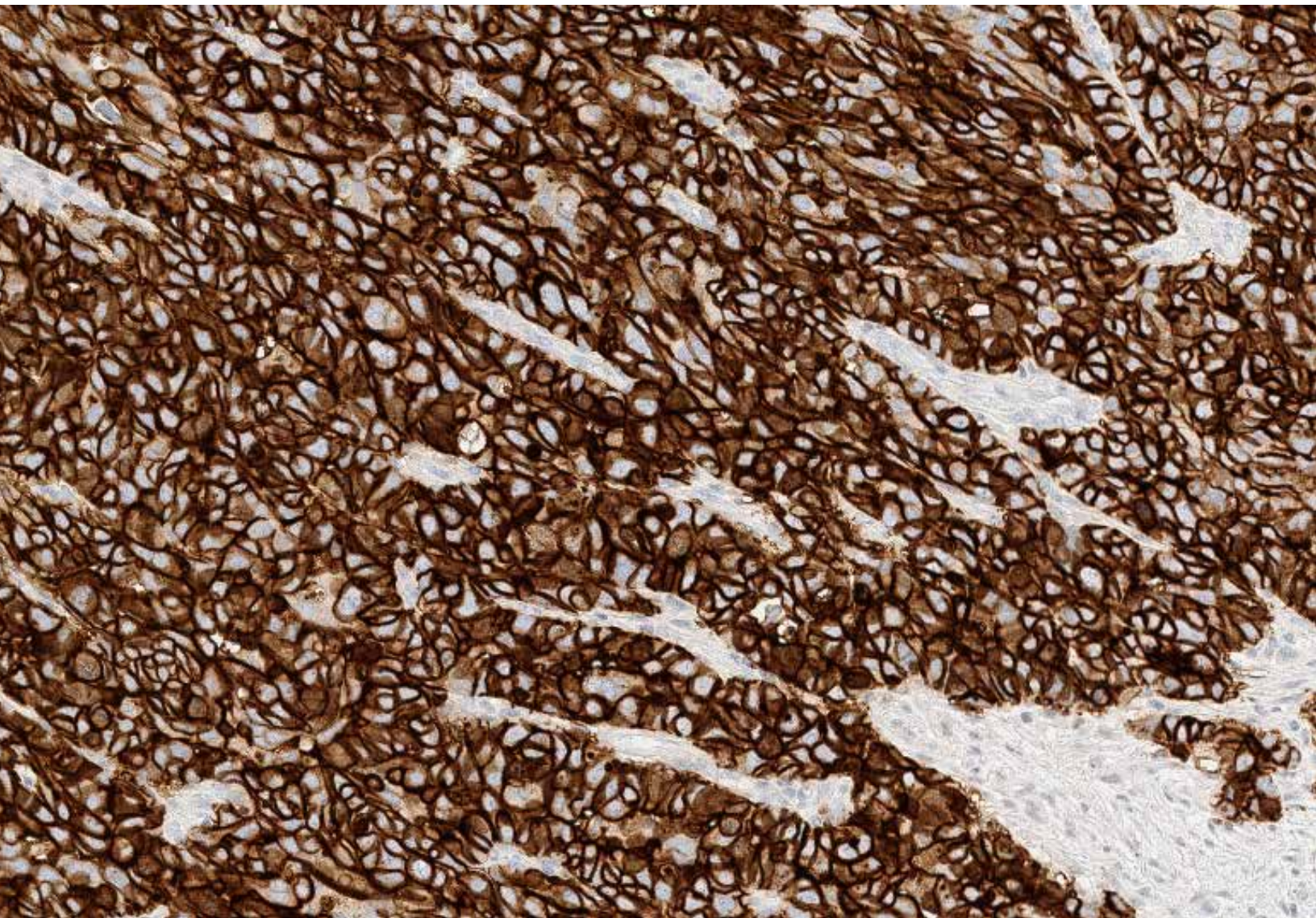
VENTANA FOLR1 (FOLR1–2.1) RxDx Assay Interpretation Guide for Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancer



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Introduction

The Folate Receptor 1 protein (FOLR1), also commonly known as Folate Receptor alpha (FRα), is a 38-40 kDA glycosylphosphatidylinositol (GPI)-anchored cell surface protein encoded by the FOLR1 gene, that is largely restricted to malignant tumors compared to normal tissue.[1] The FOLR1 protein mediates the transfer of a carbon unit necessary for the de novo synthesis of purines and thymidylate, and it is required for synthesizing DNA, RNA, and enzyme co-factors. Tumors have increased metabolic demands as a consequence of their enhanced proliferation, and thus, a higher demand for thymidylate and purines compared to normal tissues. Exploiting this biologic necessity for enhanced folate uptake may provide an opportunity for anti-folate cancer therapy.[2] FOLR1 shows limited normal tissue expression and high expression on the surface of solid tumors, particularly epithelial ovarian cancer (EOC), endometrial cancer, non-small cell lung carcinoma, and renal cell cancer. Malignancies that are managed as EOC include primary fallopian tube cancer and primary peritoneal cancer.[3] FOLR1 levels are positively associated with tumor stage and grade, which suggests that FOLR1 might confer a growth advantage to the tumor by modulating folate uptake or by generating regulatory signals.[2,4] The FOLR1 protein is either absent from normal tissues or localized to the luminal surface of certain epithelial cells, where it is inaccessible to the circulation, whereas in tumors, FOLR1 is fully accessible via circulation.[4] Therefore, FOLR1 is frequently exploited as a target for receptor specific delivery of chemotherapy and immunotherapy agents.[1,5] Since folate-linked drugs do not normally accumulate in normal tissues, this approach provides high specificity for FOLR1 expressing tumor cells.

EOC patients often present with advanced disease, and have limited prognosis. Despite considerable improvements in primary therapy, 80% of the patients with advanced EOC are expected to relapse during or after treatment with platinum-containing regimens.[6] Treatment of patients with recurrent EOC is less standardized than treatment of newly diagnosed patients. A recent retrospective study demonstrated that progression-free survival (PFS) and overall survival (OS) decrease by more than 50% as patients move from the first to the fifth relapse.[7] In addition, the study revealed that patients derived little benefit from treatment with current agents beyond the third line of therapy.[8]

Roche Tissue Diagnostics has developed the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay (hereafter VENTANA FOLR1 Assay) for use as a companion diagnostic to aid in identifying folate receptor positive patients who may benefit from FOLR1-targeted therapy. The VENTANA FOLR1 Assay is used with the OptiView DAB IHC Detection Kit as a fully automated immunohistochemistry (IHC) assay on the BenchMark ULTRA IHC/ISH instrument. The sensitivity of the IHC assay enables a reproducible, binary scoring system (Positive or Negative for FOLR1 status) for evaluating the staining results (refer to the package insert for VENTANA FOLR1 (FOLR1-2.1) RxDx Assay (Cat. No. 740-5065/ 07727917001).

Intended Use

Intended Use of Product

Refer to the corresponding VENTANA FOLR1 Assay method sheet for the detailed intended use of this product.

Purpose of Interpretation Guide

This guide is intended to:

- Provide pathologists with a tool to facilitate the clinical evaluation of formalin-fixed, paraffin-embedded (FFPE) epithelial ovarian cancer, primary peritoneal, and fallopian tube cancer sections (all managed as EOC) stained with the VENTANA FOLR1 Assay using the VENTANA FOLR1 Assay Scoring Method in accordance with the proposed product labeling.
- Provide photographic images that illustrate the staining patterns that may result from staining of ovarian carcinoma tissues with The VENTANA FOLR1 Assay. Images of primary peritoneal and primary fallopian tube cancer are not included in visual illustrations due to the rarity of these indications.
- Provide a reference for relating staining patterns and intensities to specific FOLR1 scores.
- Provide example images of challenging cases to provide guidance in their evaluation.
- Provide guidance in using the FOLR1-positive control tissue, normal fallopian tube tissue, which serves as a tissue control when stained with the VENTANA FOLR1 Assay.
- Provide guidance in using the VENTANA FOLR1 Stain Intensity Reference Slide (Cat. No. 09382780001) as a stain intensity reference for evaluation of slides stained as part of the VENTANA FOLR1 Assay.

