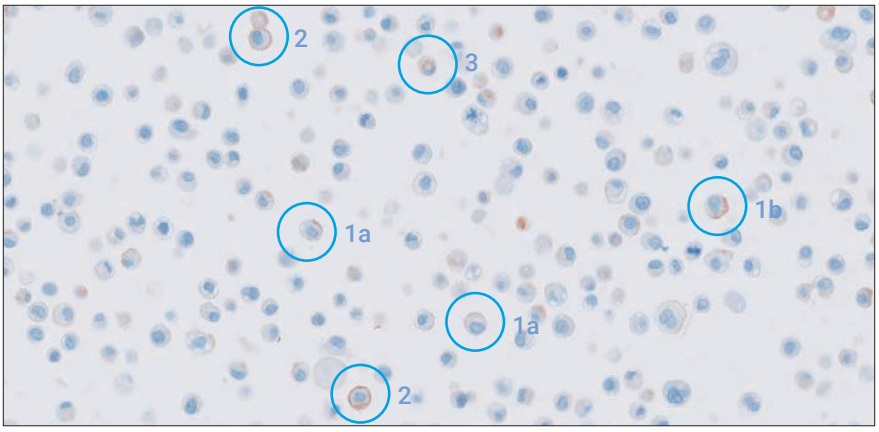


Figure 9: The control cell line with low HER2 protein expression can display different variations of specific HER2 membrane staining. Cells displaying a partial brown membrane staining, where the immunostaining is punctate and discontinuous (1a) are the true indicators of a valid staining run. In some cells, the partial brown membrane staining is more borderline (but still considered positive) consisting of a punctate and discontinuous immunostaining of both membrane and cytoplasm (1b). The borderline cells depicted here may reflect the difference in quality between digital imaging and bright field microscopy. In an IHC staining run of the control cell line with low HER2 expression, few cells with complete cell membrane staining may be present (2). In addition, dot-like immunostaining can be observed in the Golgi region of the cytoplasm in some cells (3) (20x magnification).

Medium HER2 protein expression

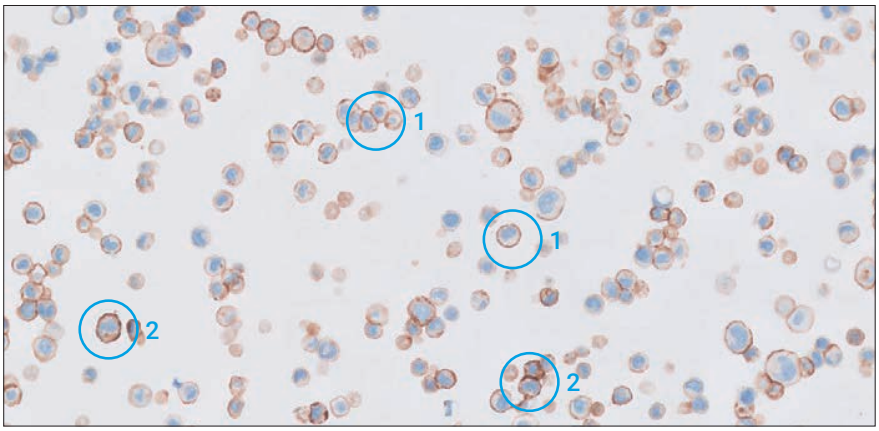


Figure 10: The control cell line with medium HER2 protein expression displays moderate to strong brown complete cell membrane staining. Moderate (1) and strong (2) (20x magnification).

High HER2 protein expression

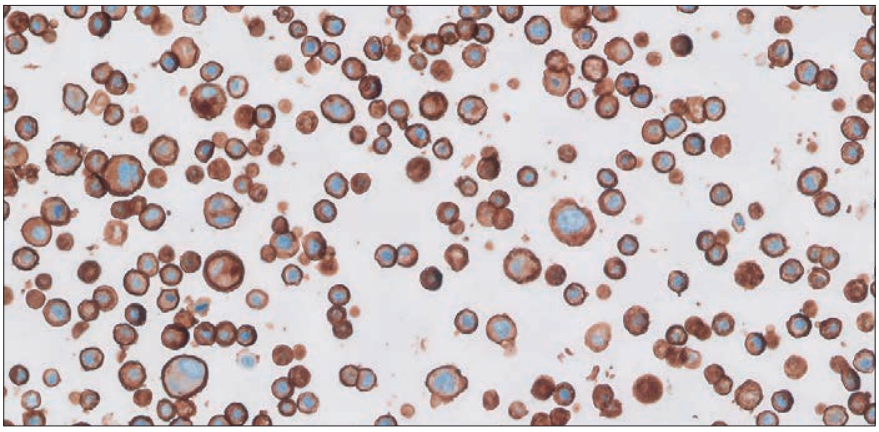


Figure 11: The control cell line with high HER2 protein expression displays strong brown complete cell membrane staining (20x magnification).

Positive and Negative Control Tissue

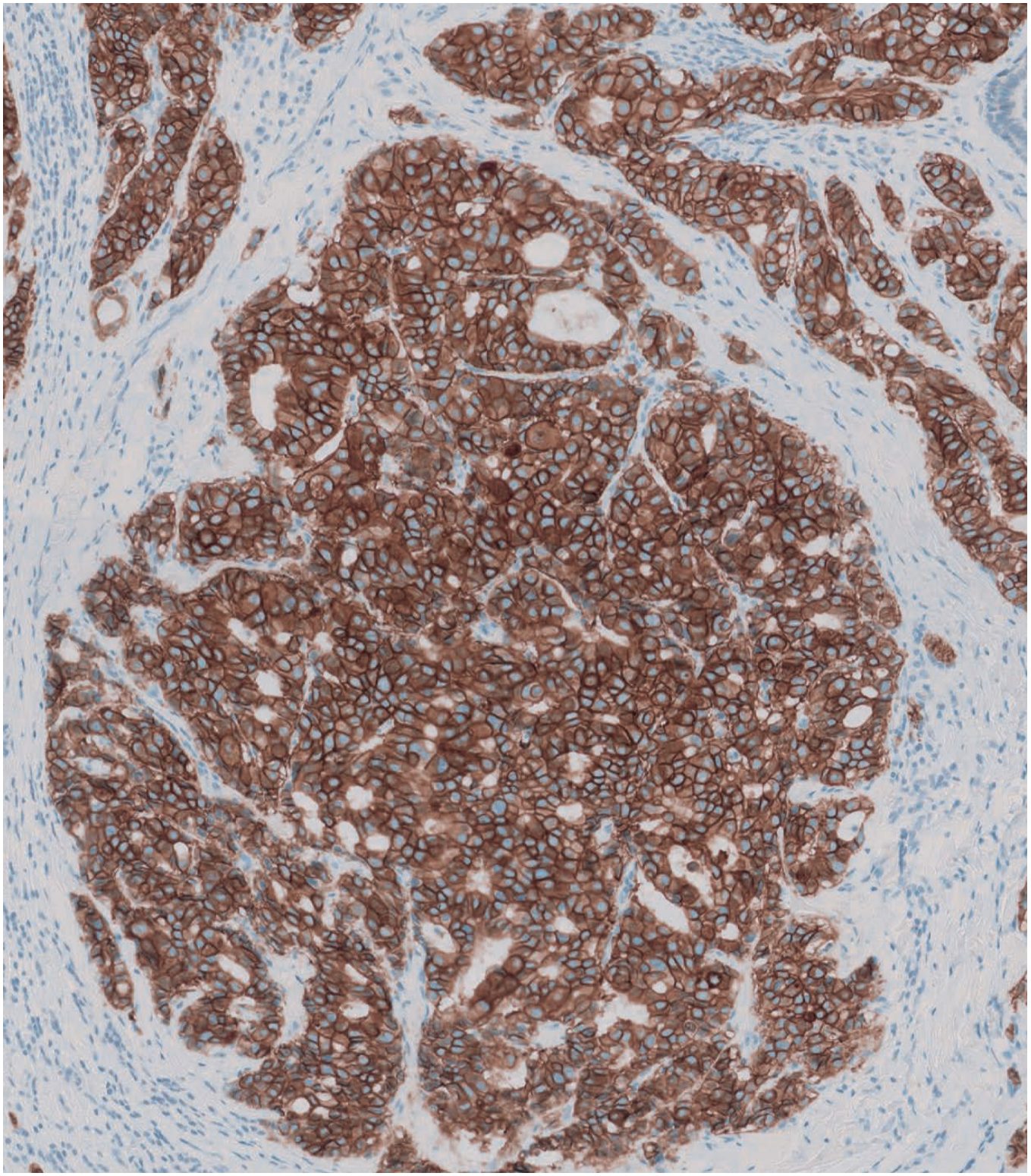
The positive control tissue verifies the fixation method, staining techniques, and reagent performance. Use undamaged cells for evaluation of staining results because necrotic or degenerated cells often stain nonspecifically. Tumor tissue should exhibit brown membrane staining. Staining of the cytoplasm and negative structures within the specimen should not be more than a 1+ staining intensity score. If the positive control tissue fails to demonstrate appropriate positive staining, then results with the patient specimens should be considered invalid.

The negative control tissue should be examined after the positive control tissue to verify the labeling specificity of the target antigen by the primary antibody. The absence of specific staining in the negative control tissue confirms the lack of cross-reactivity to cells/cellular components. Alternatively, negative portions of the positive control tissue may serve as the negative control tissue, but this should be verified by the user. A very weak reaction can be observed in most normal epithelial tissue. Nonspecific staining, if present, will be of a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. If specific staining (false positive staining) occurs in the negative control tissue, results with the patient specimens should be considered invalid.

Do not use control tissue as an aid in interpretation of patient results.

Negative Control Reagent (Optional)

Patient tissue stained with Negative Control Reagent is optional. Absence of membrane staining verifies that the labeling of the target antigen by the primary antibody is specific. Brown staining occurring in the cytoplasm of the specimen stained with the Negative Control Reagent, such as in connective tissue, leucocytes, erythrocytes, or necrotic tissue, should be considered nonspecific background staining.



Evaluating Patient Specimens

Determination of HER2 Score

For the determination of HER2 protein overexpression, only the membrane staining intensity and pattern should be evaluated using the scale presented in Table 2. For evaluation of the immunohistochemical staining and scoring, an objective of 10x magnification is appropriate. The use of a 20-40x objective magnification is recommended for confirmation of the score. Cytoplasmic staining should be considered nonspecific staining and is not to be included in the assessment of membrane staining intensity.

Only specimens from patients with invasive breast carcinoma should be scored. In cases with carcinoma in situ and invasive carcinoma in the same specimen, only the invasive component should be scored.

Table 2. Cell membrane staining intensity criteria

Staining pattern	**Score** (report to treating physician)	**HER2 protein overexpression assessment** (report to treating physician)
No staining is observed, or membrane staining is observed in less than 10% of the tumor cells	0	Negative
A 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
A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells	2+	Weakly positive
A strong complete membrane staining is observed in more than 10% of the tumor cells	3+	Strongly positive

Examples of HER2 Scores

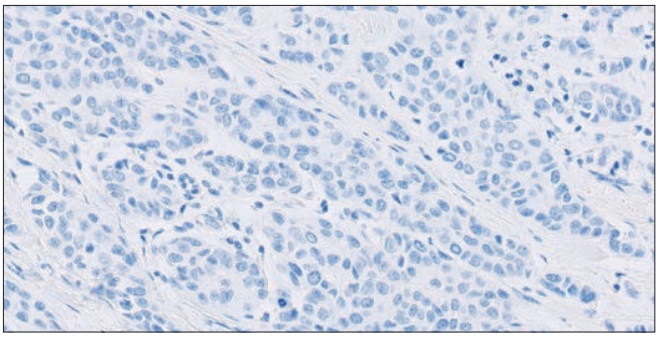


Figure 12. Breast carcinoma (FFPE) stained with HercepTest™ mAb pharmDx on Dako Omnis. Score 0.

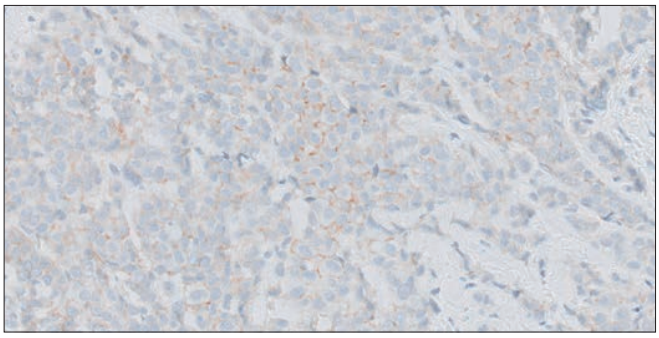


Figure 13. Breast carcinoma (FFPE) stained with HercepTest™ mAb pharmDx on Dako Omnis. Score 1+.

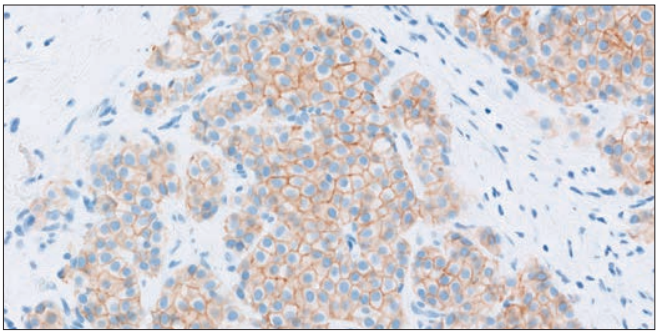


Figure 14. Breast carcinoma (FFPE) stained with HercepTest™ mAb pharmDx on Dako Omnis. Score 2+.

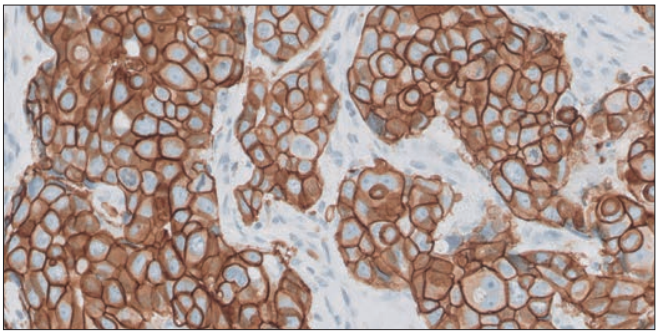


Figure 15. Breast carcinoma (FFPE) stained with HercepTest™ mAb pharmDx on Dako Omnis. Score 3+.

Key Considerations in Scoring Specimens

Scoring of HercepTest™ mAb pharmDx (Dako Omnis) must be performed in accordance with the guidelines established in the IFU and within the context of best practices and the pathologist's experience and best medical judgment. This manual highlights areas of the interpretation that are potentially challenging for users.

Most breast carcinomas tested for HER2 protein overexpression are given a score of either 0 or 3+. While the majority of these cases are clear-cut, a small percentage of the remaining 1+ and 2+ samples may be more difficult to interpret.

Note that the visual contrast between DAB and hematoxylin counterstain may affect perception of membrane staining intensity, thus adherence to the recommended interpretation guidelines is important.

Use the following additional guidelines for interpretation of HercepTest™ mAb pharmDx (Dako Omnis) staining and refer to the Image Guide starting on page 26:

1. Evaluate the patient tissue stained for HER2 protein at low magnification (10x) first. The majority of positive cases will be obvious at low magnification.
2. Well-preserved and well-stained areas of the specimen should be used to make a determination of the percent positive tumor cells.
3. To verify the presence of membrane staining, use 20-40x objective magnification.
4. If more than 10% of the tumor cells demonstrate complete membrane staining, the staining is either 2+ or 3+. Go to 20-40x objective magnification to confirm score. In a majority of the 3+ cases, at least 80% of the tumor cells are stained and the membrane staining is strong and complete.
5. If the specimen is near the cut-off of 10% tumor cells positive, it is recommended that a minimum of 100 tumor cells be counted to determine the percentage of stained cells.
6. If there is complete membrane staining at a weak to moderate intensity in greater than 10% of the tumor cells, the score of the specimen is 2+. This is usually accompanied by incomplete membrane staining of the majority of the remaining tumor cells.
7. If less than 10% of the tumor cells have complete membrane staining, although other tumor cells may demonstrate an incomplete membrane staining, the score is 1+.
8. If less than 10% of the tumor cells have complete or incomplete membrane staining, the score is 0.

The following pages provide guidance on various staining characteristics.

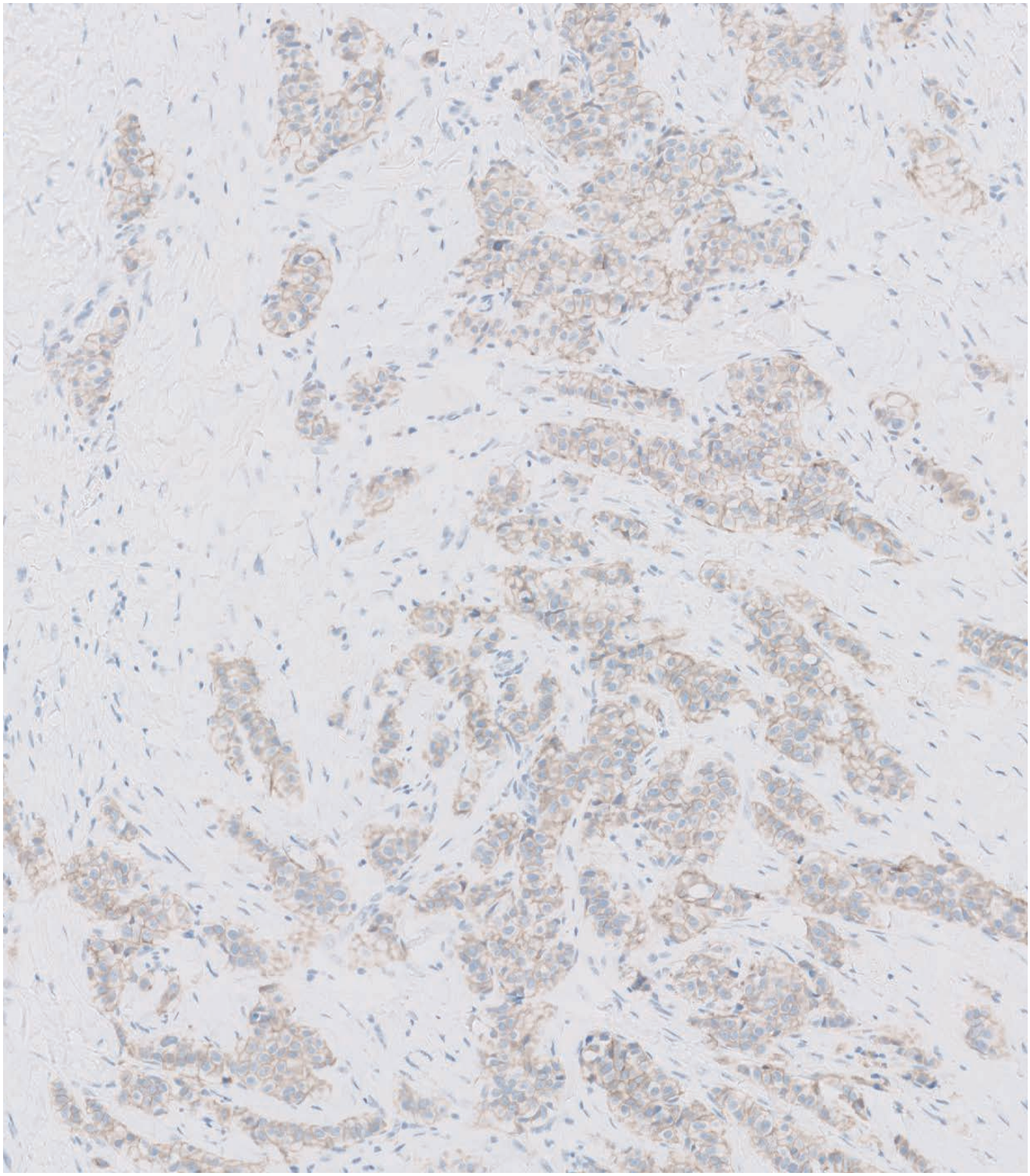


Image Guide for Interpretation of Staining

HER2 Overexpression

Evaluate the patient tissue stained for HER2 protein at low magnification (10x) first. The majority of positive cases will be obvious at low magnification.
To verify the presence of membrane staining, use 20-40x objective magnification.