Clinical Evaluation

Staining Characteristics

In EOC tissue, neoplastic cells labeled with the VENTANA FOLR1 Assay are evaluated for percent tumor cell staining of the diaminobenzidine (DAB) signal. VENTANA FOLR1 Assay staining in EOC tissue follows a cytoplasmic and membranous pattern. The signal is classified as strong, moderate, weak, or negative based on membrane localization only. The VENTANA FOLR1 Stain Intensity Reference Slide (Cat. No. 09382780001) should be used as reference for determination of moderate signal intensity.

- **Negative (0)** signal intensity is characterized by an absence of any detectable signal. Negative cases may still exhibit pale grey cytoplasmic and/or membranous discoloration.

- **Weak (1+)** signal intensity is characterized by a faint gold/light brown hue that may be partial or circumferential.

- **Moderate (2+)** or **Strong (3+)** signal intensity is characterized by a chocolate brown to thickened dark brown, black hue that may be partial or circumferential.

The signal may be distributed heterogeneously having more than one intensity level. The relative percentages of neoplastic cells staining at each of the following signal intensities: strong (3+), moderate (2+), weak (1+), and negative (0), are visually estimated and used to generate a diagnostic score.

Scoring Algorithm

Evaluating VENTANA FOLR1 (FOLR1-2.1) RxDx Assay IN EOC:

For the VENTANA FOLR1 Assay, each case is stained with the VENTANA FOLR1 (FOLR1-2.1) mouse monoclonal primary antibody and a matched negative reagent control (NRC), Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001). Neoplastic cells labeled with the VENTANA FOLR1 Assay are evaluated for presence or absence of the DAB signal. The matched NRC-stained slide is used to assess non-specific background staining and degree of background staining known to occur due to specific tissue elements. Please note: OptiView DAB IHC Detection Kit is the only detection reagent that is recommended for use with the VENTANA FOLR1 Assay.

The scoring algorithm for the VENTANA FOLR1 Assay is provided below in **Table 1**.

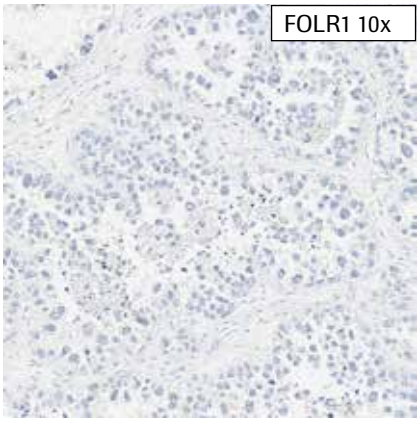
Table 1: VENTANA FOLR1 (FOLR1-2.1) RxDx Assay Scoring Algorithm for EOC

IHC Interpretation	Staining Description
Positive for FOLR1*	≥ 75% of viable tumor cells with moderate (2+) and/or strong (3+) membrane staining
Negative for FOLR1*	< 75% of viable tumor cells with moderate (2+) and/or strong (3+) membrane staining
Not Evaluable	Artifacts making interpretation not possible

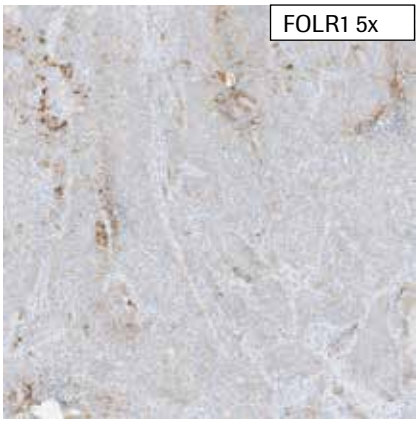
* Re-reading by Additional Pathologists for FOLR1 Scoring

To decrease variability of FOLR1 results for cases with %TC near the threshold of 75% (65% to 85%), re-reading of the slide by a second pathologist is recommended. The case result with %TC between 65-85% by a pathologist should be adjudicated by one or two independent pathologists. The patient's final result with regard to FOLR1 Positive should be obtained by either a majority rule or by consensus among the pathologists.

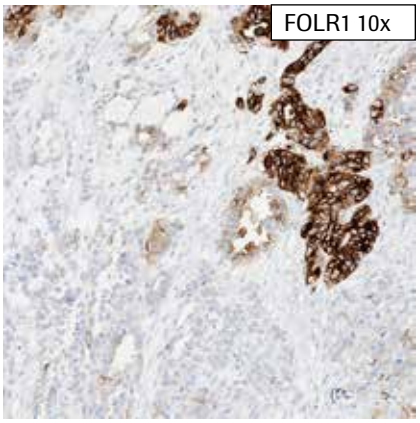
Clinical Diagnosis Negative



Exhibits no moderate or strong tumor cell membrane staining or < 75% moderate and/or strong tumor cell membrane staining

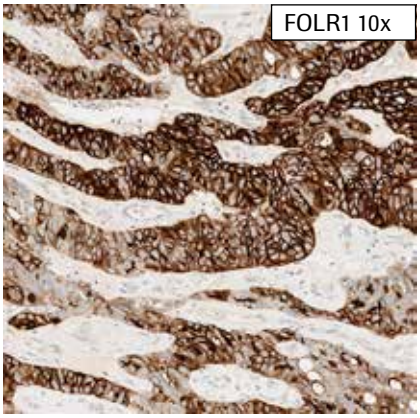


Exhibits 7% moderate and strong membrane staining or < 75% moderate and/or strong tumor cell membrane staining

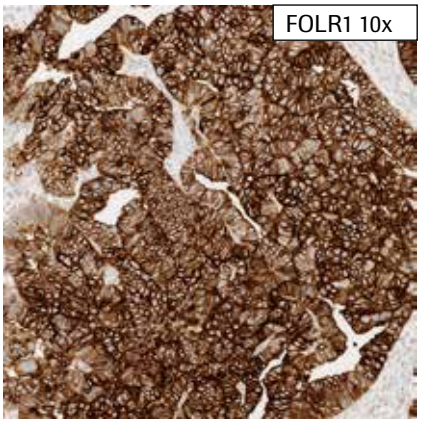


Exhibits 20% moderate and strong membrane staining or < 75% moderate and/or strong tumor cell membrane staining

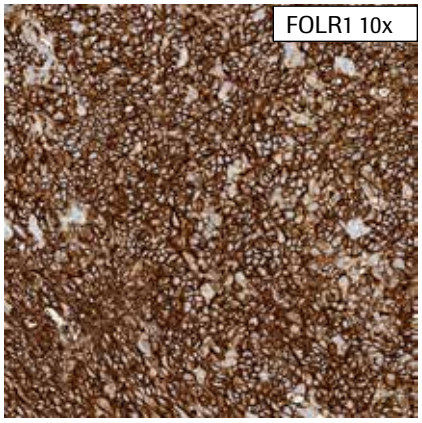
Clinical Diagnosis Positive



Exhibits 95% moderate and strong membrane staining or ≥ 75% moderate or strong membrane staining with complete circumferential pattern*



Exhibits 98% moderate and strong membrane staining or ≥ 75% tumor cells membrane staining with complete circumferential pattern*



Exhibits 98% moderate and strong membrane staining or ≥ 75% tumor cells membrane staining with complete circumferential pattern*

*Note: Partial circumferential membrane staining patterns are also acceptable for FOLR1 positivity. FOLR1 positivity does not require complete circumferential membrane staining.