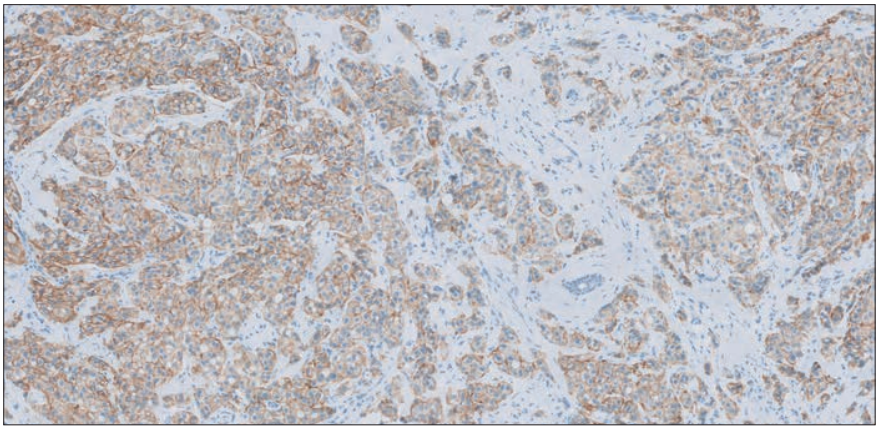


Figure 16: Breast carcinoma with 2+ score (10x magnification).

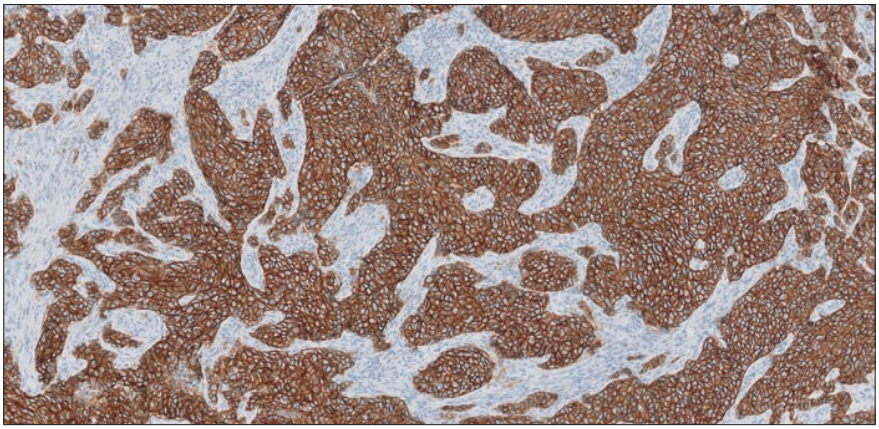


Figure 17: Breast carcinoma with 3+ score (10x magnification).

Key point

The majority of positive cases will be obvious at low power magnification.

Incomplete and Complete Membrane Staining

Verify membrane staining by using 20-40x objective magnification.

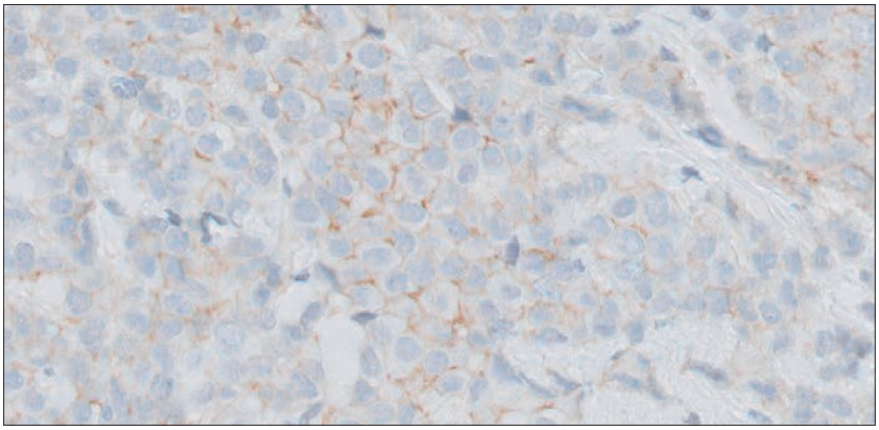


Figure 18: Breast carcinoma exhibiting incomplete membrane staining, the cells are only stained in part of their membrane, score 1+ (40x magnification).

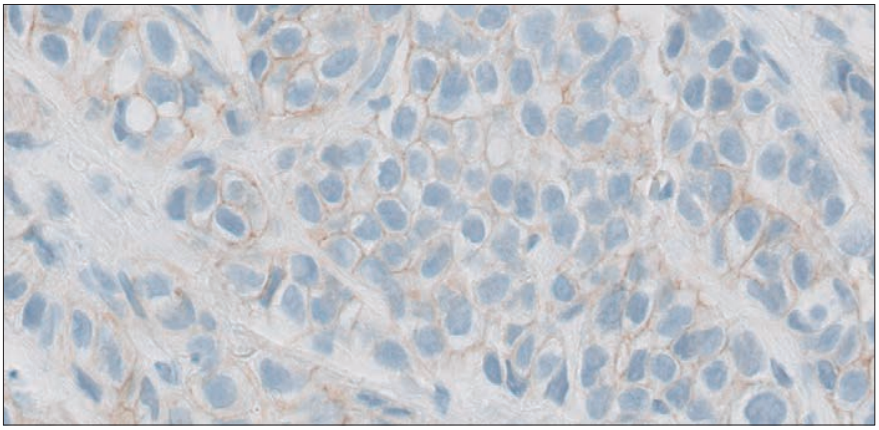


Figure 19: Breast carcinoma exhibiting a weak to moderate complete membrane staining, score 2+ (40x magnification).

Key point

Evaluate if staining of the membranes is complete. Use 20-40x objective magnification to confirm staining pattern and intensity.

Intensity of Complete Membrane Staining

If more than 10% of tumor cells demonstrate complete membrane staining, the staining is either 2+ or 3+. Go to 20-40x objective magnification to confirm score. If there is complete membrane staining at a weak to moderate intensity in greater than 10% of the tumor cells, the score of the specimen is 2+. If a strong complete membrane staining is observed in more than 10% of the tumor cells, the score is 3+.

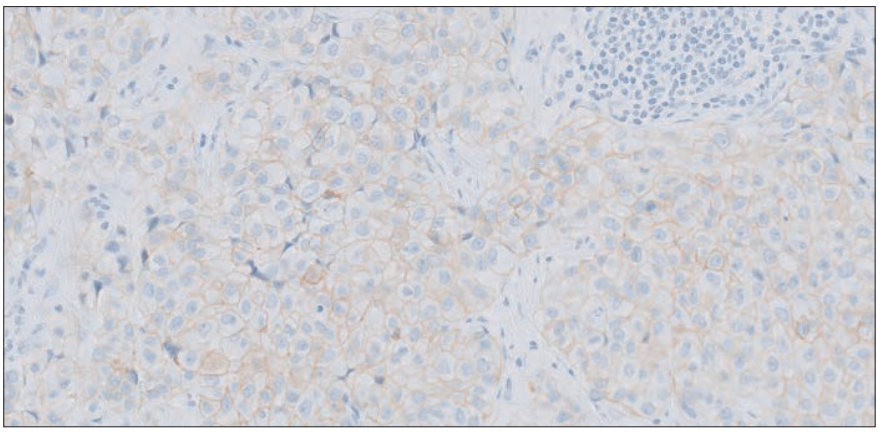


Figure 20: Breast carcinoma exhibiting complete membrane staining with weak intensity, score 2+ (20x magnification).

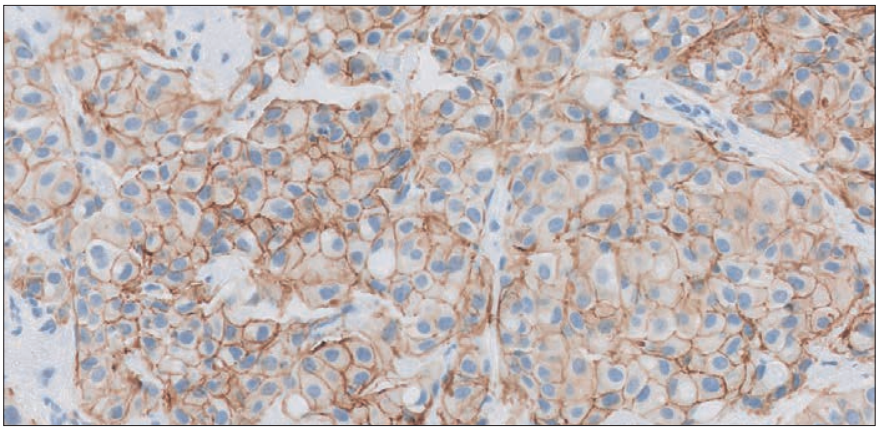


Figure 21: Breast carcinoma exhibiting complete membrane staining with moderate intensity, score 2+ (20x magnification).

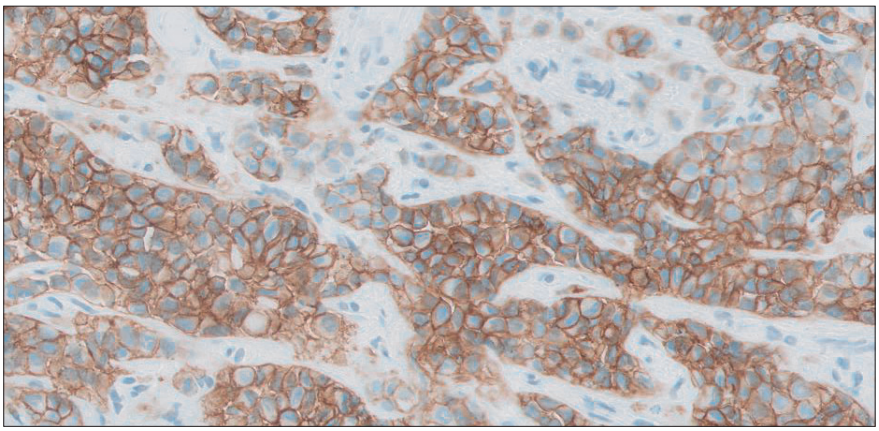


Figure 22: Breast carcinoma exhibiting complete membrane staining with strong intensity, score 3+ (20x magnification).

Key point

Use 20-40x objective magnification to confirm staining pattern and intensity.

Percentage

If the specimen is near the cut-off of 10% positive tumor cells, it is recommended that a minimum of 100 tumor cells be counted to determine the percentage of stained cells.

If less than 10% of the tumor cells have complete or incomplete membrane staining, the score is 0.

If more than 10% of tumor cells demonstrate membrane staining, the score is either 1+, 2+ or 3+, based on the cell membrane staining intensity criteria presented in Table 2.

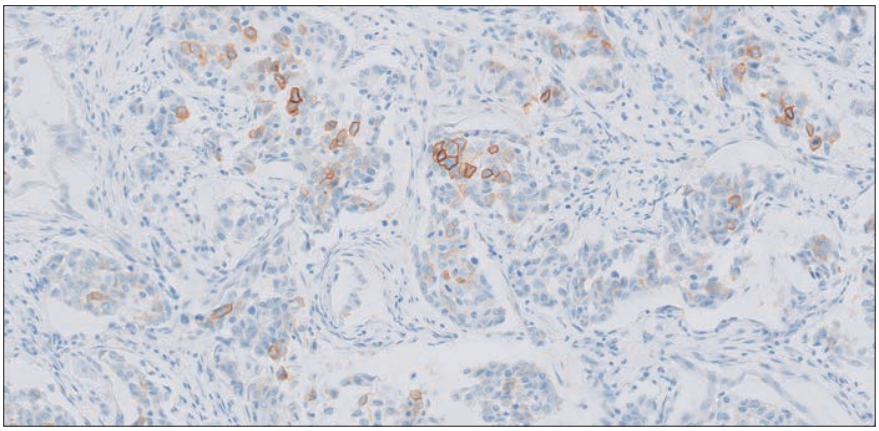


Figure 23: Breast carcinoma exhibiting complete membrane staining with strong intensity i.e. 'hot spots' in less than 10% tumor cells, score 0 (20x magnification).

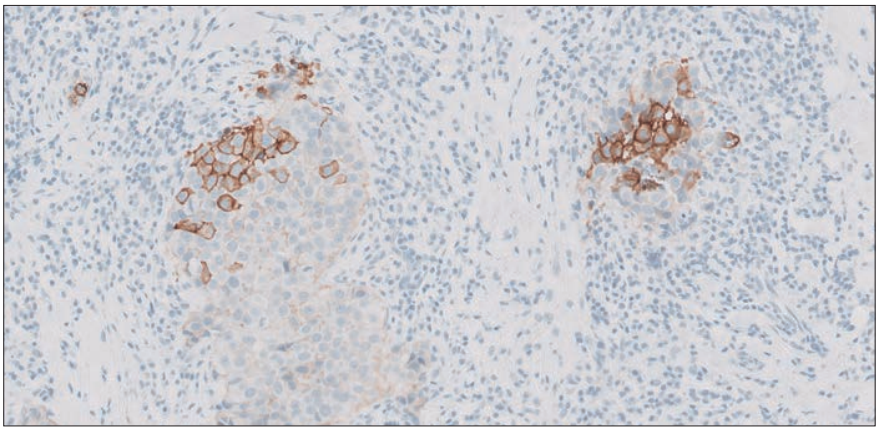


Figure 24: Breast carcinoma exhibiting complete membrane staining with strong intensity in more than 10% tumor cells, score 3+ (20x magnification).

Key point

If the specimen is near the cut-off of 10% positive tumor cells, it is recommended that a minimum of 100 tumor cells be counted to determine the percentage of stained cells.

Key point

Strong focal staining (3+), i.e. 'hot spots', may occasionally be seen. Immunostaining of a second tissue block from the same specimen is recommended.

If less than 10% of the tumor cells have complete membrane staining, although other tumor cells may demonstrate an incomplete membrane staining, the score is 1+.

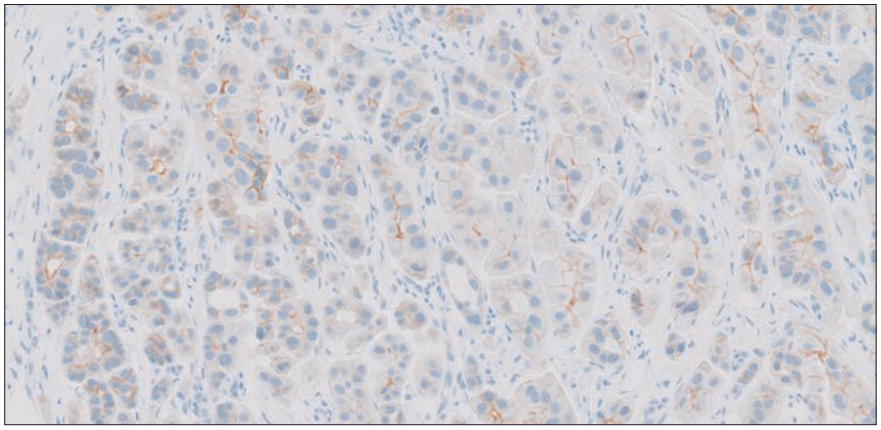


Figure 25: Breast carcinoma exhibiting complete membrane staining in less than 10% tumor cells, while the majority demonstrate an incomplete membrane staining, score 1+ (20x magnification).

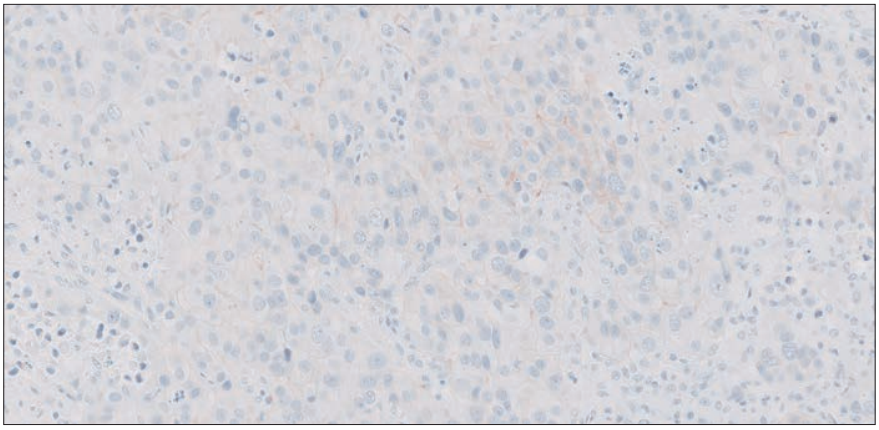


Figure 26: Breast carcinoma exhibiting complete membrane staining in less than 10% tumor cells, while the majority demonstrate an incomplete membrane staining, score 1+ (20x magnification).

Key point

Evaluate if the membranes are complete.
Use 20-40x objective magnification to confirm score.

Non-tumor and Early Stages

Only specimens from patients with invasive breast carcinoma should be scored. In cases with carcinoma in situ and invasive carcinoma in the same specimen, only the invasive component should be scored. Below are examples (Fig. 27-29) of non-tumor tissue and early stages, all to be excluded from scoring.

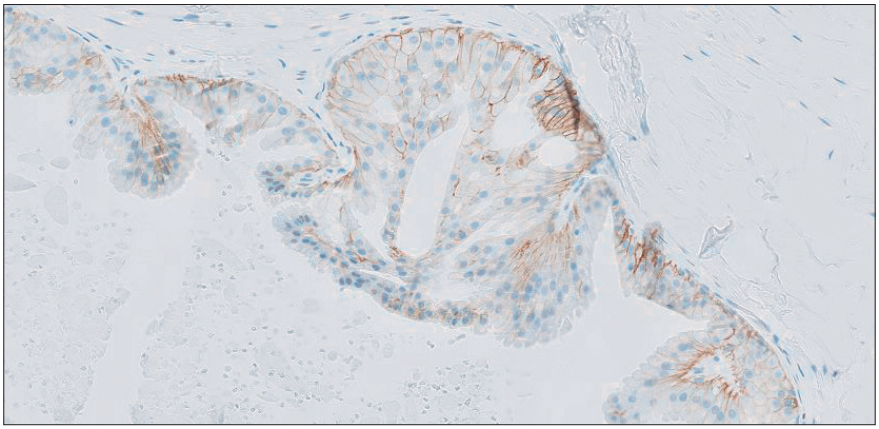


Figure 27: Example of apocrine metaplasia (20x magnification), exclude from scoring.

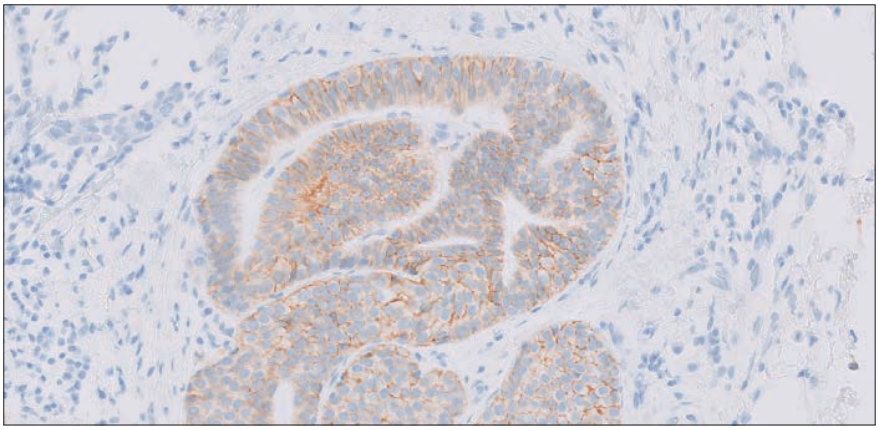


Figure 28: Example of hyperplasia (20x magnification), exclude from scoring.

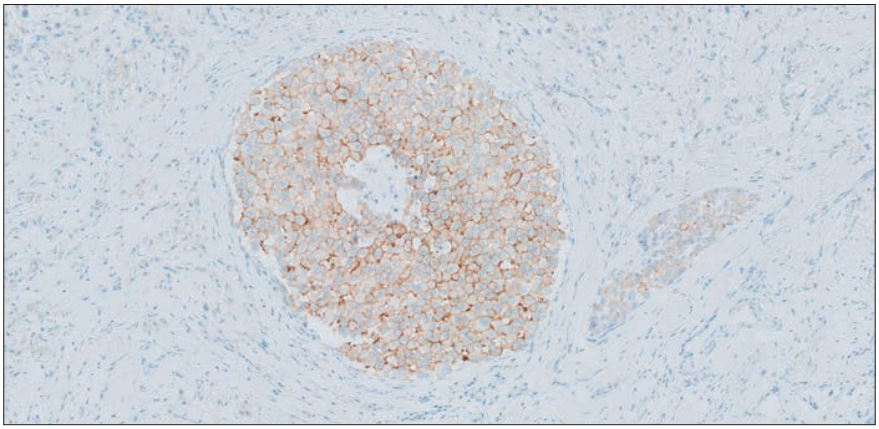


Figure 29: Example of intraductal carcinoma (ductal carcinoma in situ) (20x magnification), exclude from scoring.

Key point

Benign and proliferative breast epithelia may show HER2 positivity and should be excluded from scoring.

Breast Epithelium

Epithelium in breast tissue is usually negative (Fig. 30), but can in some cases show weak staining (Fig. 31).

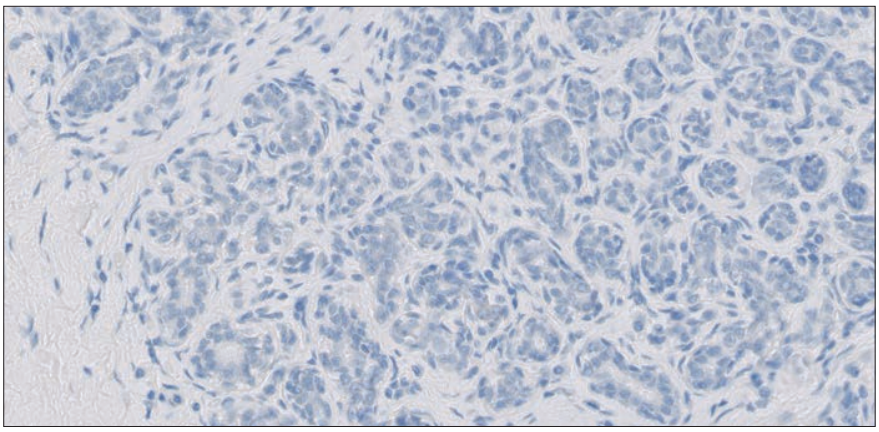


Figure 30: Example of normal breast epithelium with no staining (40x magnification).

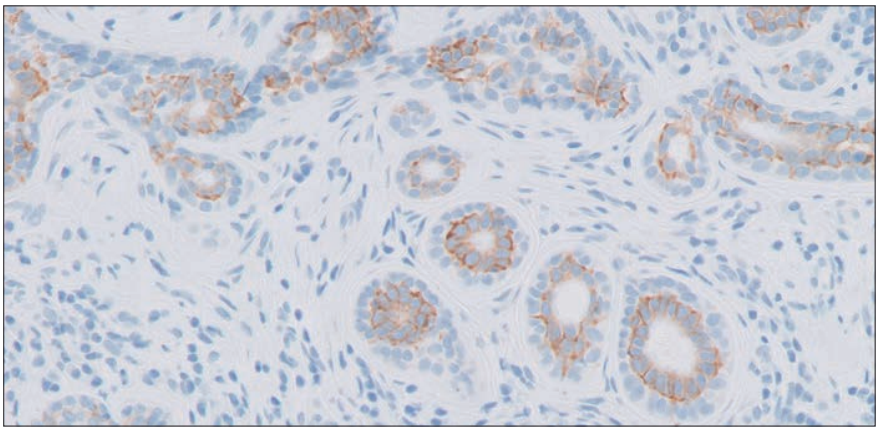


Figure 31: Example of normal breast epithelium with weak staining (40x magnification).

Key point

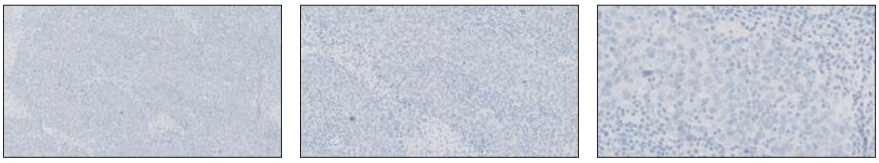
Normal breast epithelial tissue may stain for HER2.

Case Examples

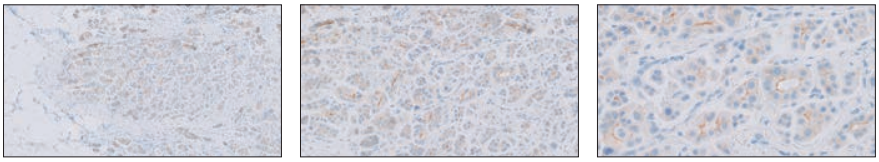
Pages 34-52 include case examples of breast cancer tissue stained with HercepTest™ mAb pharmDx (Dako Omnis).

Each case is displayed at 10x magnification, 20x magnification and 40x magnification. All four HER2 scores (0, 1+, 2+, 3+) are represented. For an overview, please see below:

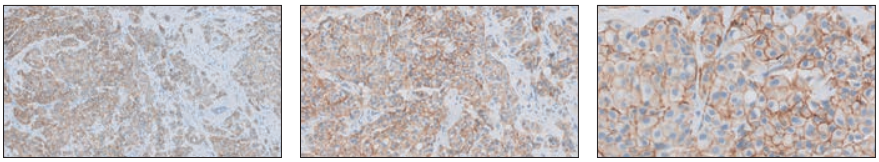
HER2 Score 0: Cases 1 and 2, pages 34-35



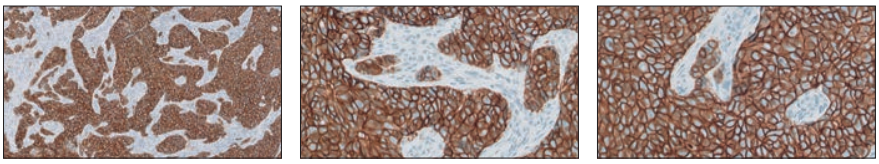
HER2 Score 1+: Cases 3-7, pages 36-40



HER2 Score 2+: Cases 8-14, pages 41-47



HER2 Score 3+: Cases 15-19, pages 48-52



Case Examples

Case 1: Score 0

No staining is observed, or membrane staining is observed in less than 10% of the tumor cells.

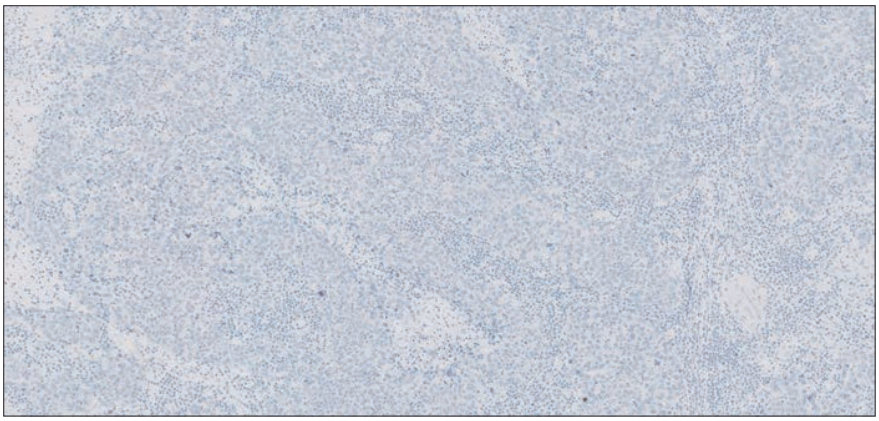


Figure 32a: 10x magnification.

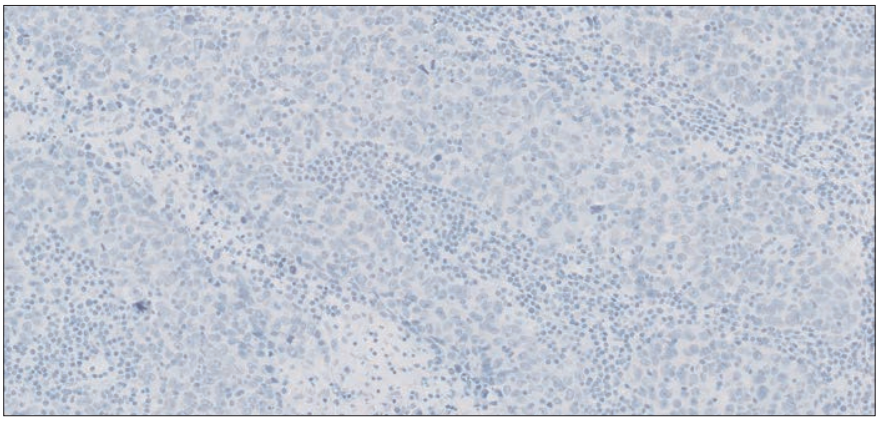


Figure 32b: 20x magnification.

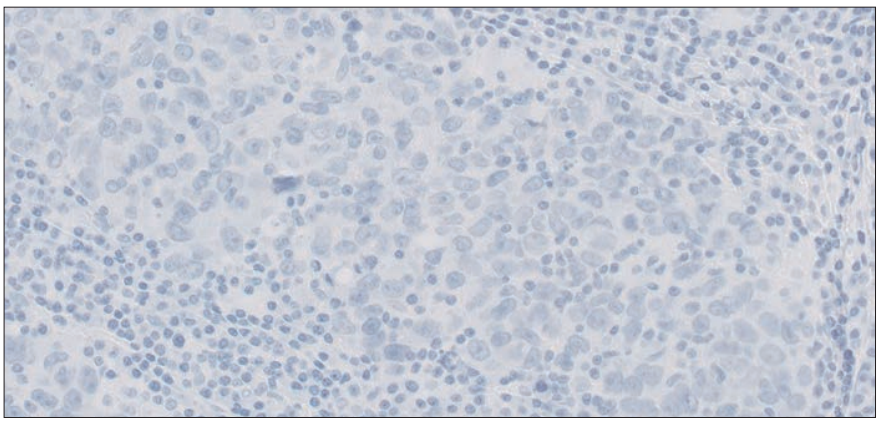


Figure 32c: 40x magnification.

Case 2: Score 0

No staining is observed, or membrane staining is observed in less than 10% of the tumor cells.

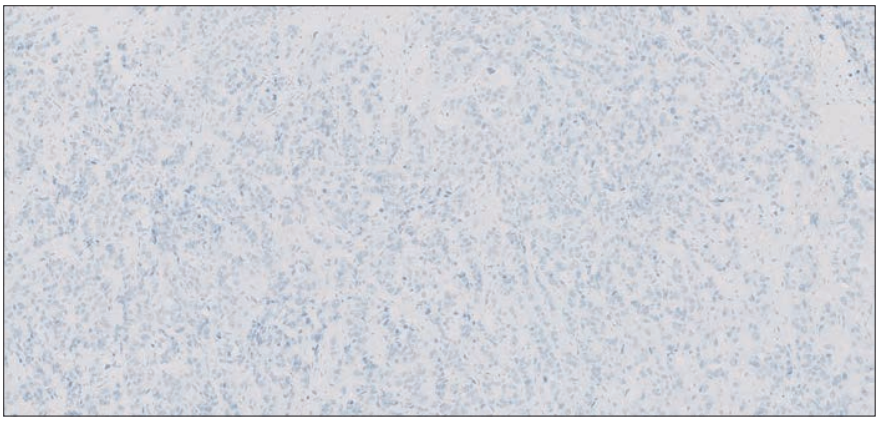


Figure 33a: 10x magnification.

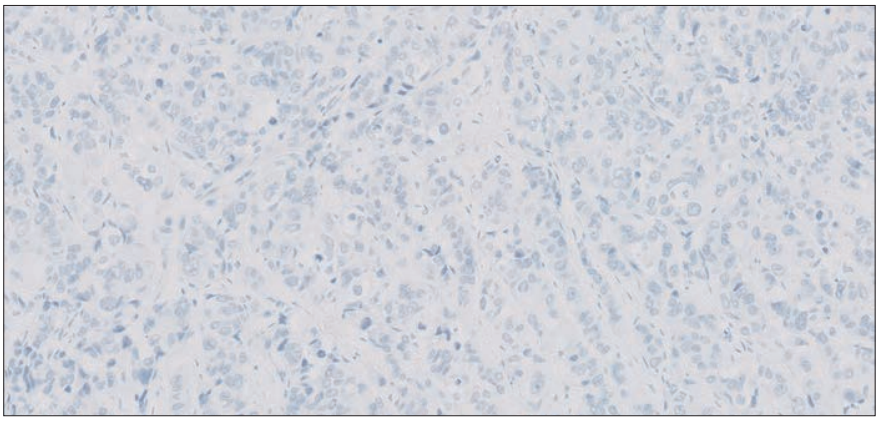


Figure 33b: 20x magnification.

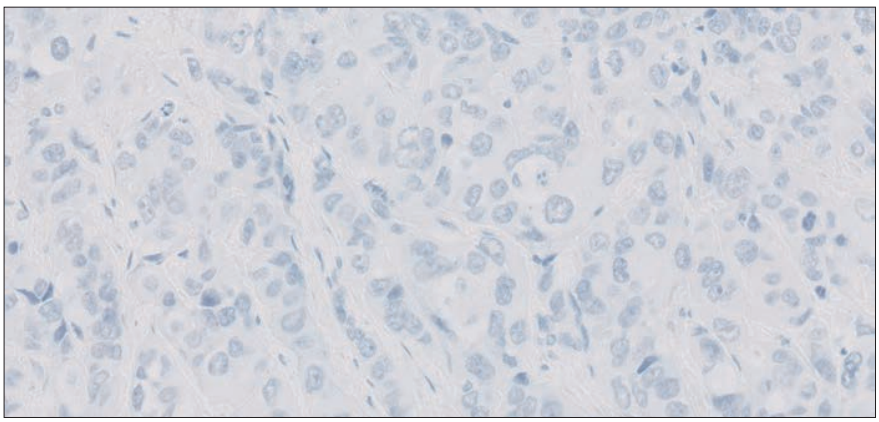


Figure 33b: 40x magnification.

Case 3: Score 1+

A 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.

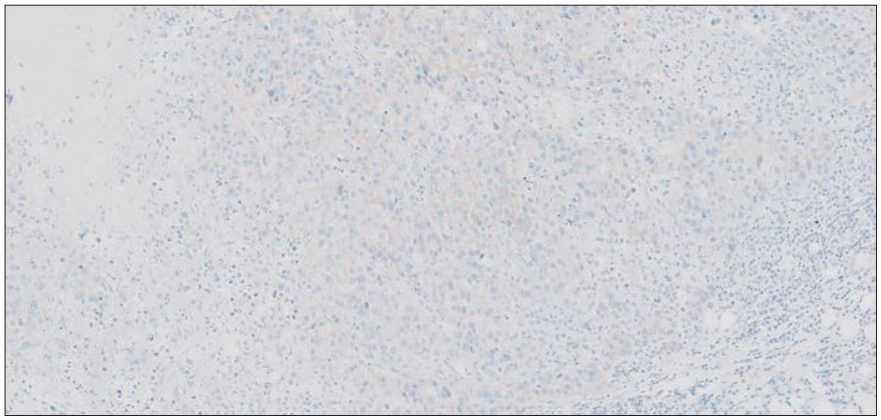


Figure 34a: 10x magnification.

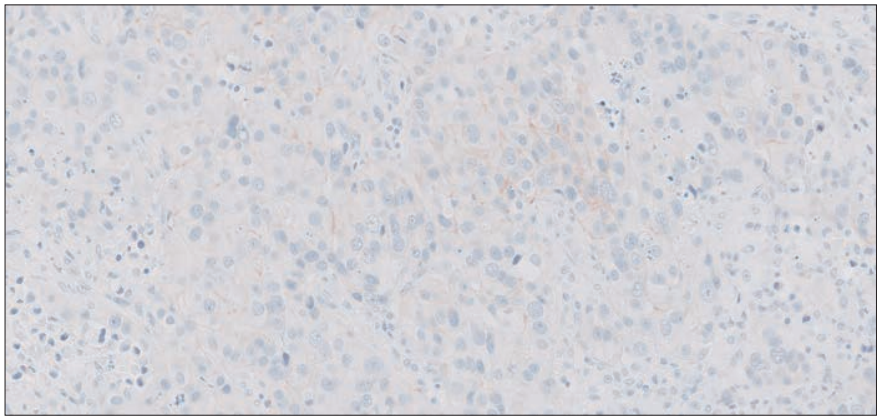


Figure 34b: 20x magnification.