Scoring Method

VENTANA FOLR1 Assay staining can be observed in tumor cells of EOC tissues, which exhibit a cytoplasmic and membranous staining pattern with varying ranges of stain intensity; only membranous staining is evaluated for the assay. Membrane staining pattern may be apical or circumferential (partial or complete).

Tissue morphology and background acceptability are assessed for each case using the criteria described in **Tables 2** and **3**.

The percentage of tumor cells staining at each stain intensity (negative, weak, moderate, strong) will be assessed from specimens containing a minimum of approximately 100 viable tumor cells. Only moderate and strong stain intensities will contribute to the FOLR1 status determination using the scoring method. If the H&E evaluation indicates that the patient specimen is inadequate (for example, if less than 100 tumor cells are present), then a new specimen should be obtained and stained with the VENTANA FOLR1 Assay. EOC tissue cases are considered positive for FOLR1 status if ≥ 75% of viable tumor cells demonstrate moderate and/or strong membrane staining. FOLR1 staining percentage at each intensity is determined by a trained pathologist using the stain intensity reference slide as the baseline for moderate stain intensity. Viewing a case at multiple levels of magnification may be useful in visually estimating the relative percentages of tumor cells staining and differentiating tumor cells from normal stained cells. Because the scoring method for EOC utilizes stain intensity, a pre-stained stain intensity reference slides is used as a required adjunct tool in the interpretation of moderate FOLR1 stain intensity. Image on **Page 8** shows an example of moderate stain intensity that users should refer to on the VENTANA FOLR1 Stain Intensity Reference Slide when evaluating FOLR1-stained slides. The VENTANA FOLR1 Assay Scoring Method is described in **Table 1**.

Table 2: Morphology Acceptance Criteria

Interpretation	Microscope Observation
Acceptable	Cellular elements of interest are visualized allowing clinical interpretation of the stain.
Not Acceptable	Cellular elements of interest are not visualized compromising the clinical interpretation of the stain.

Table 3: Background Acceptance Criteria

Interpretation	Microscope Observation
Acceptable	Non-specific staining is not obtrusive to interpretation of specific staining.
Not Acceptable	Non-specific staining is obtrusive to interpretation of specific staining.

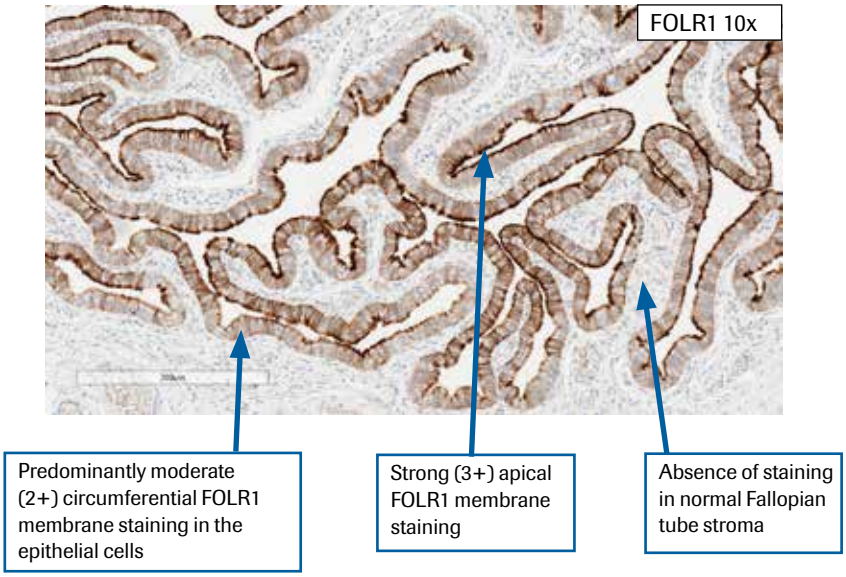
Stain Intensity Reference

VENTANA FOLR1 Stain Intensity Reference Slide (Cat. No. 09382780001)

Pre-stained, formalin-fixed, paraffin-embedded normal human fallopian tube tissue evaluated for FOLR1 stain intensity according to the criteria described in the figure below will be provided for use as a stain intensity reference when evaluating FOLR1-stained slides. Because the scoring method for EOC utilizes stain intensity, a pre-stained stain intensity reference slide is used as a required adjunct tool in the interpretation of moderate FOLR1 stain intensity.

An appropriate and acceptable range of staining on the stain intensity reference slide must be present to proceed with the evaluation of FOLR1-stained slides. An effective stain intensity reference slide must exhibit at least one area with moderate circumferential membrane staining (region needs to contain at least 10 contiguous cells).

Since the scoring method for EOC is based on staining that is of moderate or greater intensity, the stain intensity reference slide is used as an example of moderate staining to aid readers as they evaluate EOC specimens. The moderate (2+) circumferential staining seen in normal fallopian tube tissue serves as a representation of the intensity expected in moderate intensity staining of tumor cell membranes in EOC tissues. While stronger staining components may be present on the stain intensity reference slide, they are not used as a reference when scoring EOC specimen samples. Image below shows an example of moderate stain intensity that users should refer to on the VENTANA FOLR1 Stain Intensity Reference Slide when evaluating FOLR1-stained slides.



FOLR1 Stain Intensity Criteria for Normal Fallopian Tube and EOC Tissues

Controls

Normal fallopian tube tissue with positive and negative staining elements is recommended for use as a run control tissue that can be used to detect out-of-specification issues that might be instrument-related. The luminal membrane of the normal fallopian tube tissue shows specific membranous staining for the FOLR1 protein. When qualifying a normal fallopian tube tissue to serve as a system-level control tissue, the tissue must exhibit predominately moderate circumferential membrane staining and strong apical membrane staining when stained with the VENTANA FOLR1 Assay as described in **Table 4** and depicted on the following page.

When qualifying a normal fallopian tube tissue to serve as a system-level control tissue, the tissue must also exhibit an absence of staining in the stroma.

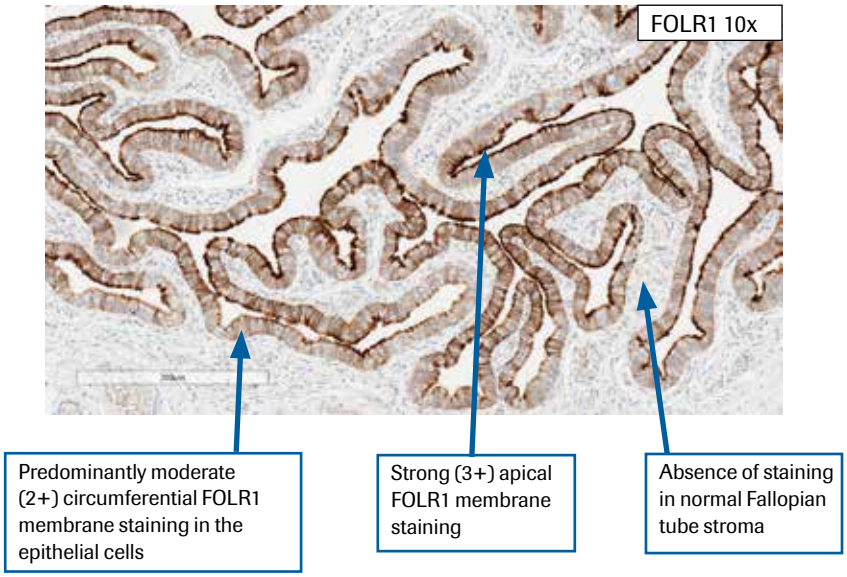
A positive control tissue should be a fresh autopsy/surgical specimen that is fixed and processed in the same manner as the patient specimens and should be run for each set of test conditions with every VENTANA FOLR1 Assay staining procedure performed. This tissue is provided by the end user and may be used to monitor all steps of specimen processing and staining. A tissue section fixed or processed differently from the test specimen can be used as a control for reagents and staining, but not for fixation or tissue preparation. Positive predominately moderate circumferential membrane staining and strong apical membrane staining and absence of specific staining in the stroma of the normal fallopian tube specimen confirms that the VENTANA FOLR1 Assay was applied and the instrument functioned properly. The positive tissue control should only be used to monitor performance and it should not be used to aid the clinical diagnosis of patient samples, as per CLSI I/LA28-A2.

Before use as a tissue control, the normal fallopian tube specimens should be qualified by the end user for appropriate staining, according to the interpretation criteria described in **Table 4**, using the VENTANA FOLR1 Assay with the OptiView DAB IHC Detection Kit and recommended staining protocol. Multiple fallopian tube specimens may be required to select an appropriate system-level control candidate.

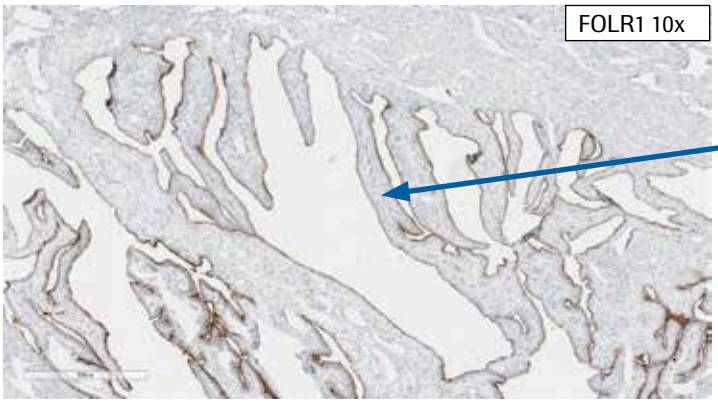
Table 4: Acceptance Criteria for FOLR1 Staining in Normal Fallopian Tube Tissue

System-level Control Tissue Interpretation	Staining Pattern
Acceptable	Predominately moderate circumferential* FOLR1 membrane staining in the epithelium of normal fallopian tube. AND Absence of specific staining in normal fallopian tube stroma.
Not Acceptable	Absence of staining, or predominately weak or strong circumferential* FOLR1 membrane staining in the epithelium of normal fallopian tube. AND/OR Non-specific FOLR1 background staining that interferes with interpretation.
*Note: Apical staining of the first layer of the luminal cells must not be considered in evaluating the acceptability of normal fallopian tube FOLR1 staining.	

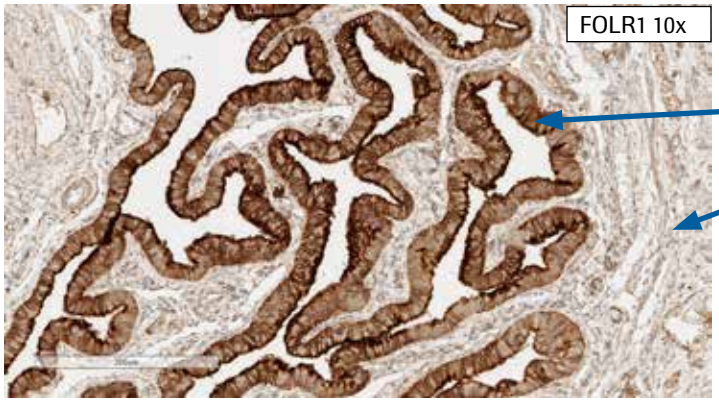
Acceptable Staining of Control Normal Fallopian Tube Tissue



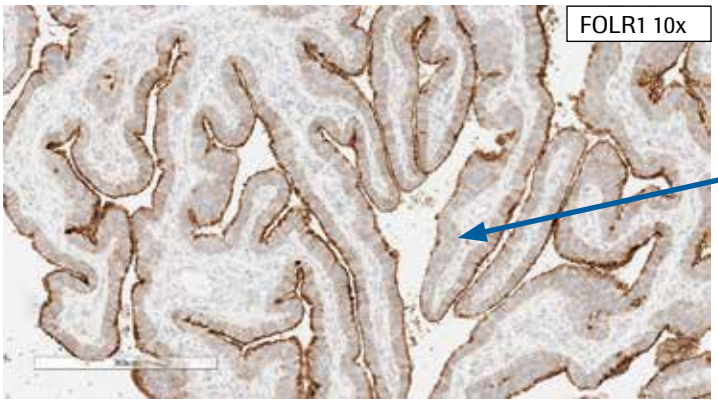
Not Acceptable Staining of Control Normal Fallopian Tube Tissue



Not acceptable normal fallopian tube system level control case exhibits absence of moderate circumferential staining in the epithelium of normal fallopian tube tissue.



Not acceptable normal fallopian tube system-level control case exhibits strong circumferential staining in the epithelium of normal fallopian tube tissue and exhibits background staining that interferes with interpretation.



Not acceptable normal fallopian tube system-level control case exhibits weak membrane staining in the epithelium of normal fallopian tube tissue.

Specimen Workflow

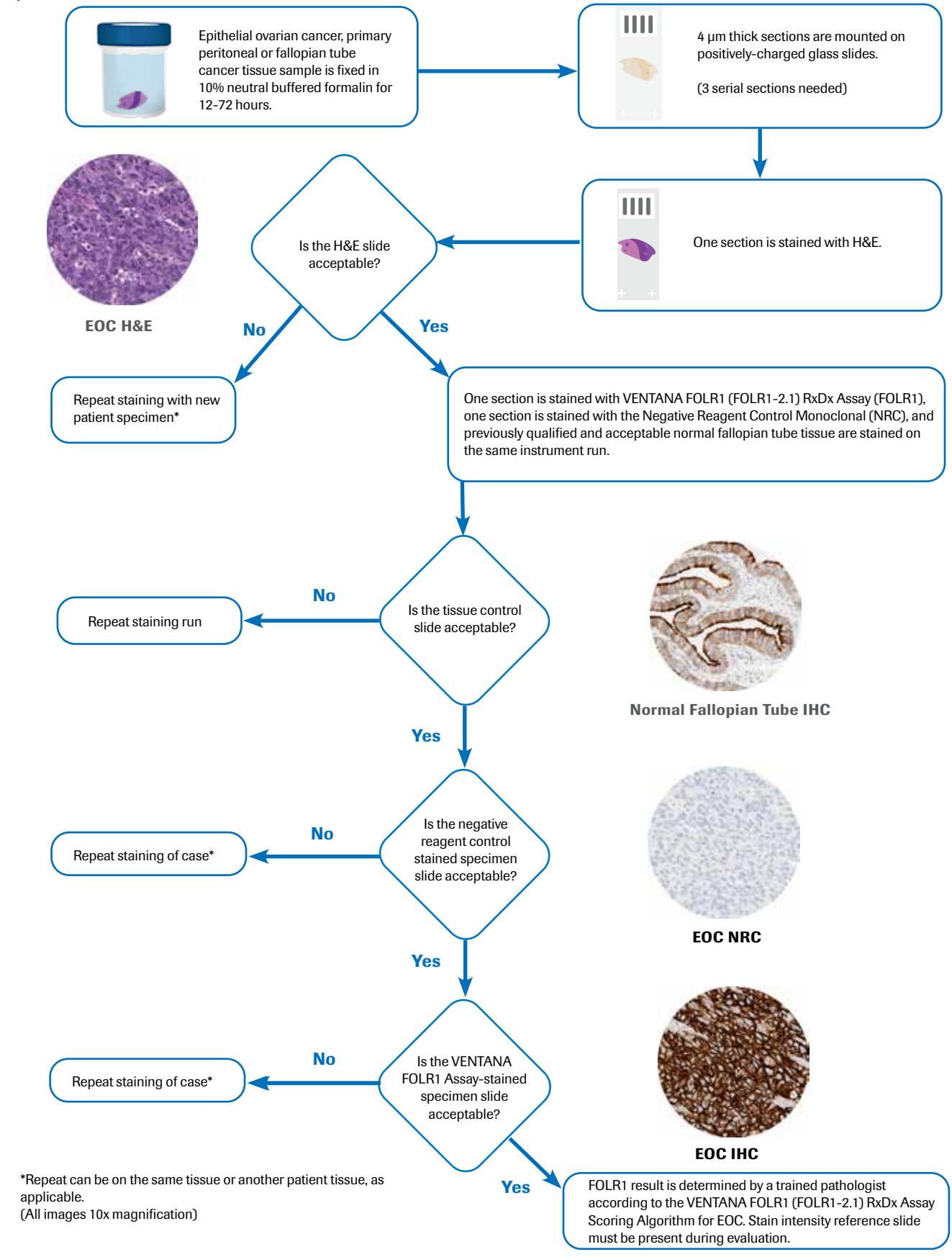
Staining requires three sections from each case, one serial tissue section for hematoxylin and eosin (H&E) staining, a second serial tissue section for NRC staining, and a third serial tissue section for VENTANA FOLR1 Assay staining. As with any IHC assay, it is recommended that specific staining is evaluated on viable tumor cells. Ideally, patient specimens will have a minimum of 100 viable tumor cells identified on the H&E in order to determine FOLR1 status. A normal fallopian tube tissue with moderate stain intensity is to be used as the stain intensity reference slide (VENTANA FOLR1 Stain Intensity Reference Slide (Cat. No. 09382780001)) and is used when evaluating clinical samples. If the H&E evaluation indicates that the patient specimen is inadequate (for example, if less than 100 tumor cells are present), then a new specimen should be obtained and stained with the VENTANA FOLR1 Assay.