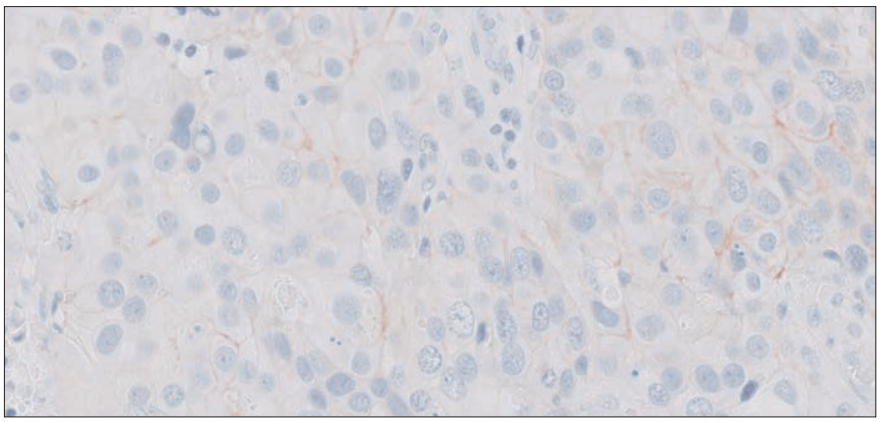


Figure 34c: 40x magnification.

Case 4: Score 1+

A 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.

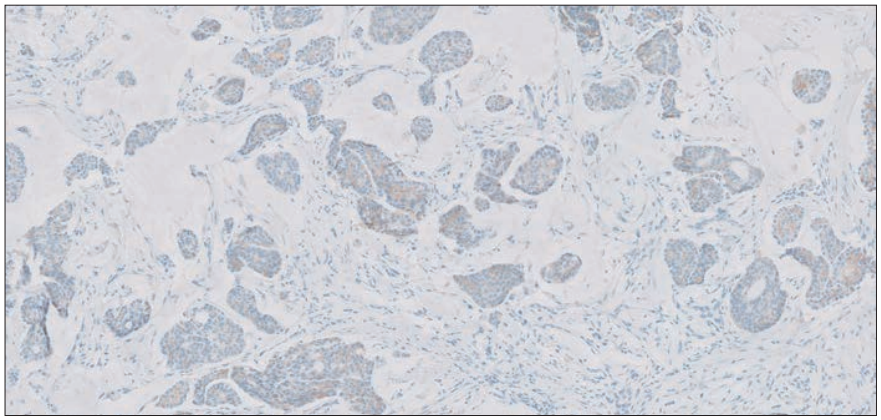


Figure 35a: 10x magnification.

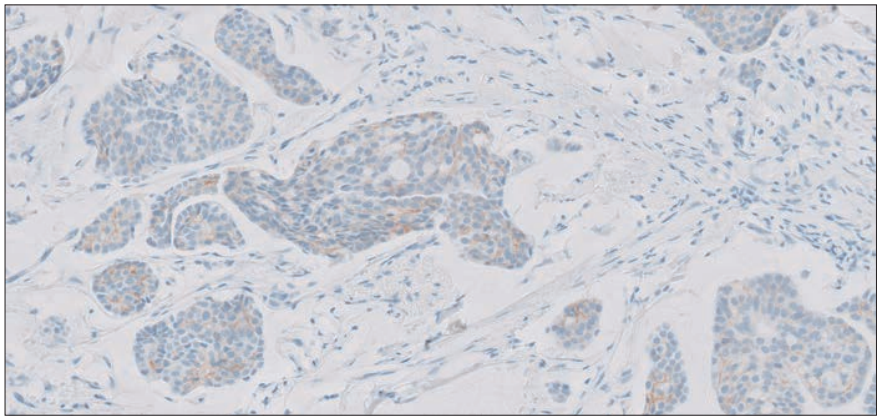


Figure 35b: 20x magnification.

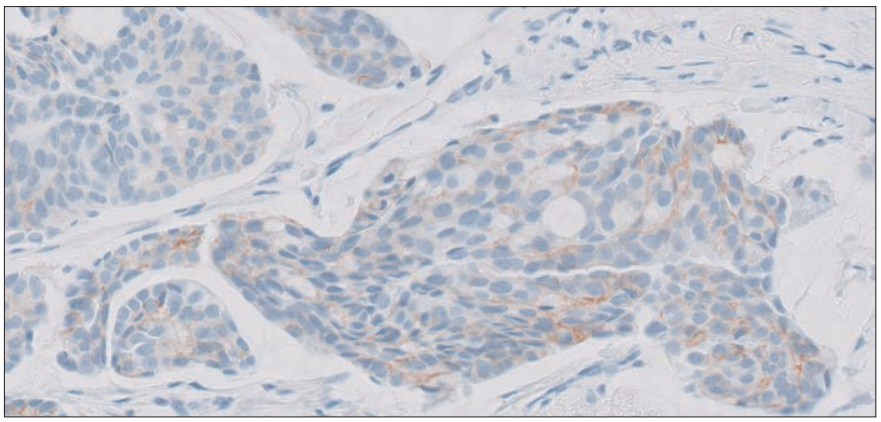


Figure 35c: 40x magnification.

Case 5: Score 1+

A 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.

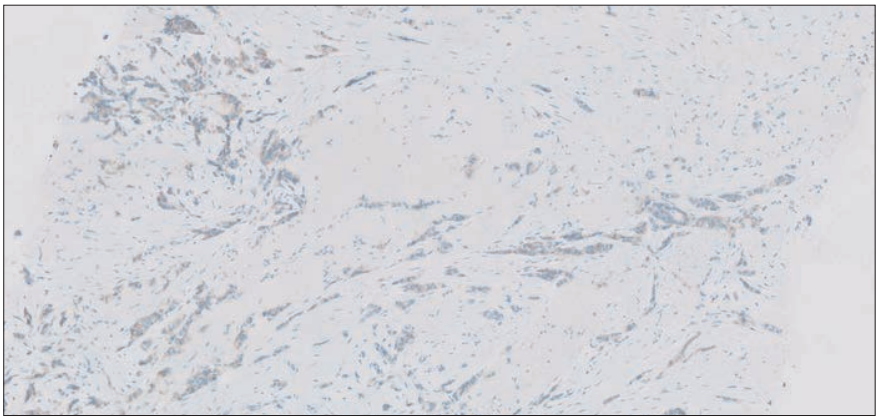


Figure 36a: 10x magnification.

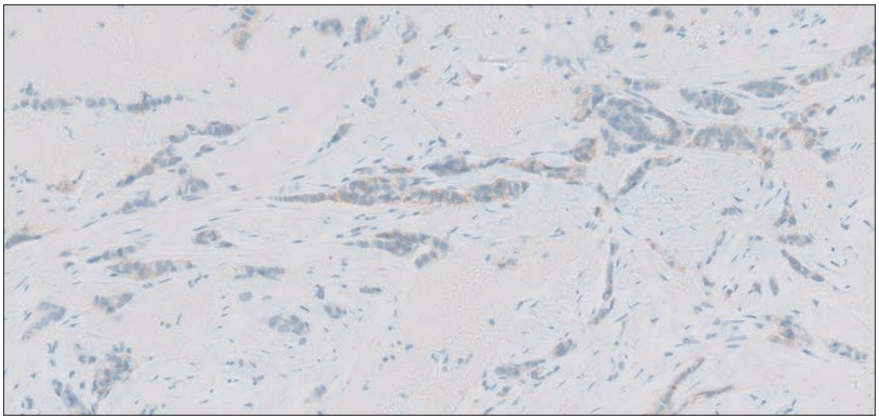


Figure 36b: 20x magnification.

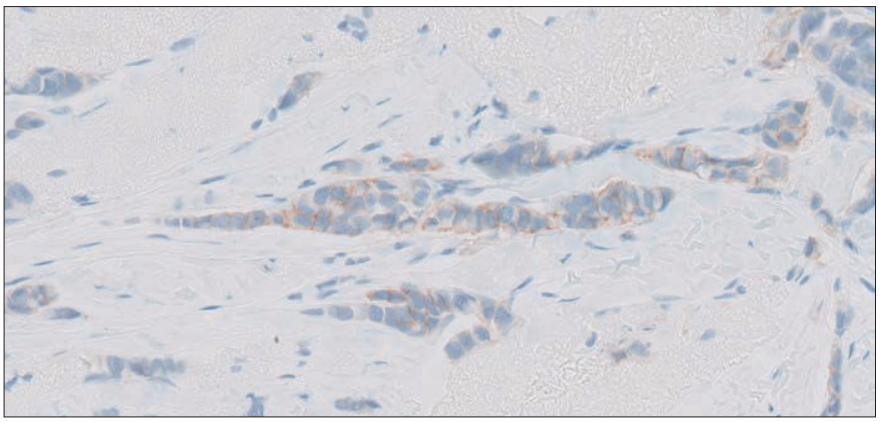


Figure 36c: 40x magnification.

Case 6: Score 1+

A 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.

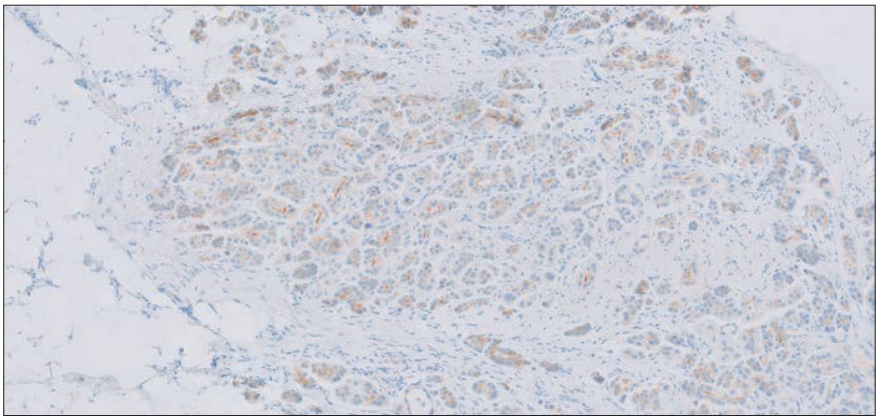


Figure 37a: 10x magnification.

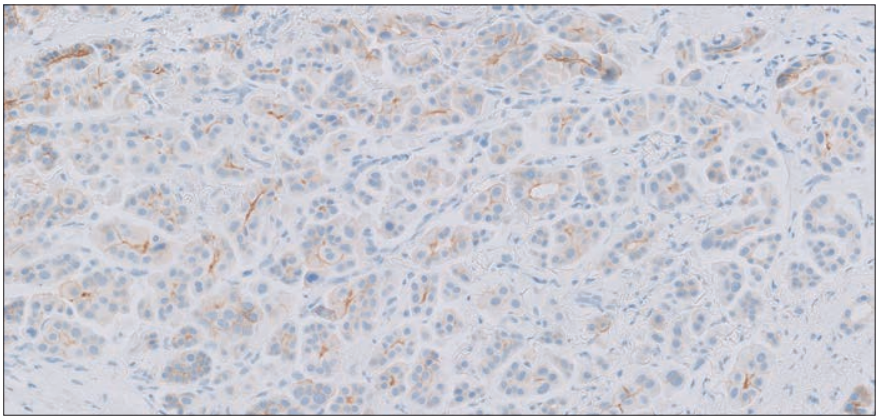


Figure 37b: 20x magnification.

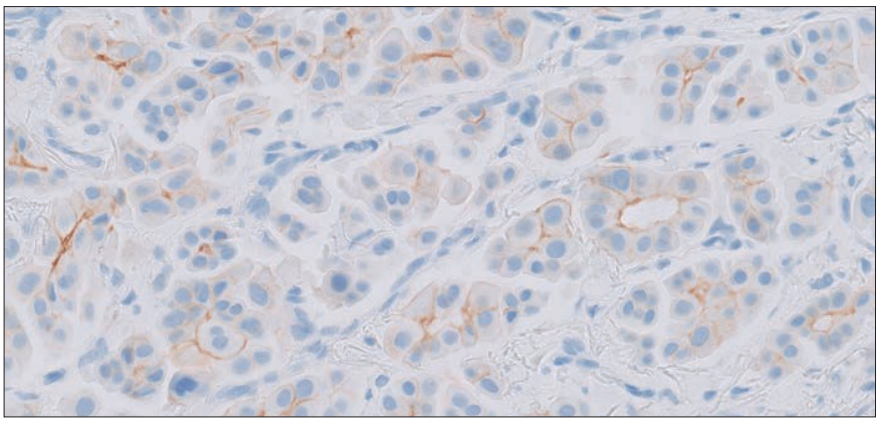


Figure 37c: 40x magnification.

Case 7: Score 1+

A 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.

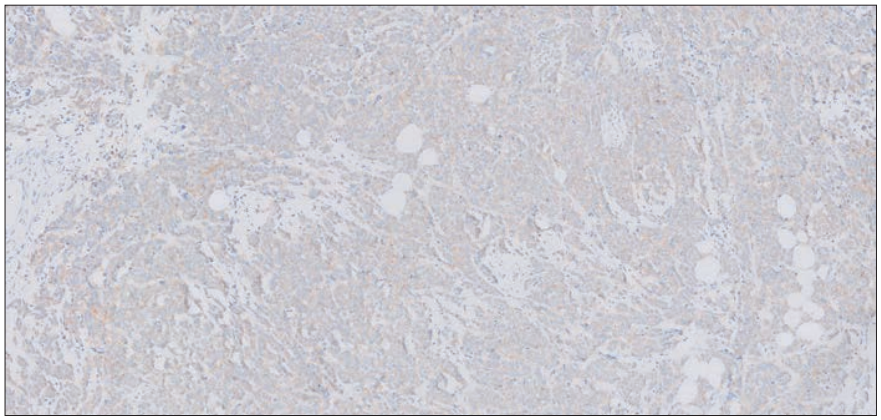


Figure 38a: 10x magnification.

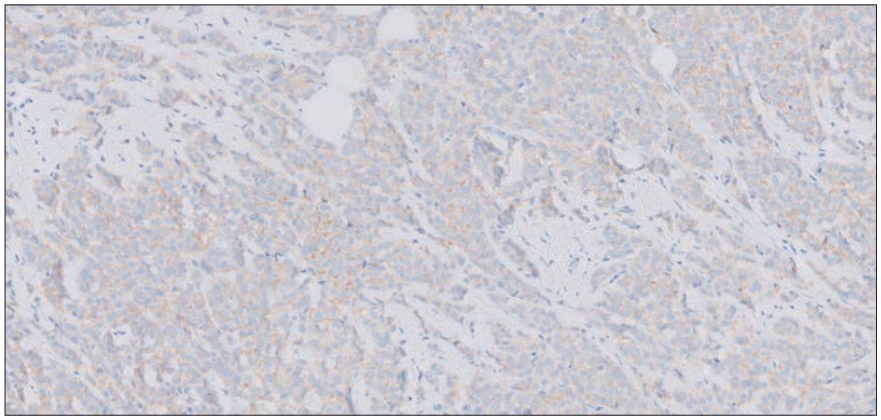


Figure 38b: 20x magnification.

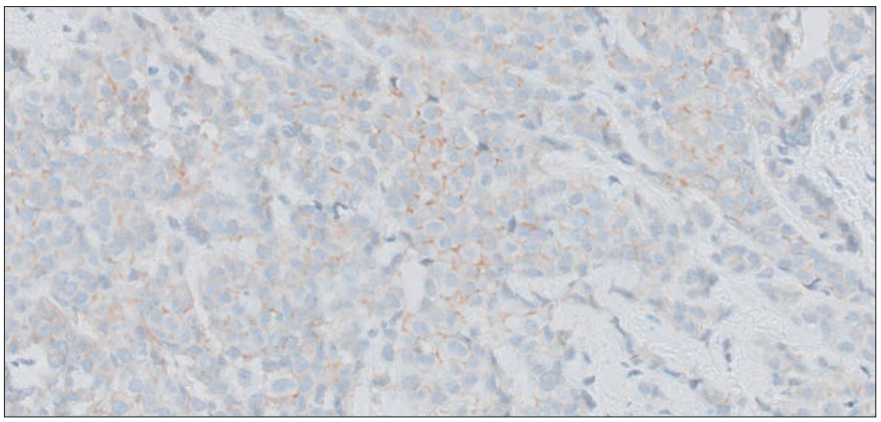


Figure 38c: 40x magnification.

Case 8: Score 2+

A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.

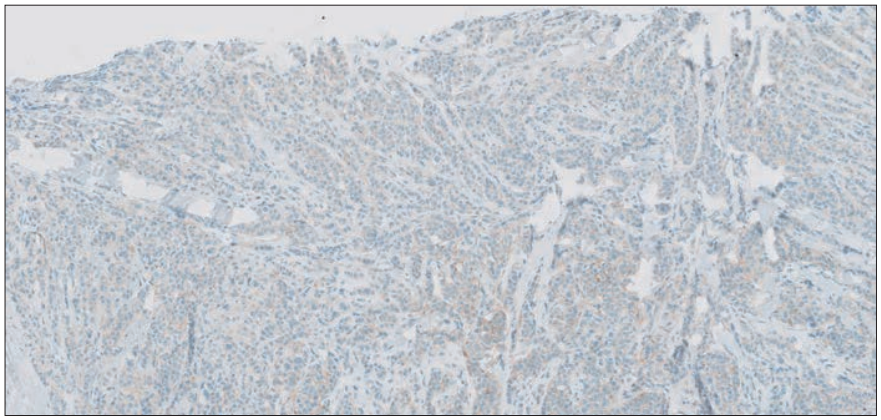


Figure 39a: 10x magnification.

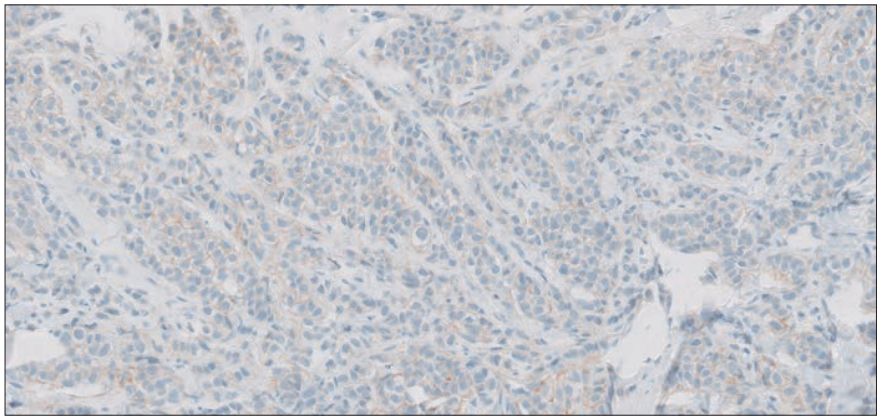


Figure 39b: 20x magnification.

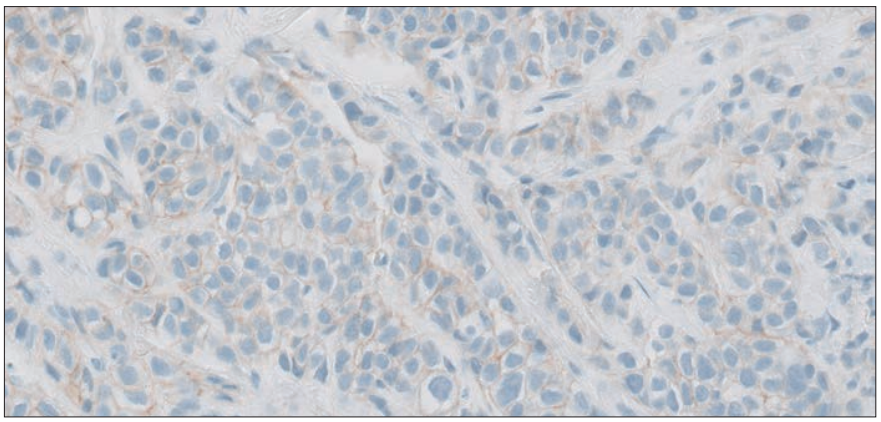


Figure 39c: 40x magnification.