A user-supplied normal fallopian tube tissue is recommended as a system-level control for the assay to monitor the proper functioning of the reagents and staining run. Both positive and negative elements must be stained appropriately as defined by the acceptance criteria for normal fallopian tube tissue (**Table 4**) on each run for the run to be considered valid.

A matched NRC slide must be run for every specimen to evaluate nonspecific staining and aid in the interpretation of results.

The FOLR1-stained specimen slides should be assessed by a trained pathologist using the described scoring criteria. If either the FOLR1-stained tissue control slide or the NRC-stained specimen slide is not acceptable, staining of patient samples should be repeated. Repeat may be on the same tissue or another patient tissue, as applicable. A non-evaluable VENTANA FOLR1 Assay-stained slide would mean that determination of reactivity is not possible due to necrosis, absent tissue, or artifacts and the slide cannot be used for clinical evaluation.

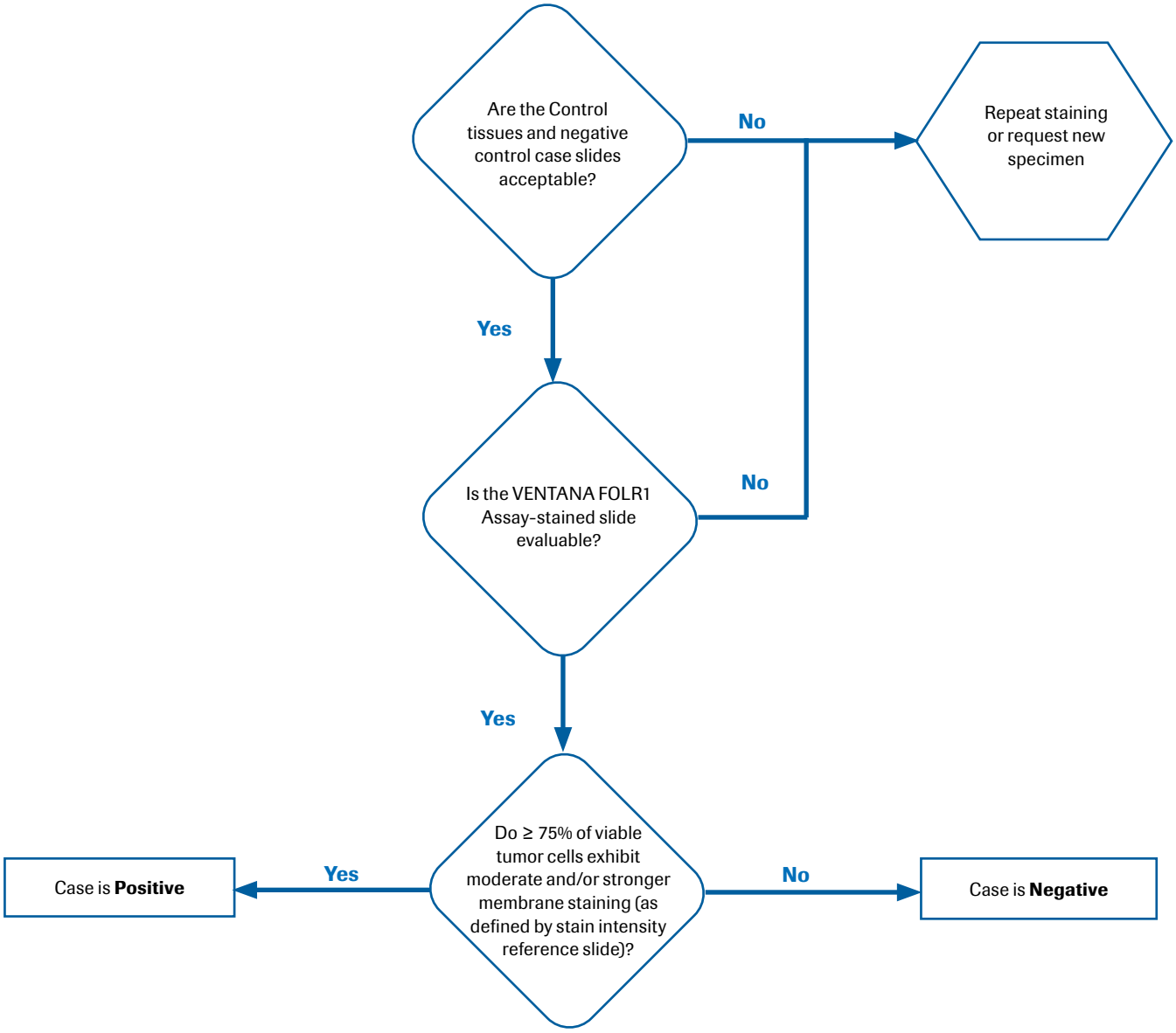
Specimen Flow



*Repeat can be on the same tissue or another patient tissue, as applicable.
(All images 10x magnification)

Decision Tree

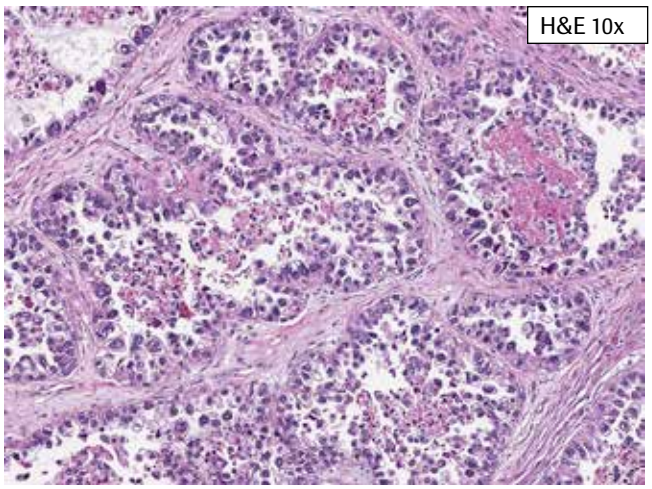
Slides stained with the VENTANA FOLR1 Assay should be evaluated using the approach noted in the figure below