Case 9: Score 2+

A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.

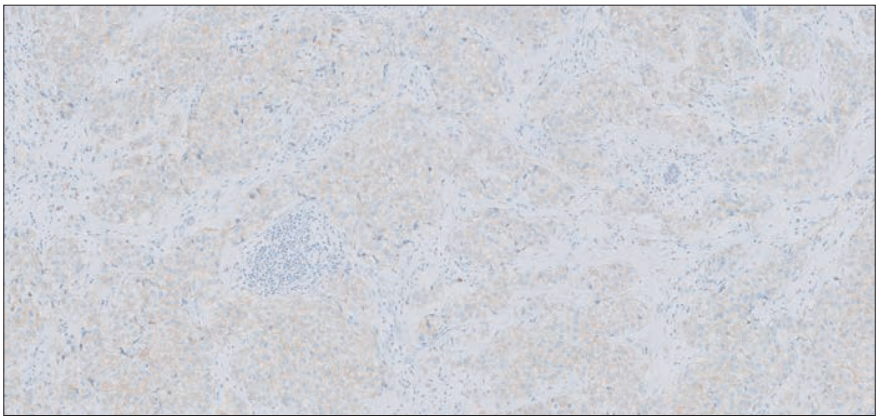


Figure 40a: 10x magnification.

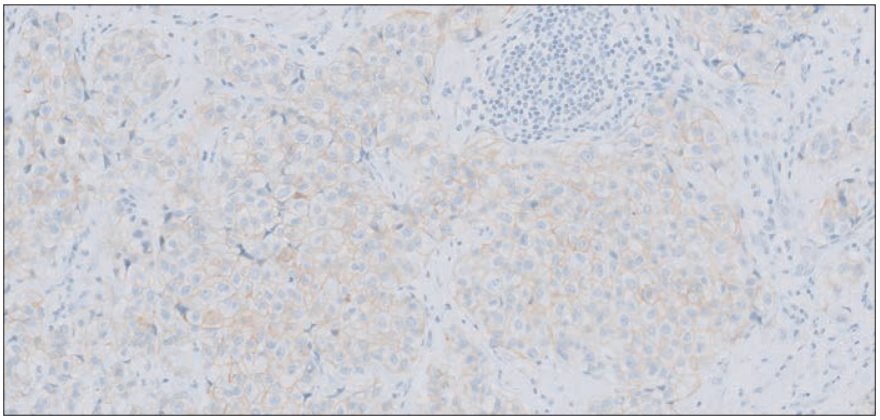


Figure 40b: 20x magnification.

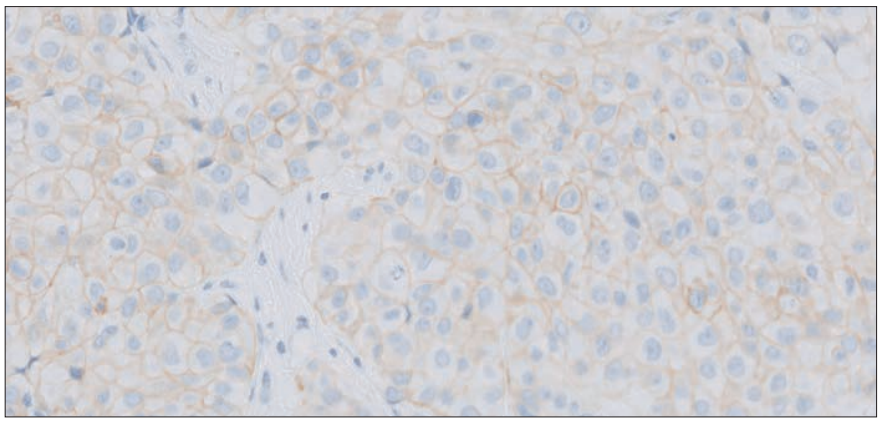


Figure 40c: 40x magnification.

Case 10: Score 2+

A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.

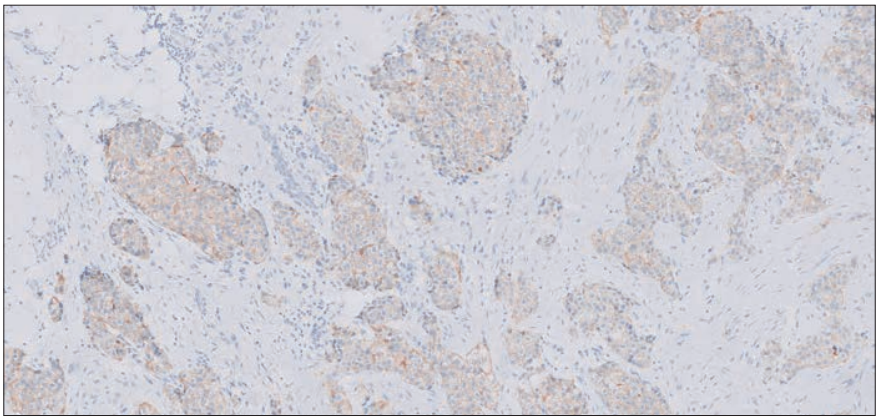


Figure 41a: 10x magnification.

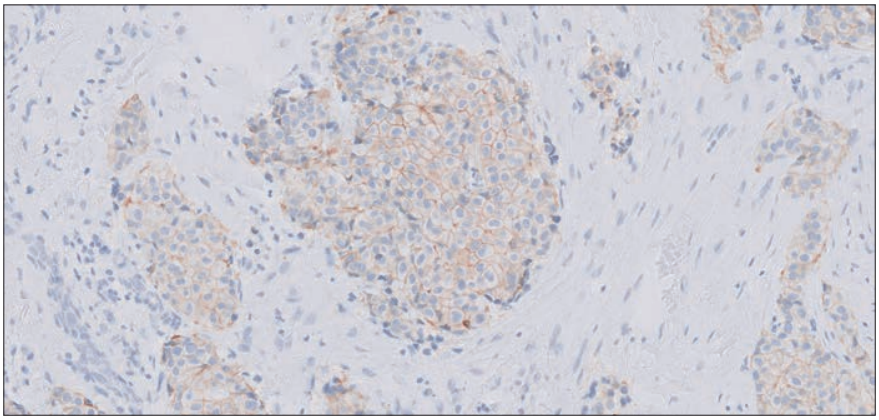


Figure 41b: 20x magnification.

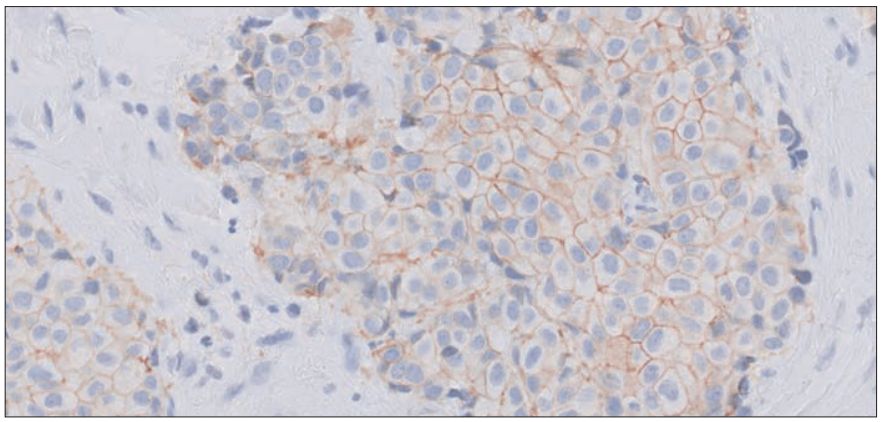


Figure 41c: 40x magnification.

Case 11: Score 2+

A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.

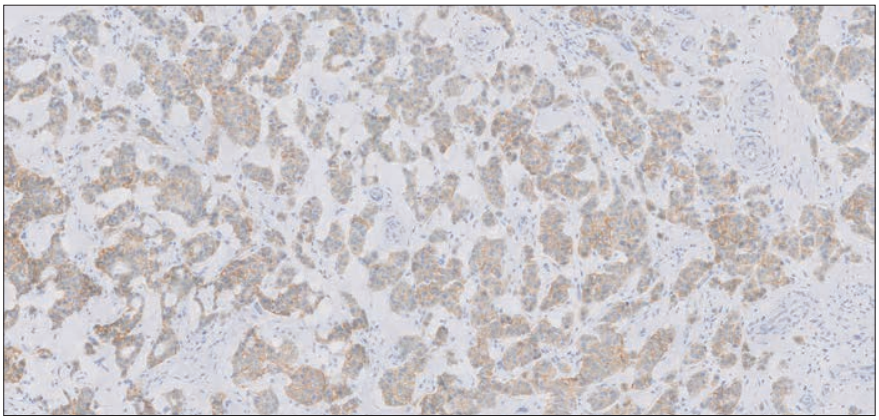


Figure 42a: 10x magnification.

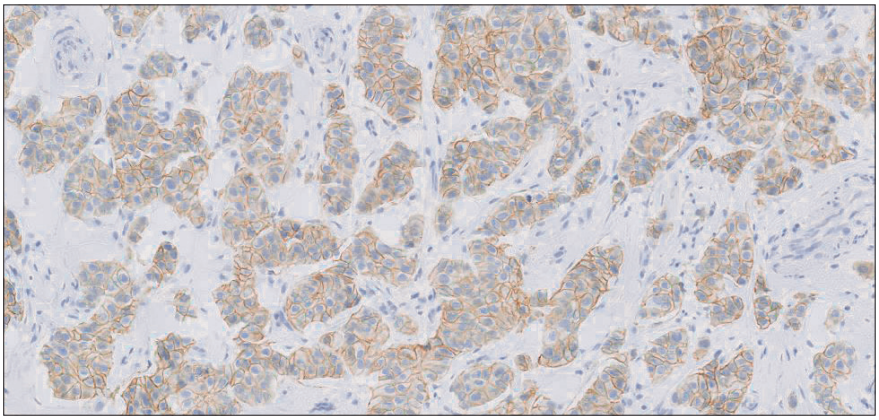


Figure 42b: 20x magnification.

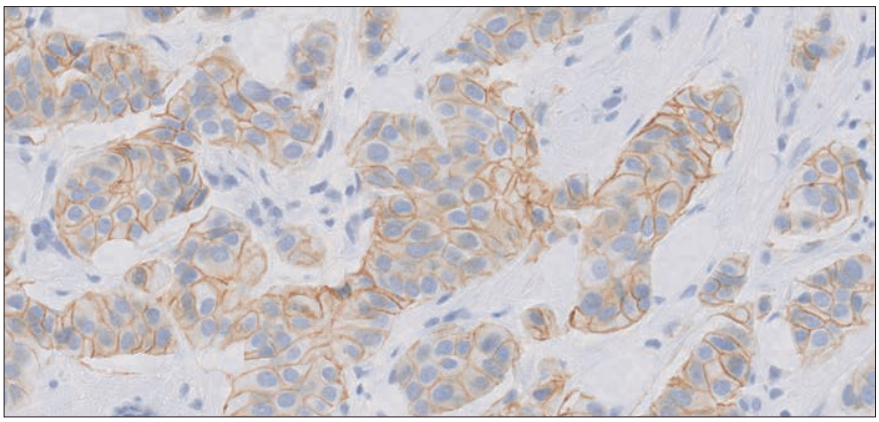


Figure 42c: 40x magnification.

Case 12: Score 2+

A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.

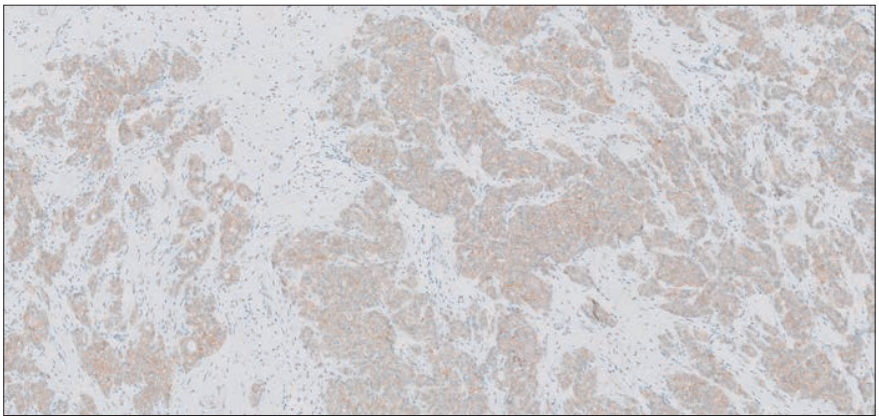


Figure 43a: 10x magnification.

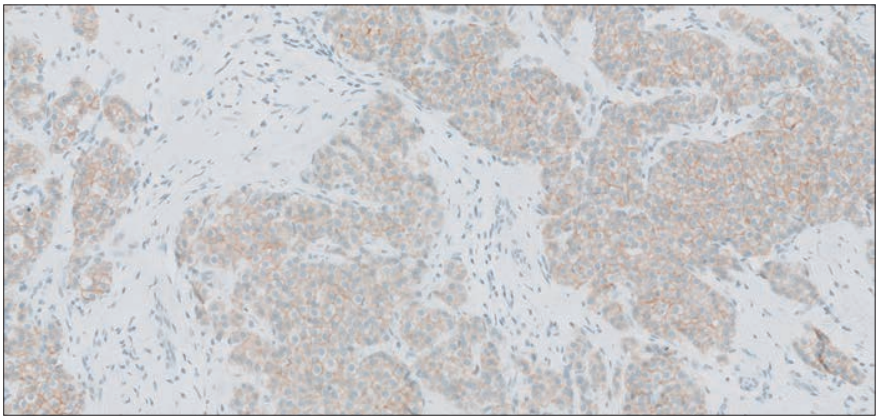


Figure 43b: 20x magnification.

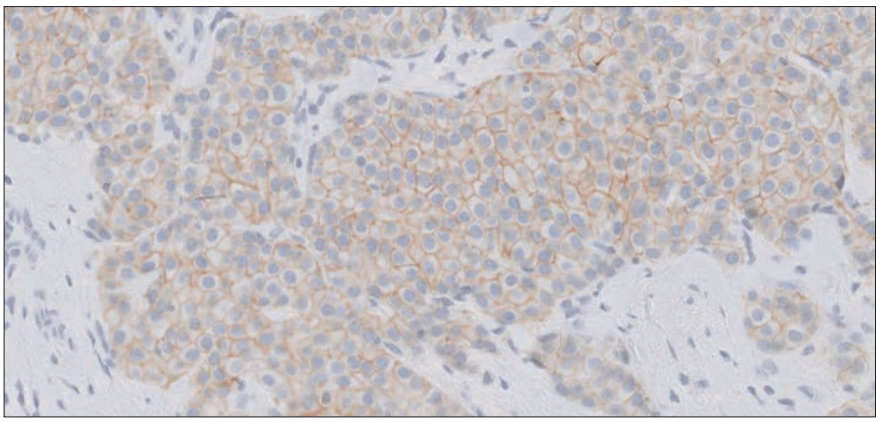


Figure 43c: 40x magnification.

Case 13: Score 2+

A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.

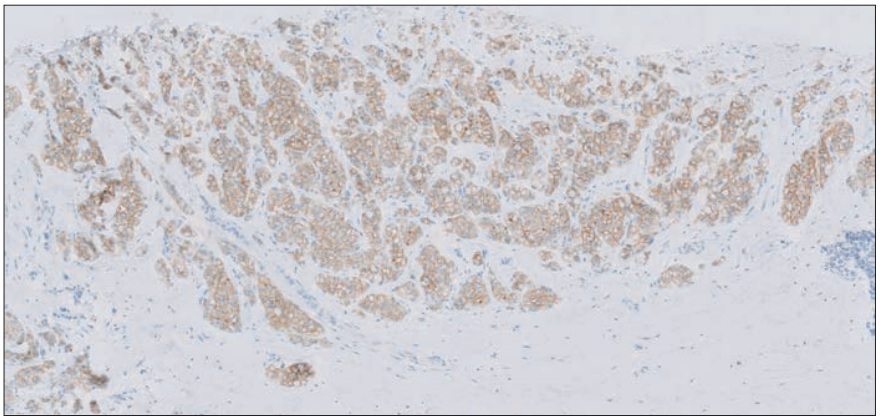


Figure 44a: 10x magnification.

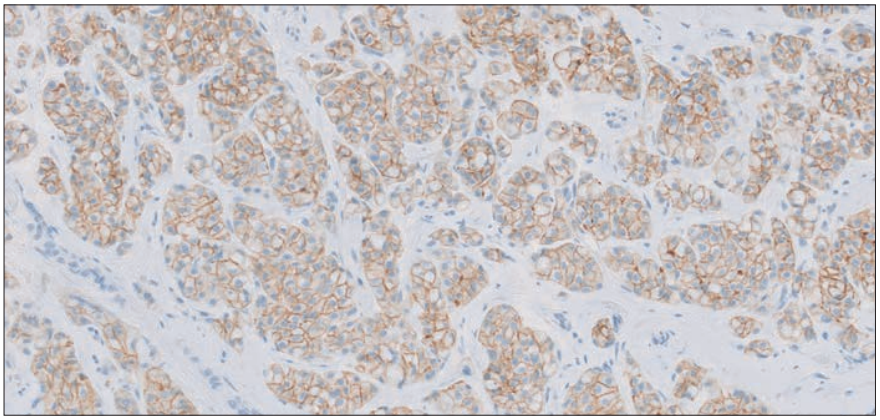


Figure 44b: 20x magnification.

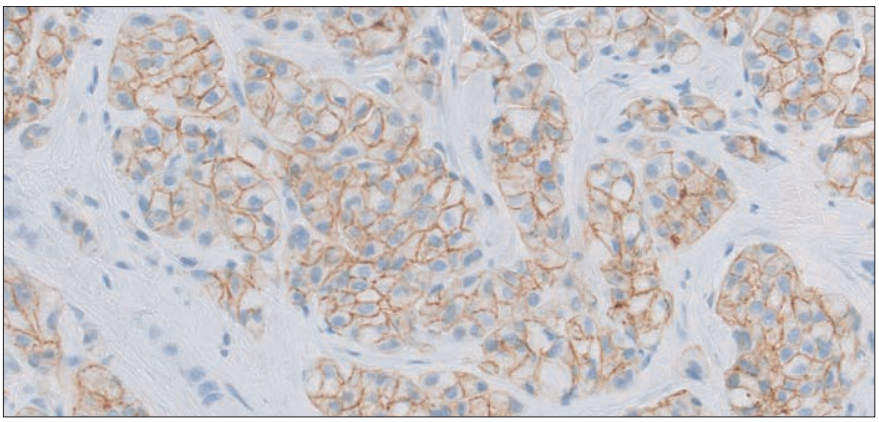


Figure 44c: 40x magnification.

Case 14: Score 2+

A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.