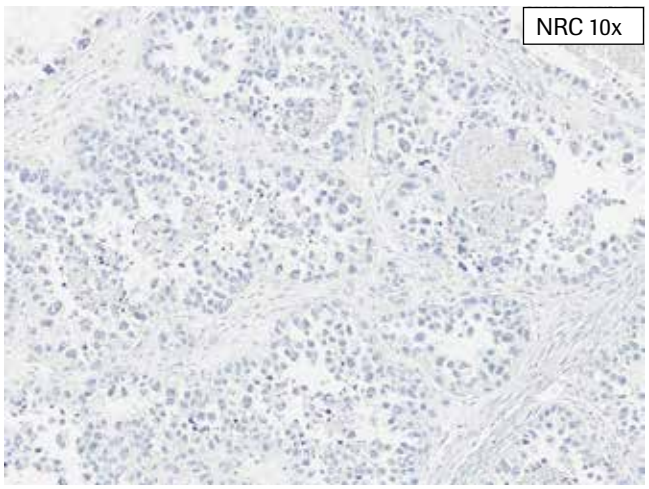


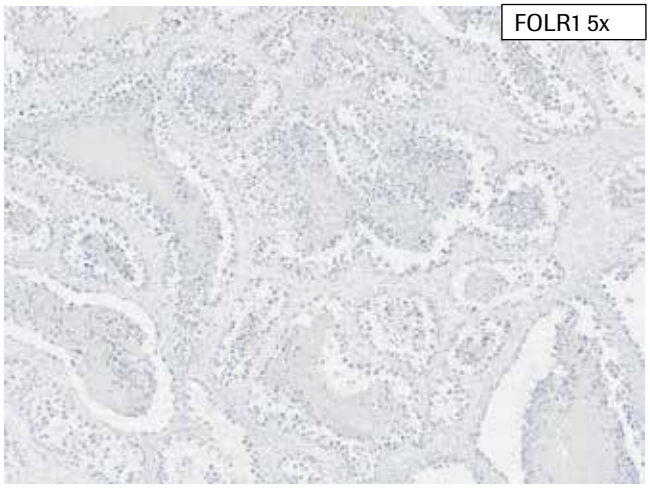
Reference Images

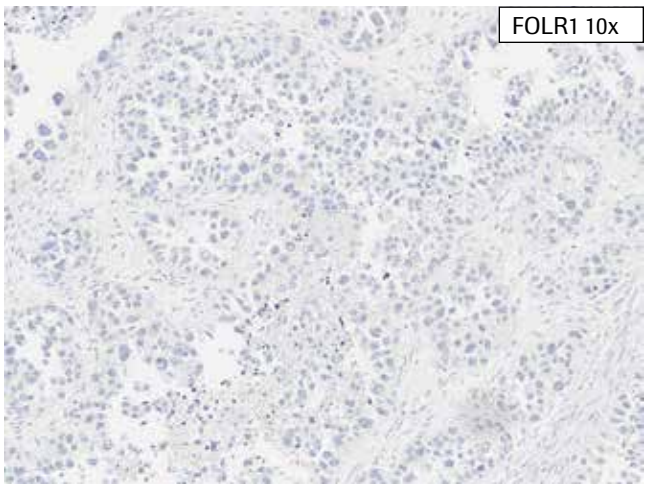
Negative Cases

A case assigned negative for FOLR1 status is characterized by an absence of any detectable signal or less than 75% of neoplastic cells exhibiting moderate and/or strong membrane staining. Scores for FOLR1-stained images were determined using the lower magnification image.

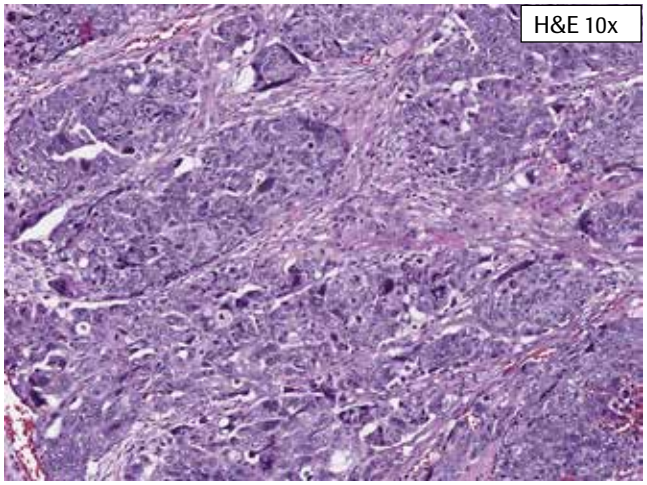


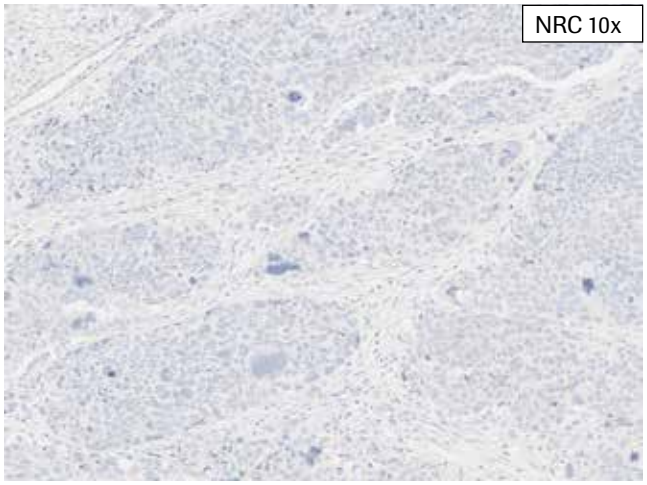


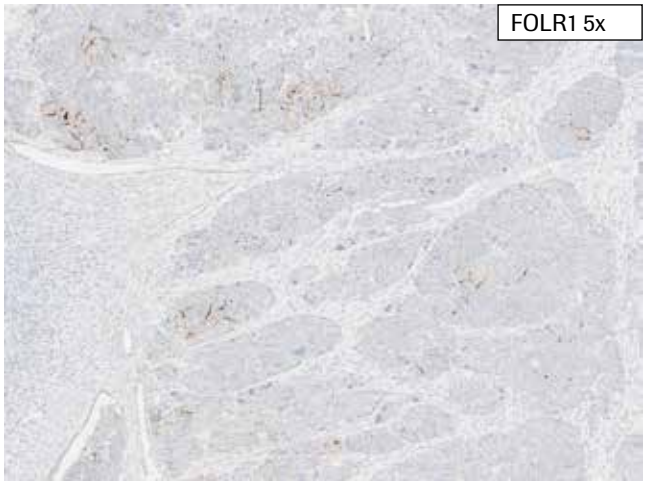


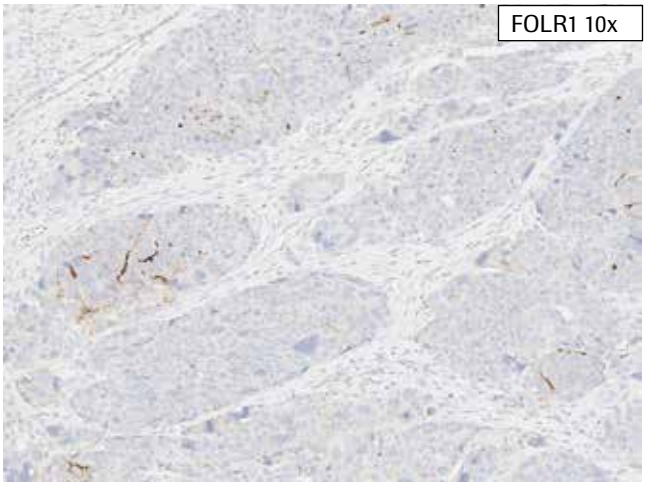


Negative EOC Case 1 exhibits 0% moderate and strong tumor cell membrane staining or < 75% of tumor cells with moderate and/or strong tumor cell membrane relative to the negative control slide. This case is assigned a Negative FOLR1 status.

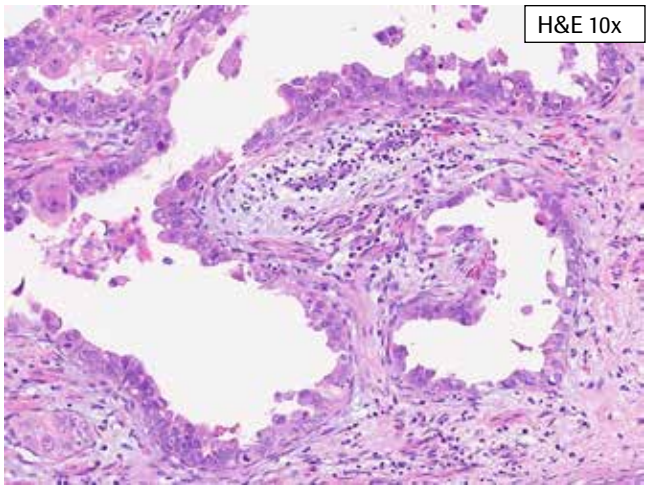


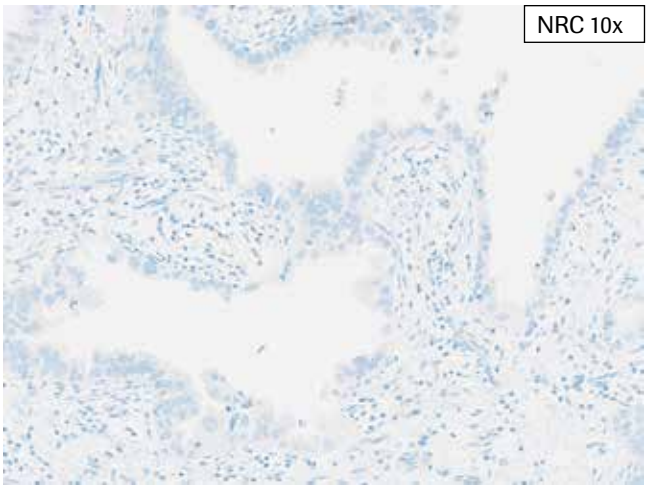


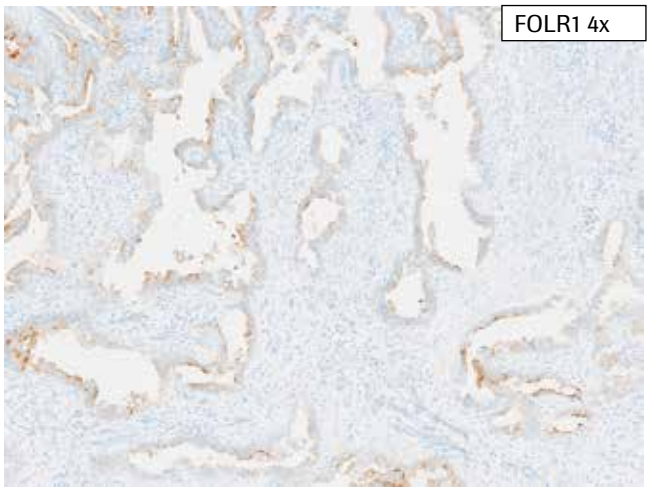


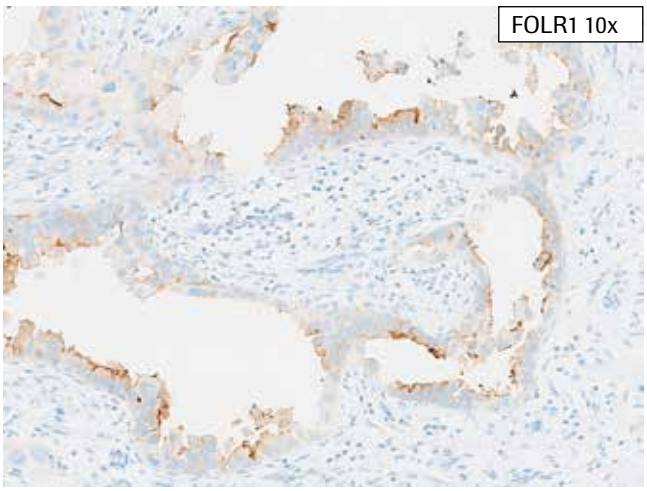


Negative EOC Case 2 exhibits 5% moderate, and strong apical membrane tumor cell staining, or < 75% of tumor cells with moderate and/or strong apical membrane staining relative to the negative control slide. Apical staining is defined as staining in the portion of the cells oriented towards the lumen of the gland/tubule. This case is assigned a Negative FOLR1 status.