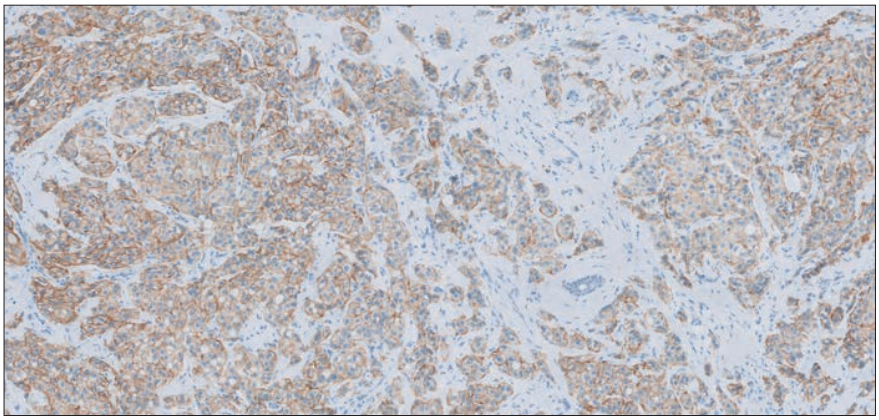


Figure 45a: 10x magnification.

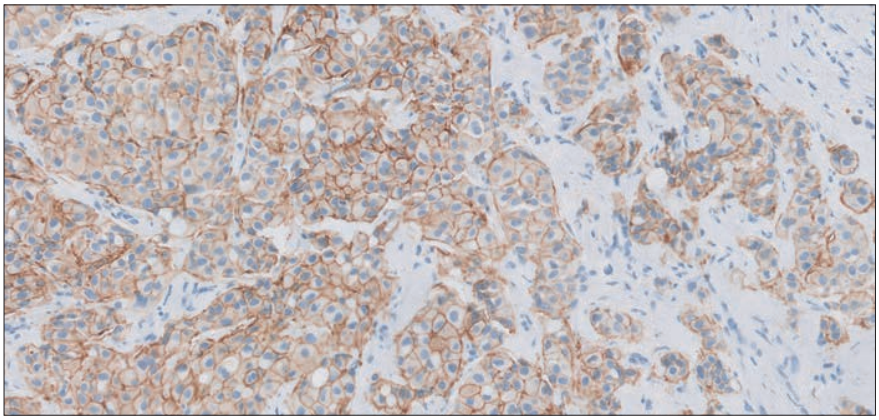


Figure 45b: 20x magnification.

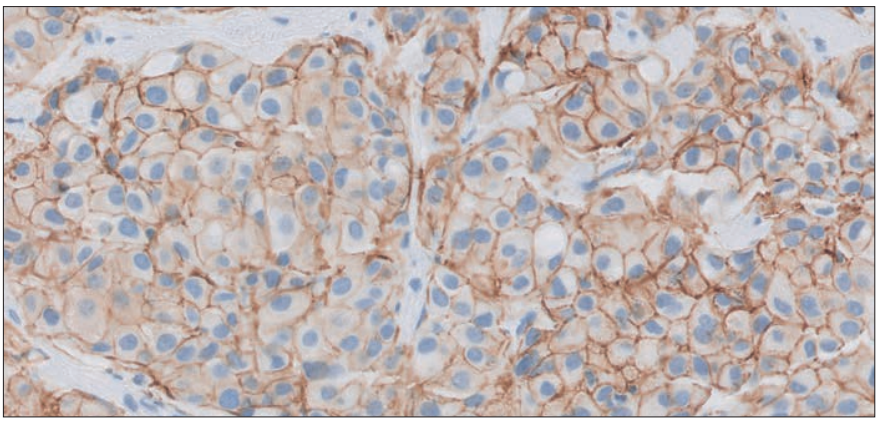


Figure 45c: 40x magnification.

Case 15: Score 3+

A strong complete membrane staining is observed in more than 10% of the tumor cells.

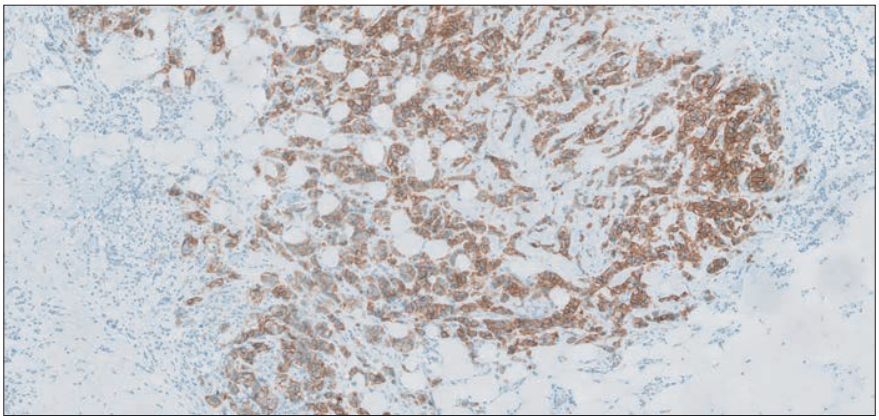


Figure 46a: 10x magnification.

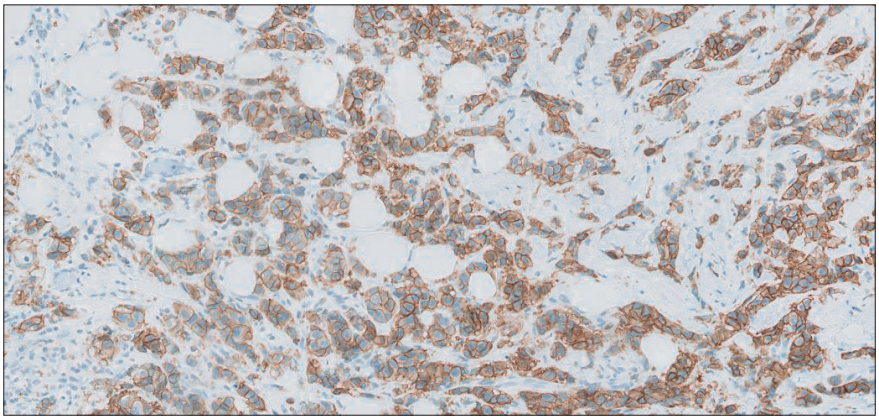


Figure 46b: 20x magnification.

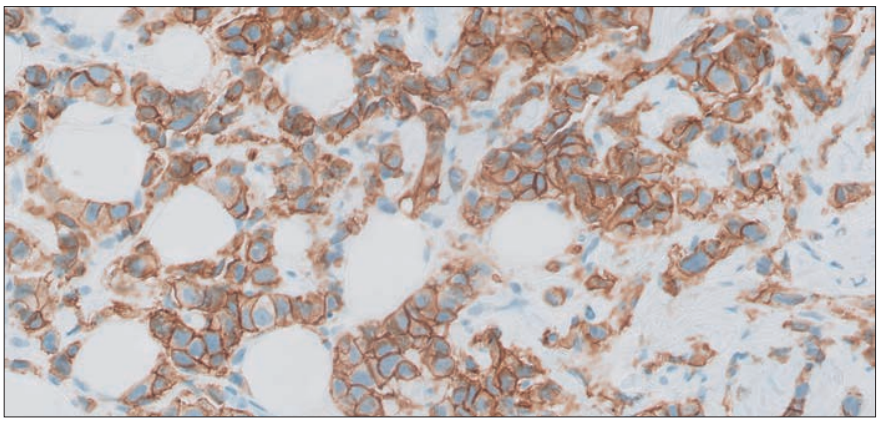


Figure 46c: 40x magnification.

Case 16: Score 3+

A strong complete membrane staining is observed in more than 10% of the tumor cells.

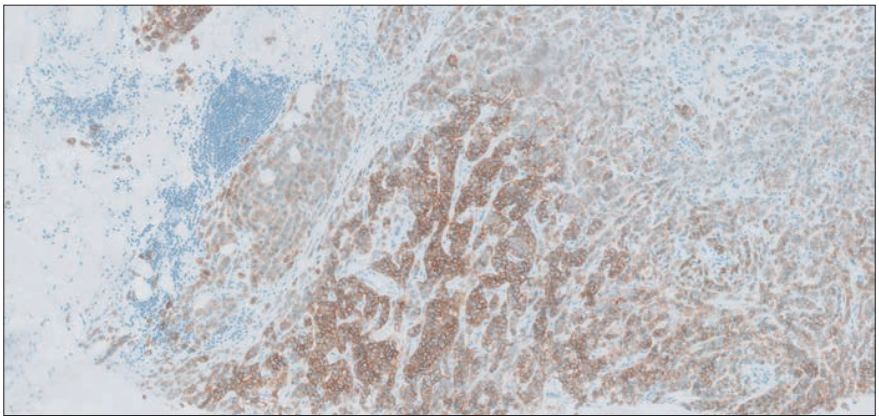


Figure 47a: 10x magnification.

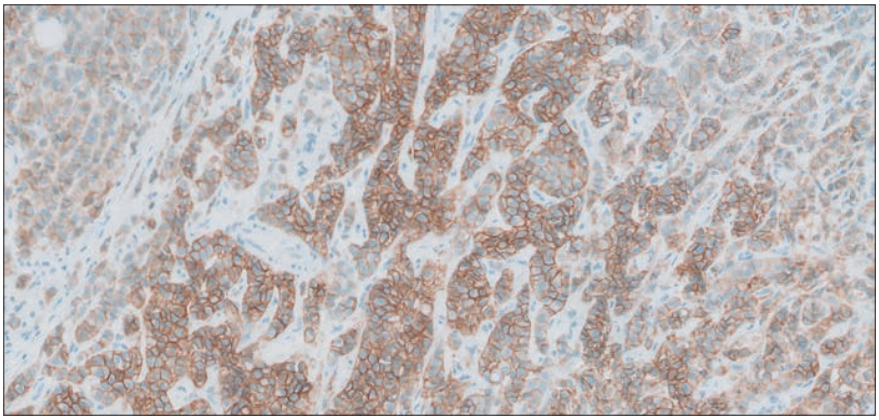


Figure 47b: 20x magnification.

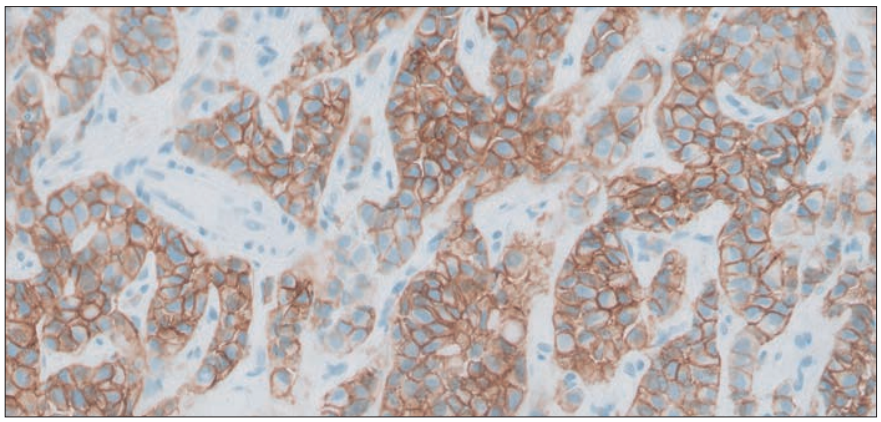


Figure 47c: 40x magnification.

Case 17: Score 3+

A strong complete membrane staining is observed in more than 10% of the tumor cells.

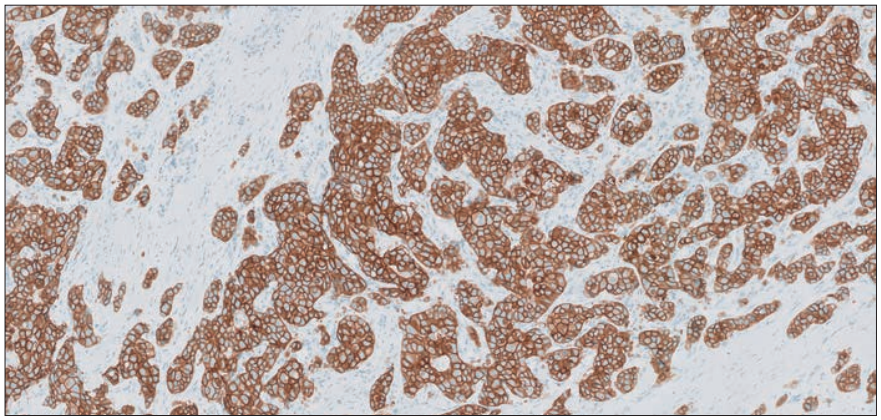


Figure 48a: 10x magnification.

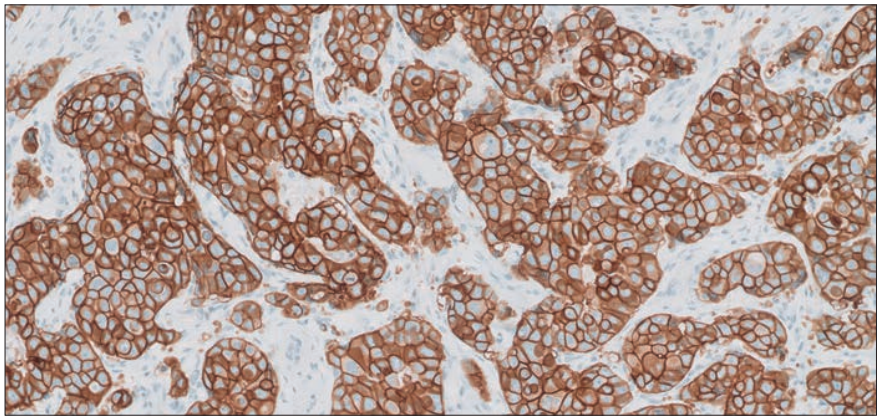


Figure 48b: 20x magnification.

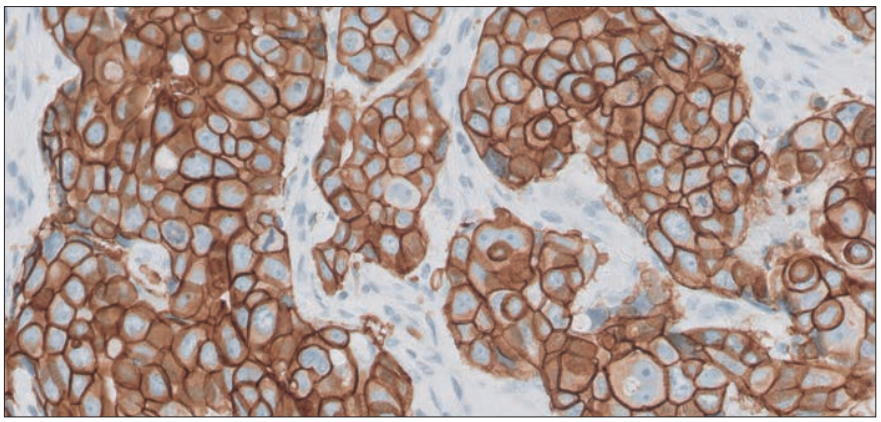


Figure 48c: 40x magnification.

Case 18: Score 3+

A strong complete membrane staining is observed in more than 10% of the tumor cells.

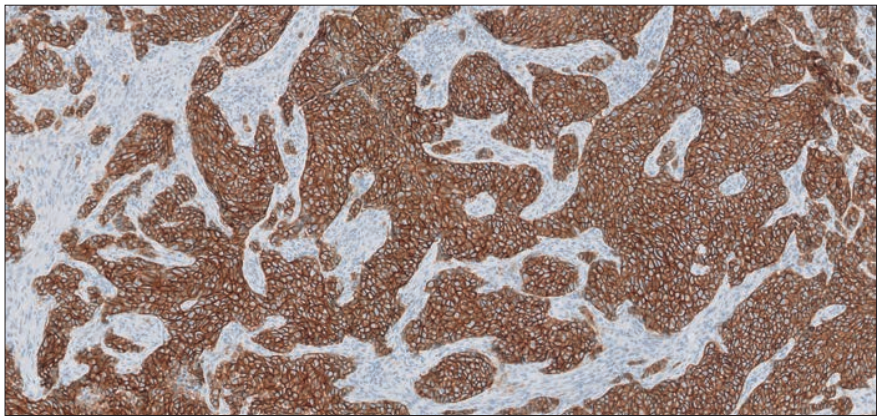


Figure 49a: 10x magnification.

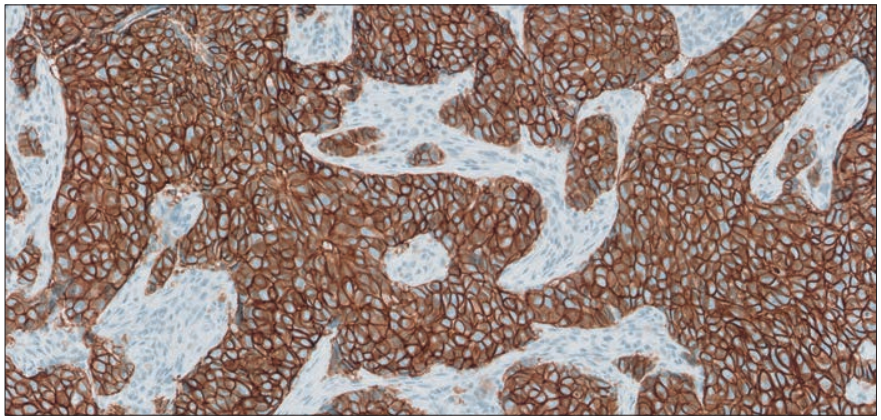


Figure 49b: 20x magnification.

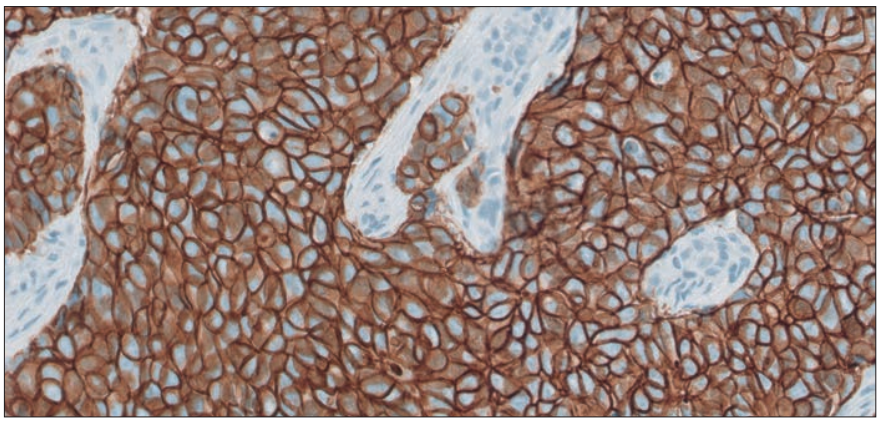


Figure 49c: 40x magnification.

Case 19: Score 3+

A strong complete membrane staining is observed in more than 10% of the tumor cells.

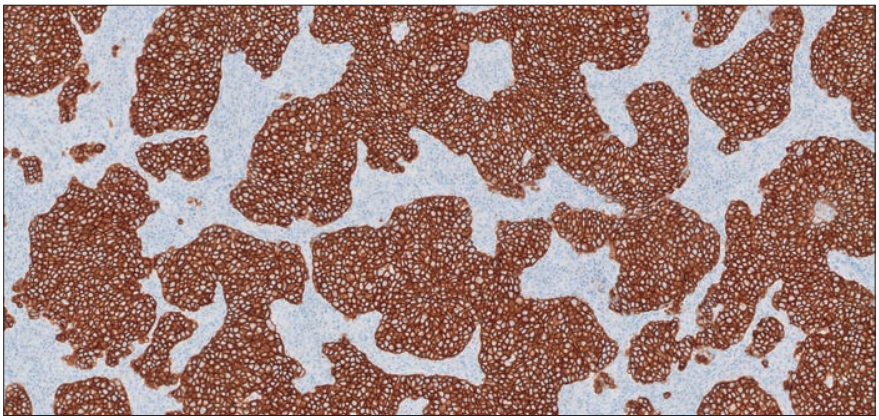


Figure 50a: 10x magnification.

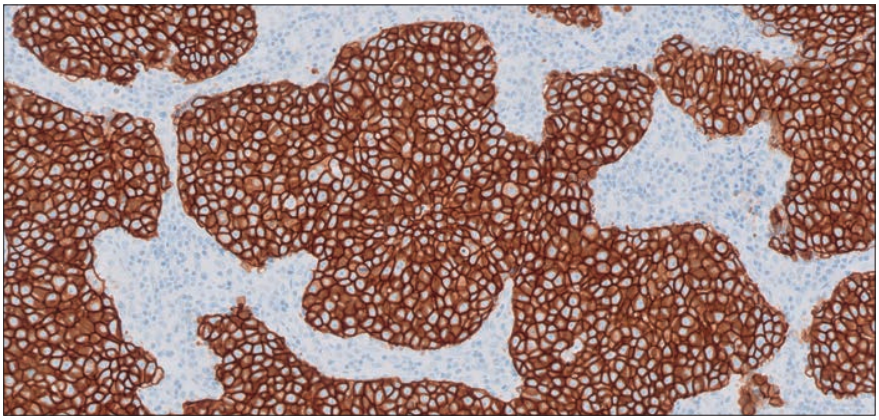


Figure 50b: 20x magnification.

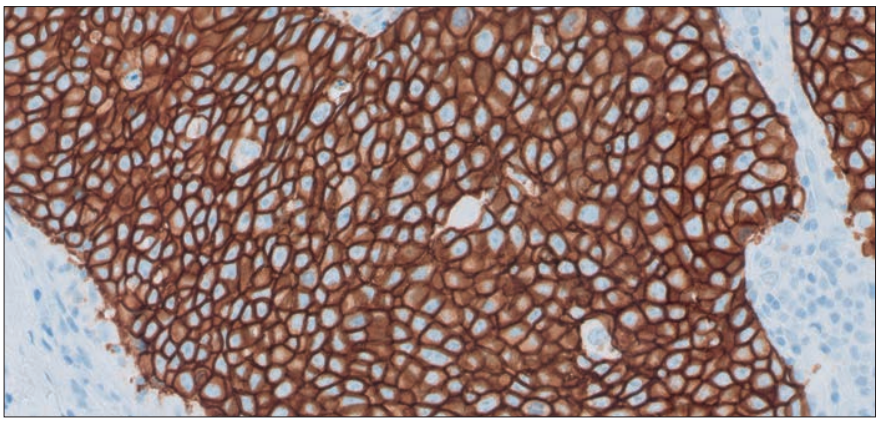
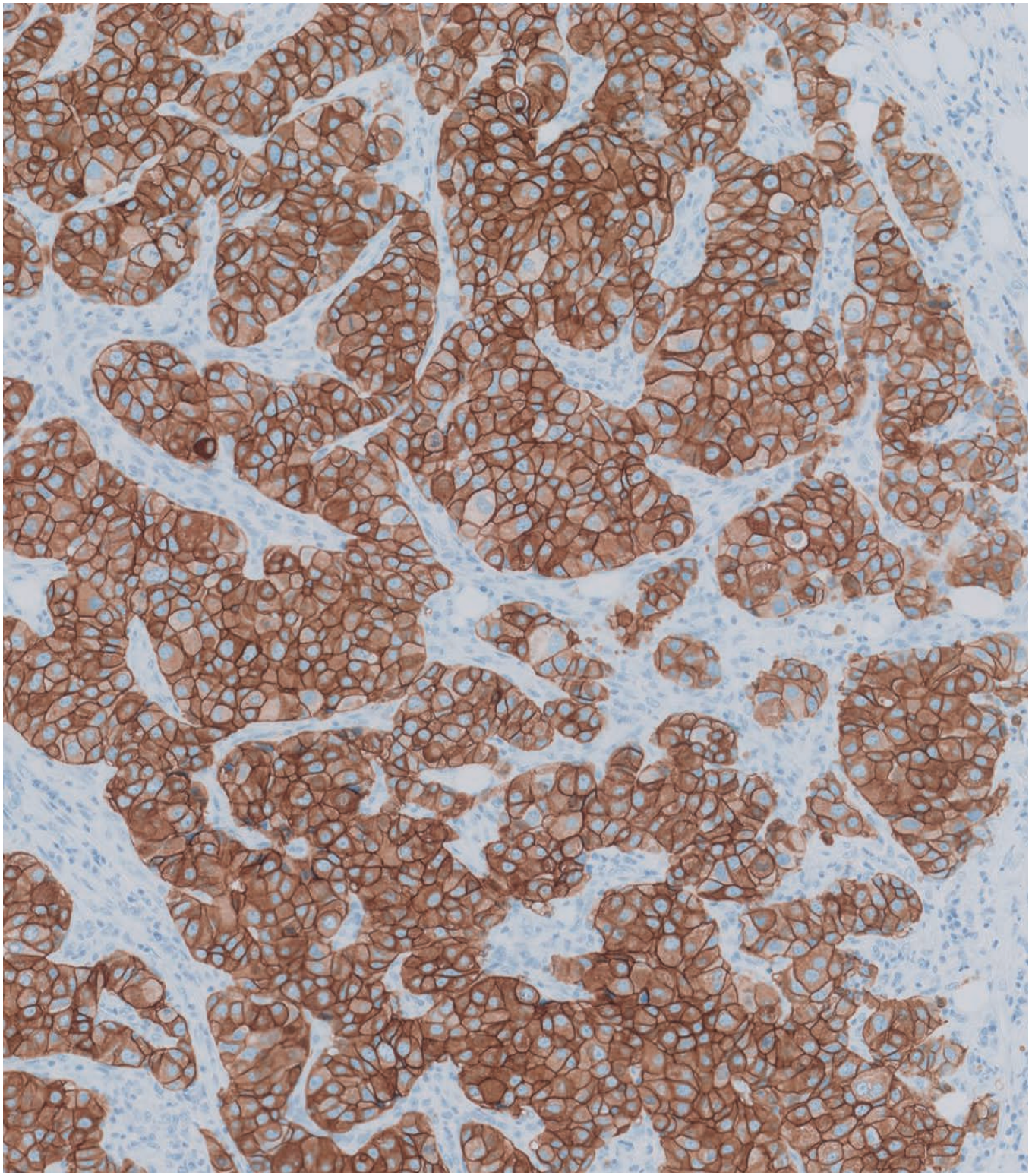


Figure 50c: 40x magnification.



Artifacts

Nonspecific Cytoplasmic Background Staining

Cytoplasmic staining should be considered nonspecific and is not to be included in the assessment of membrane staining intensity. Background staining is defined as diffuse, nonspecific cytoplasmic staining of a specimen. It can be caused by several factors including, but not limited to, pre-analytic fixation, processing of the specimen, incomplete removal of paraffin from sections, and incomplete rinsing of slides.

Possible cause of background

- Inappropriate fixation method used
- Incomplete removal of paraffin
- Incomplete rinsing of slides
- Re-use of mixing strip
- Starch additives used for mounting sections to slides
- Drying of slides (ensure that slides remain wet after staining prior to coverslipping)

If background staining is significant, the specific staining must be interpreted with caution.

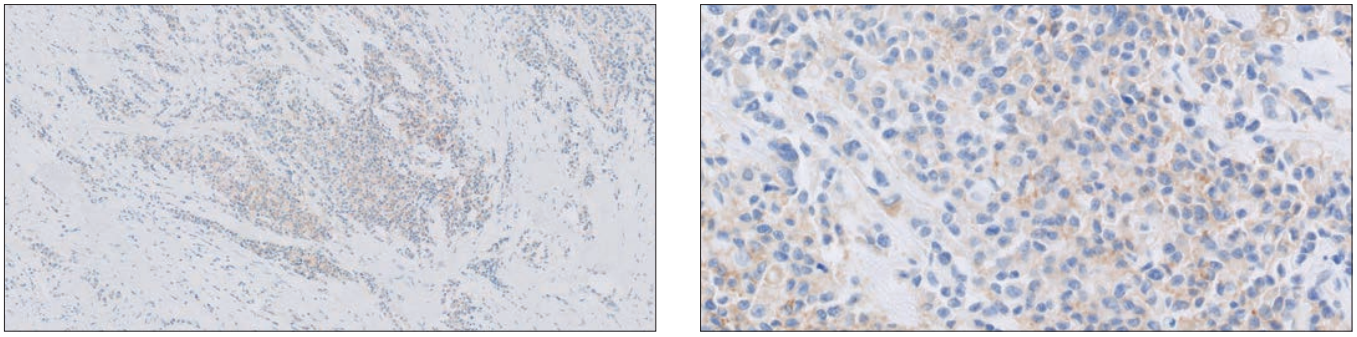


Figure 51: Breast carcinoma demonstrating cytoplasmic nonspecific staining, shown at two magnifications, 10x magnification (left), and 40x magnification (right).

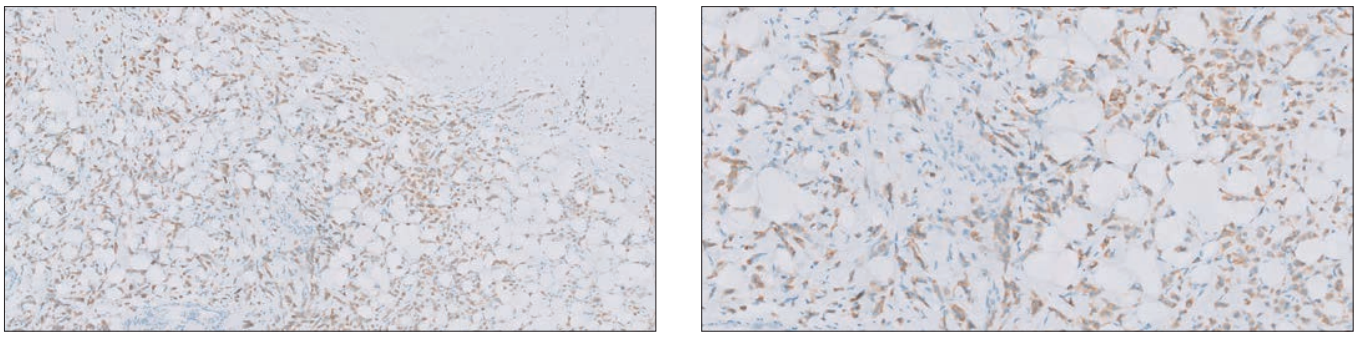


Figure 52: Breast carcinoma demonstrating cytoplasmic nonspecific staining, shown at two magnifications, 10x magnification (left), and 20x magnification (right).

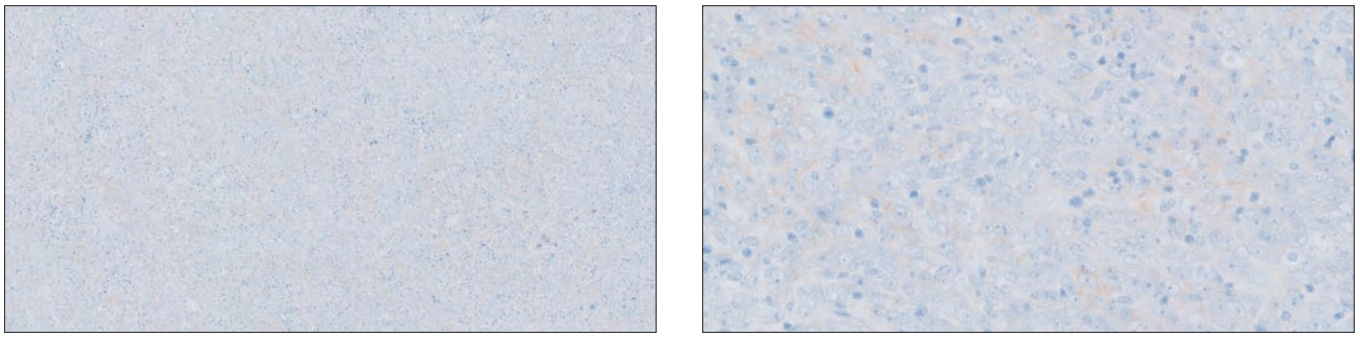


Figure 53: Breast carcinoma demonstrating diffuse cytoplasmic nonspecific staining, shown at two magnifications, 10x magnification (left), and 40x magnification (right).

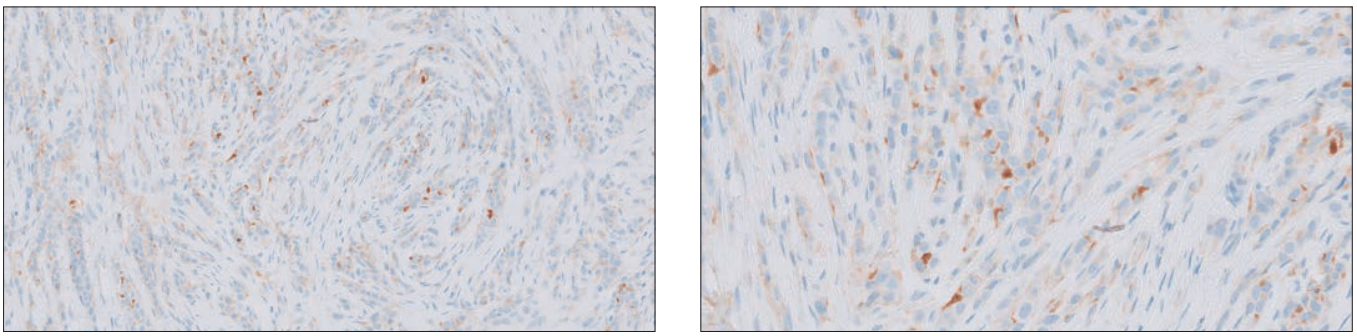


Figure 54: Breast carcinoma demonstrating dot-like artifact and cytoplasmic nonspecific staining. The dot-like artifact is specific to the cytoplasm. Shown at two magnifications, 20x magnification (left), and 40x magnification (right).

Key point

Evaluate if staining is nonspecific cytoplasmic staining.

Use 20-40x objective to evaluate for presence/ absence of membrane staining.

Edge Artifact

Frequently, edge artifacts are linked to the pre-analytic handling of the tissue and the method of surgical extraction is often the cause. This artifact is more frequently observed in stereotactic needle biopsies. Increased staining is observed around the periphery of the tissue specimen, known as 'edge effect'. If the positive reaction is only at the edge of the tissue section, i.e. a few layers of staining at the periphery and ending abruptly with penetration into the centrally located tumor, scoring at the edge of the tissue specimen should be avoided.

One possible reason for edge artifacts could be tissue drying prior to fixation. Another reason could be inadequate fixation of thick tissue samples, which mimics edge artifacts by rendering the central portion of the tissue suboptimally fixed relative to the peripheral areas. In these circumstances, the immunoreactivity based on the suboptimal central portion may be mistakenly interpreted as false-negative.

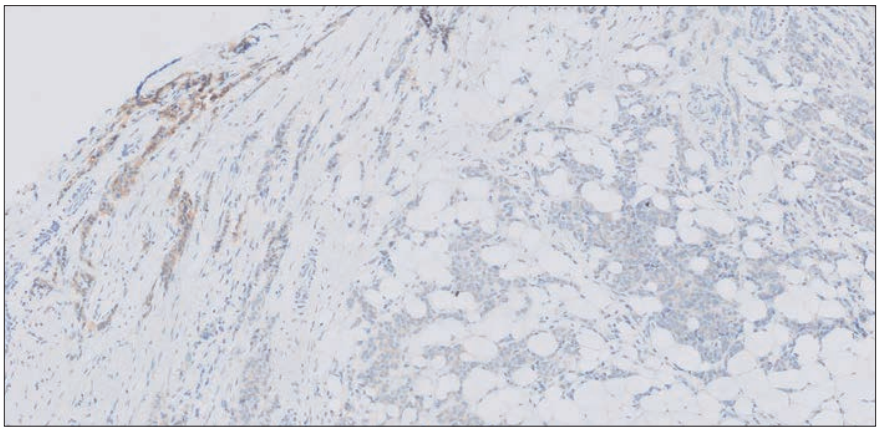


Figure 55: Example of edge artifact due to tissue drying prior to fixation (10x magnification).

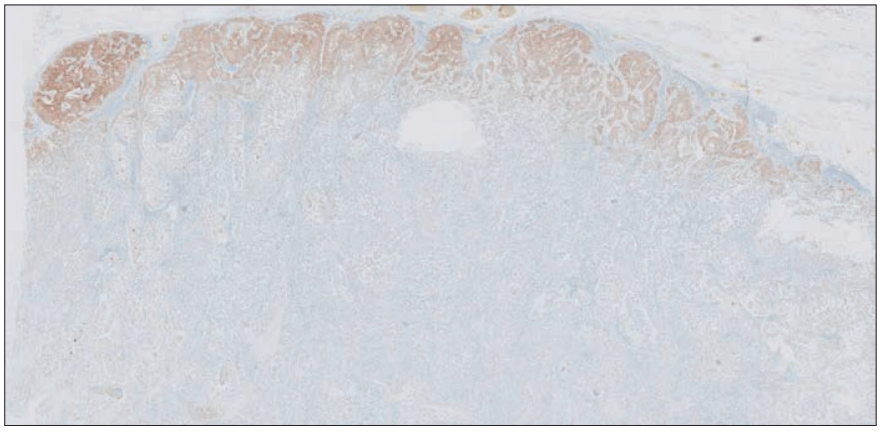


Figure 56: Example of edge artifact due to suboptimal fixation (1x magnification).

Key point

Interpret edge artifact with the specimen type and whole slide staining pattern in mind.

Crush Artifact

Crush artifact is similar to edge artifact as they both show increased staining at the periphery of the tissue. This artifact may be encountered more often in stereotactic needle biopsies. It is presumed that the tissue injury occurs during the extraction of the tissue from the needle rather than from the actual biopsy process. The compression of the tissue along the edges produces morphologically distorted cellular architecture that should be interpreted as artifact.

- Inadvertent crushing of the tissue occasionally occurs during sectioning, also resulting in peripheral crush artifact.
- When compared to surrounding cells, crushed cells often demonstrate stronger staining due to compression of cell membranes. Crushed cells should be avoided when scoring.

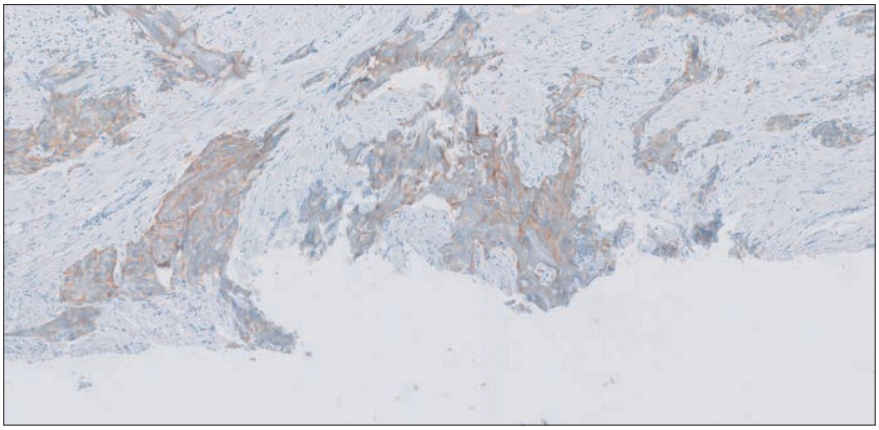


Figure 57: Example of crush artifact (10x magnification).

Key point

Scoring of crush artifact should be avoided if staining is inconsistent with entire specimen.

Heterogenous Staining

Heterogeneous staining pattern caused by biological events is an infrequent issue. Consequently, when present, this staining pattern may represent artifacts of tissue preparation. See also Edge Artifact section.

- The pathologist's experience and judgment are important in the evaluation of heterogeneous staining.
- In the absence of clear histologic evidence of biological heterogeneity, the most well-preserved and well-stained areas should be scored.
- Review these cases at a low magnification. Use high power (20x and/or 40x) to confirm staining pattern, if necessary.
- There must be more than 10% of the infiltrating tumor cells demonstrating complete membrane staining for the score to be at least 2+ or greater.

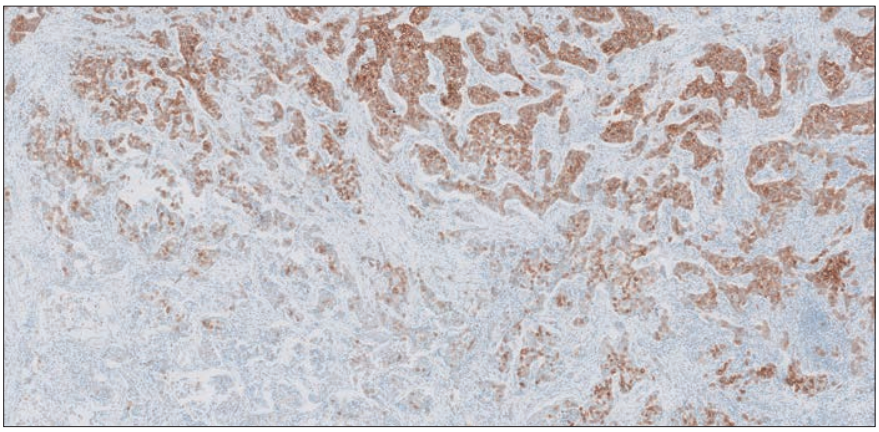


Figure 58: Breast carcinoma with example of heterogeneous staining. Characteristic feature: 3+ score at the top and 0 score in the lower left, with an intermingling of tumor cell subsets in between (>10% of the infiltrative tumor cells demonstrate complete membrane staining) (5x magnification).

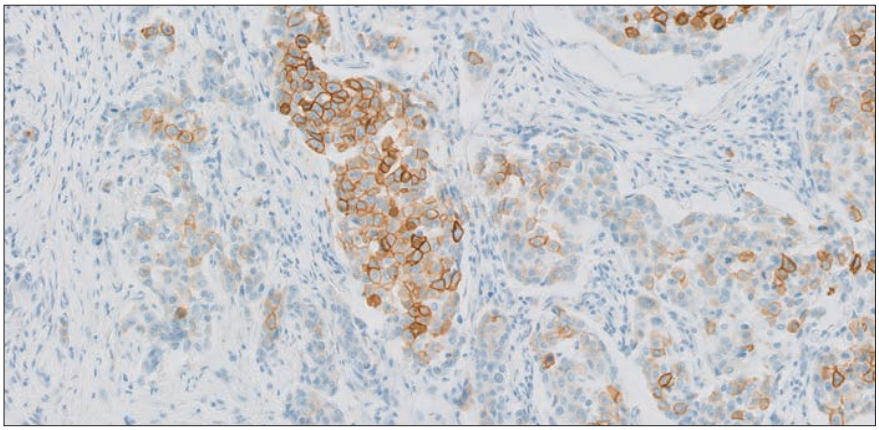


Figure 59: Breast carcinoma with example of heterogeneous staining (10x magnification).

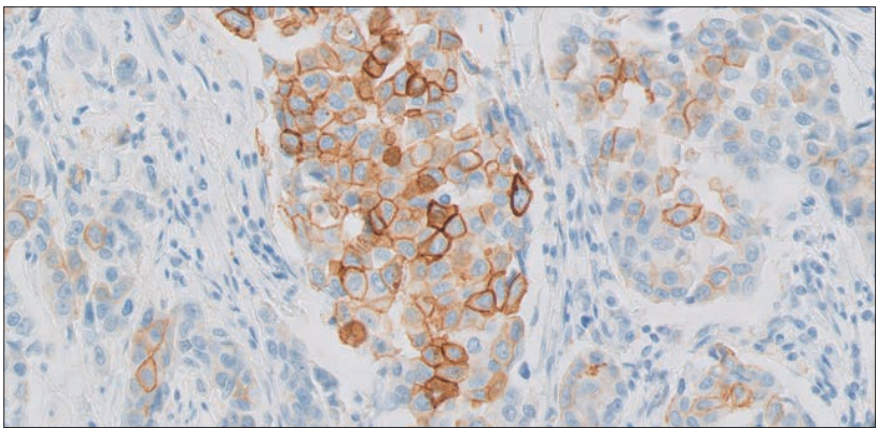


Figure 60: Breast carcinoma with example of heterogeneous staining (40x magnification).

Key points

Determine if staining pattern is an artifact. Consider re-staining or repeating staining using another sample from the same patient.

Evaluate the percentage of cells showing membrane staining.

Evaluate if the membrane staining is complete or incomplete.

Determine the intensity of the membrane staining.

Necrosis

Necrosis is irreversible cellular injury with histologically undefined/poorly defined cellular details and associated inflammation. Necrotic areas should be excluded from scoring.

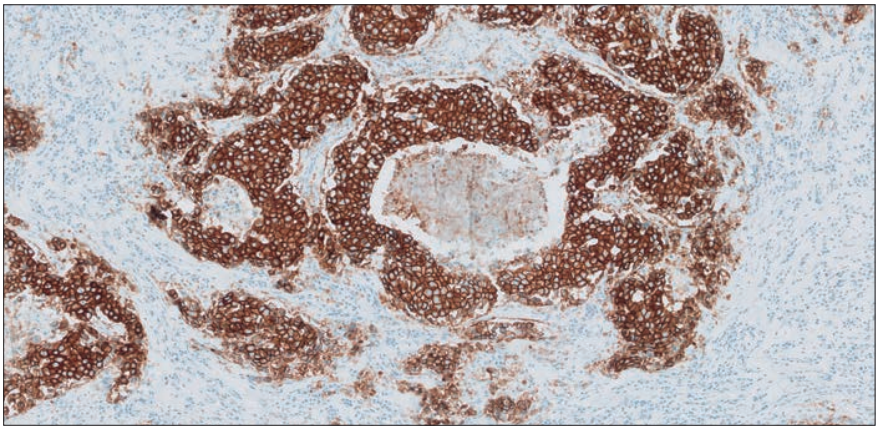


Figure 61: Example of necrosis (10x magnification).

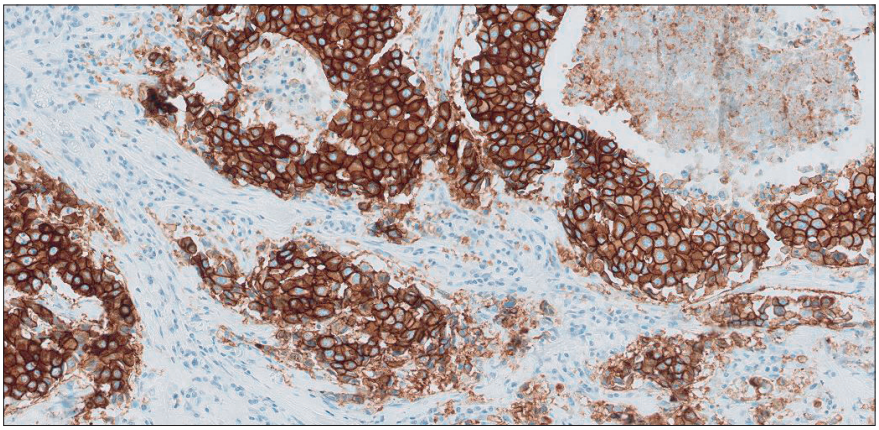


Figure 62: Example of necrosis (20x magnification).

Key point

Necrotic areas should be excluded from scoring.

Retraction Artifact

Retraction artifacts may sometimes be observed. This may be due to poor fixation. It is also often seen in mucin-producing tumors due to mucin shrinkage during tissue processing.

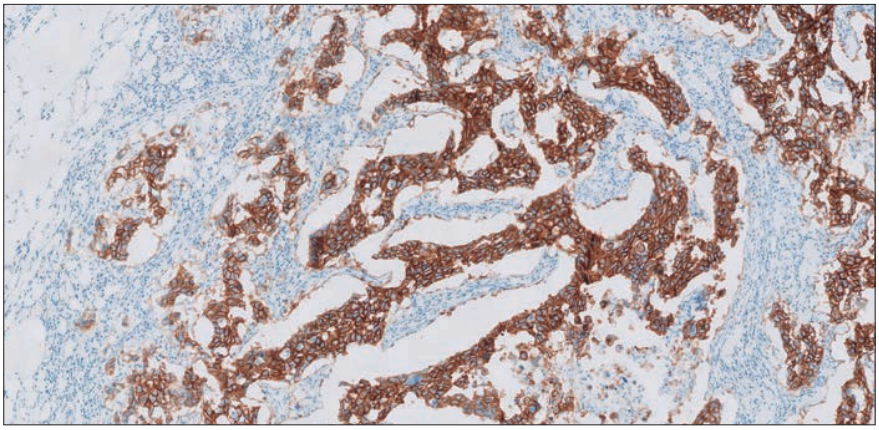


Figure 63: Example of how retraction artifact caused by poor fixation may look (10x magnification).

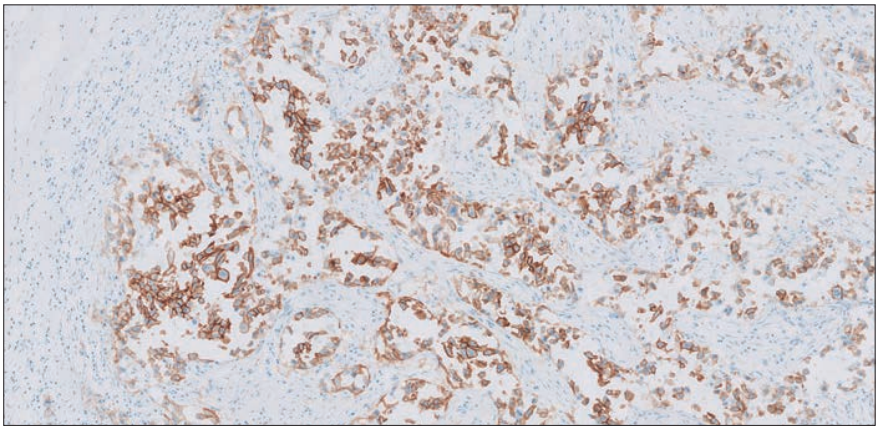


Figure 64: Example of how retraction artifact caused by mucus may look (10x magnification).

Key point

Proper fixation is important for accurate diagnosis.

Pathologist experience and judgment are necessary to determine whether the stain can be evaluated as is or should be repeated, perhaps using a second tissue block from the same specimen.

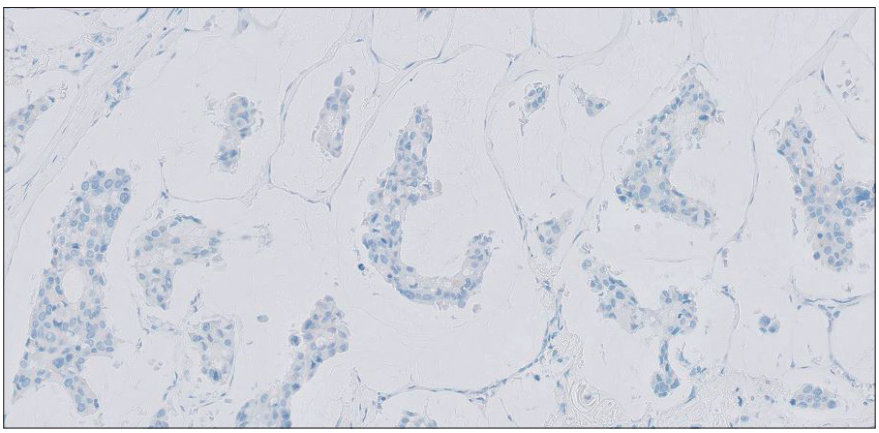


Figure 65: Example of how retraction artifact caused by mucus may look (10x magnification).

Troubleshooting Guide

Problem	Probable Cause	Suggested Action
1. No or weak staining of slides	1a. Wrong protocol and reagents selected.	1a. Check slide history to verify that correct protocol was selected.
	1b. Excessive heating of mounted tissue sections prior to loading on Dako Omnis may lead to loss of immunoreactivity and morphology.	1b. Dry the tissue sections at 60 °C for a maximum of one hour. Drying of tissue sections at elevated temperatures must only be performed in a calibrated oven with uniform heat distribution.
	1c. Wrong storage conditions used for reagents or tissue sections.	1c. Check that reagents have been stored correctly according to listed storage conditions. Ensure that tissue sections are stained within cut section storage recommendations..
	1d. Inappropriate fixation method used.	1d. Ensure that patient tissue is not fixed for too short or too long a time period, or that an alternative fixative was not used.
	1e. Reagent is used past its expiration date.	1e. Ensure that reagent is not used past its expiration date.
	1f. Reagent is used past its onboard stability.	1f. Ensure that reagent is not used past its onboard stability
	1g. Incorrect placement of dynamic gap lids in stainer modules.	1g. Check placement of dynamic gap lids.
	1h. Damaged dynamic gap lids.	1h. Check integrity of dynamic gap lids.
	1i. Reagent-grade quality (distilled or de-ionized) water is not used to dilute the Target Retrieval Solution concentrate.	1i. Ensure that reagent-grade quality (distilled or de-ionized) water is used to prepare 1x Target Retrieval Solution. Please note that not all sources of distilled or de-ionized water have sufficient quality/purity for IHC reagent preparation. Agilent recommends reagent-grade quality distilled or de-ionized water or water similar in purity to be used for reagent preparation.
	1j. Incorrect Target Retrieval Solution is used.	1j. Ensure that Target Retrieval Solution specified in Materials provided, Substitutions for kit components and/or Reagent Preparation sections is used.
2. Unapproved staining of the positive control tissue or Control Slide	2a. Degradation of Control Slide or reagents or run artifacts.	2a. Restain positive control tissue/Control Slide to confirm results. Check that reagents, including Control Slides and positive control tissue, have been stored correctly according to listed storage conditions. Ensure Control Slide is not used past its expiration date. Ensure that Control Slide has the same lot number as primary antibody and visualization reagent.
3. Excessive background staining of slides	3a. Starch additives used in mounting sections to slides.	3a. Avoid using starch additives for adhering sections to glass slides. Many additives are immunoreactive.
	3b. Sections dried out after staining procedure onboard Dako Omnis.	3b. Verify that the unloading station is filled with sufficient amount of water.
	3c. Sections dried prior to coverslipping.	3c. Avoid stained slides drying out after unloading from Dako Omnis and before coverslipping.
	3d. Inappropriate fixation method used.	3d. Ensure that approved fixative was used. Alternative fixative may cause excessive background staining.
	3e. Paraffin incompletely removed.	3e. Check appearance of solvent coupling. Gently scrub the couplings to remove impurities. Check the integrity of the couplings on the backside of the bulk bottles after cleaning. Refer to Dako Omnis Basic User Guide for details.
	3f. Nonspecific binding of reagents to tissue.	3f. Ensure that correct fixation method of the specimen is used and avoid large areas of necrosis.
	3g. Re-use of mixing strip.	3g. Ensure that new mixing strips are used.
4. Tissue detaches from slides	4a. Use of incorrect slides.	4a. Use FLEX IHC Microscope Slides (Code K8020), or SuperFrost Plus slides.

Problem	Probable Cause	Suggested Action
5. Excessively strong specific staining	5a. Inappropriate fixation method used.	5a. Ensure that only approved fixatives and fixation methods are used.
	5b. Reagent-grade quality (distilled or de-ionized) water is not used to dilute the Target Retrieval Solution concentrate.	5b. Ensure that reagent-grade quality (distilled or de-ionized) water is used to prepare 1x Target Retrieval Solution. Please note that not all sources of distilled or de-ionized water have sufficient quality/purity for IHC reagent preparation. Agilent recommends reagent-grade quality distilled or de-ionized water or water similar in purity to be used for reagent preparation.
6. Target Retrieval Solution (concentrate or working solution) is cloudy in appearance prior to loading on Dako Omnis	6a. The solution has been incorrectly stored or the expiration date of the solution has passed.	6a. Check the expiration date and kit storage conditions outside of package. Discard the Target Retrieval Solution.
7. Slide is flagged	7a. Reagent is used past its expiration date.	7a-c. Flagged slides should be evaluated by qualified personnel. Contact a Dako representative if further action is needed.
	7b. Lot-restricted reagent is stored onboard Dako Omnis beyond its validated onboard stability.	
	7c. Maintenance overdue or other factors.	

Note: If the problem cannot be attributed to any of the above causes, or if the suggested corrective action fails to resolve the problem, please contact Agilent Pathology Support for further assistance. Additional information on staining techniques and specimen preparation can be found in Dako Education Guide: Immunohistochemical Staining Methods (available from www.agilent.com), Atlas of Immunohistology and Immunoperoxidase Techniques. A Practical Approach to Tumor Diagnosis.

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Reporting Results

Suggested information to include when reporting results with HercepTest™ mAb pharmDx (Dako Omnis).

HercepTest™ mAb pharmDx (Dako Omnis) Summary of Sample Tested

Date of Run: _____

HercepTest™ mAb pharmDx (Dako Omnis) Lot: _____

Patient Identifiers: _____

Specimen ID: _____

Slide ID: _____

Comments: _____

HercepTest™ mAb pharmDx (Dako Omnis) Controls Results

HercepTest™ mAb pharmDx Control Slides (Dako Omnis):	Pass: ☐	Fail: ☐	
Positive Control Tissue:	Pass: ☐	Fail: ☐	
Negative Control Tissue	Pass: ☐	Fail: ☐	
Patient Specimen, Negative Control Reagent (optional):	Pass: ☐	Fail: ☐	Not included: ☐

HercepTest™ mAb pharmDx (Dako Omnis) Result to Treating Physician

Score/HER2 protein overexpression assessment:

0, Negative: ☐

1+, Negative: ☐

2+, Weakly positive: ☐

3+, Strongly positive: ☐

Other comments to Treating Physician: _____

_____

_____

Date and signature: _____

Notes

Notes

Notes

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