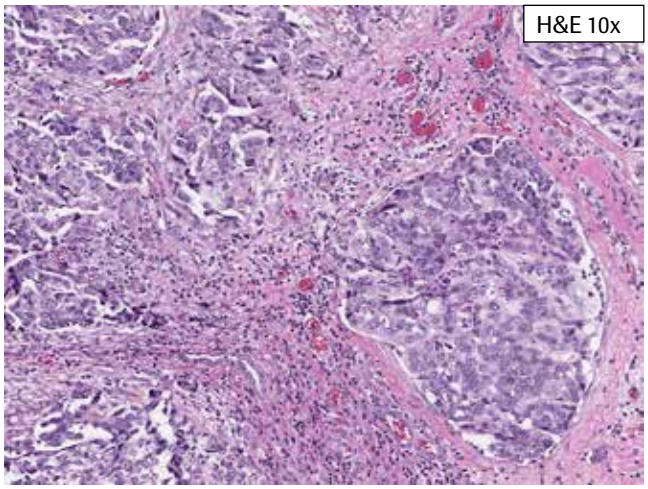


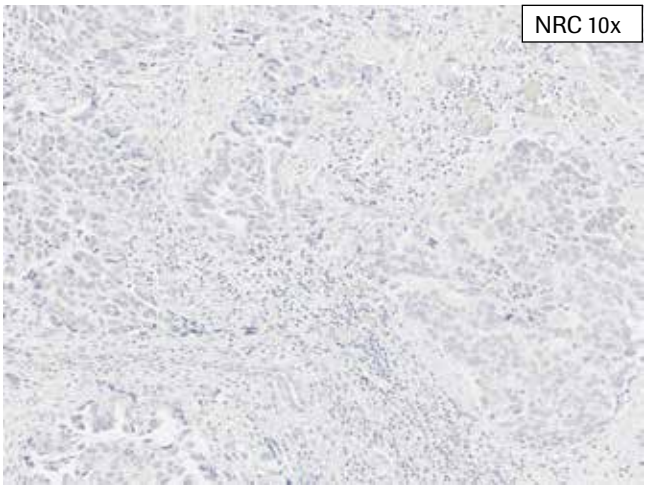


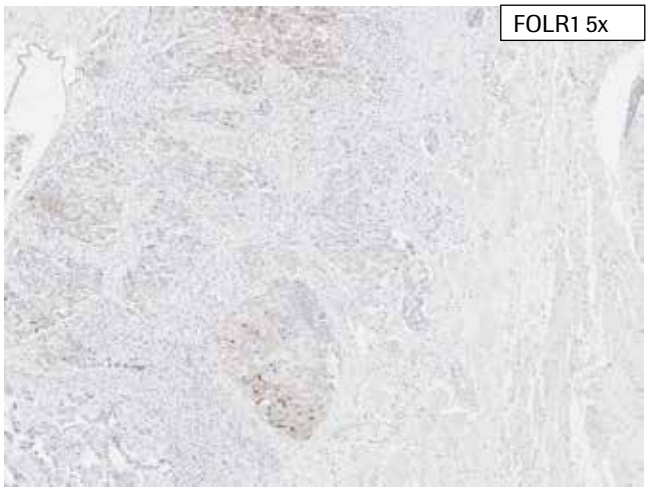


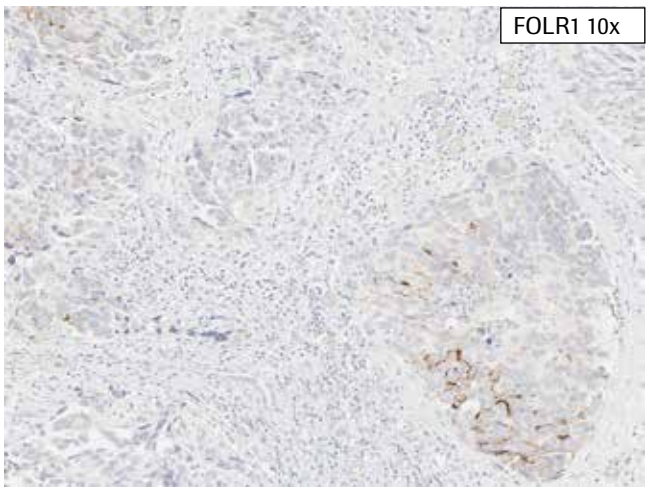


Negative EOC Case 3 exhibits 40% moderate and strong tumor cell membrane staining or < 75% of tumor cells with moderate and/or strong tumor cell membrane relative to the negative control slide. This case is assigned a Negative FOLR1 status.

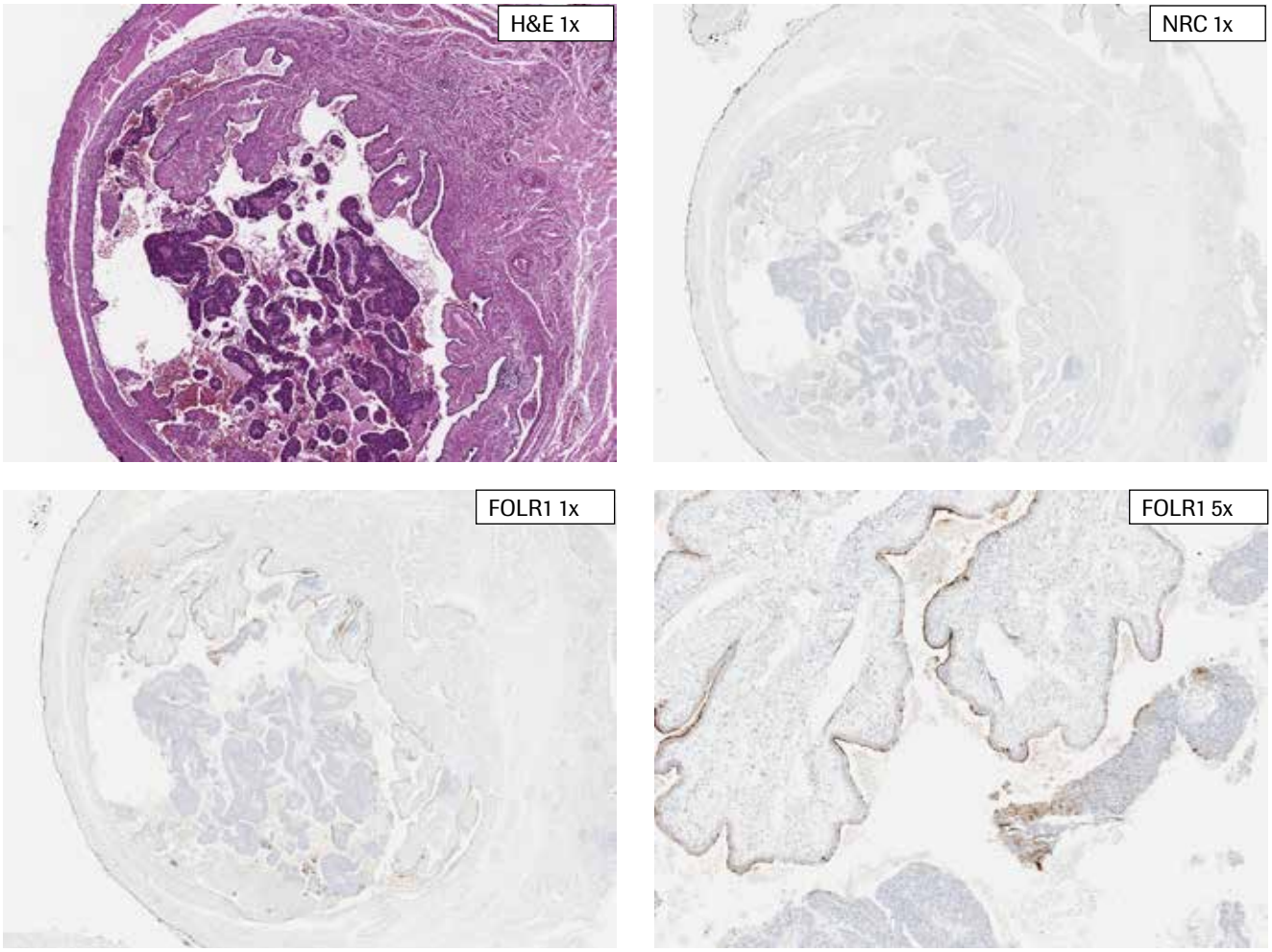








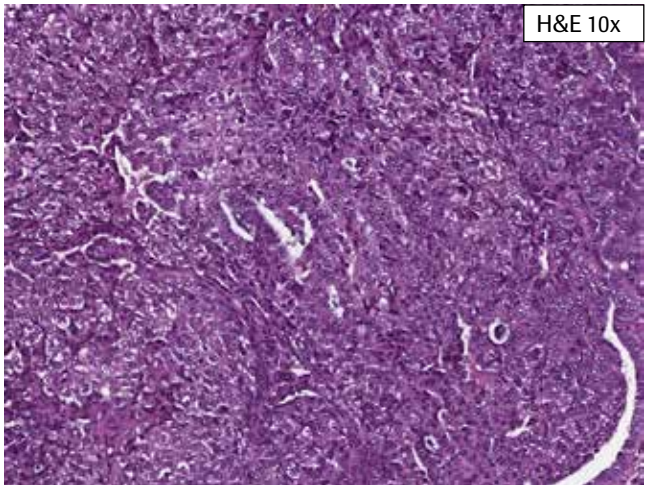
Negative EOC Case 4 exhibits 5% moderate and strong tumor cell membrane staining or < 75% of tumor cells with moderate and/or strong tumor cell membrane relative to the negative control slide. This case is assigned a Negative FOLR1 status.

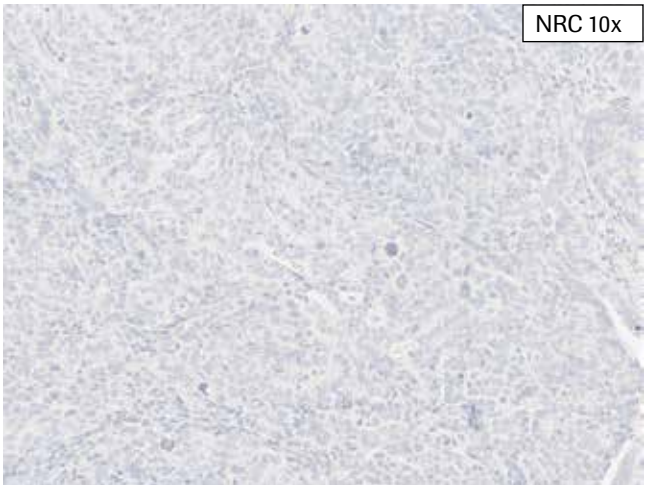


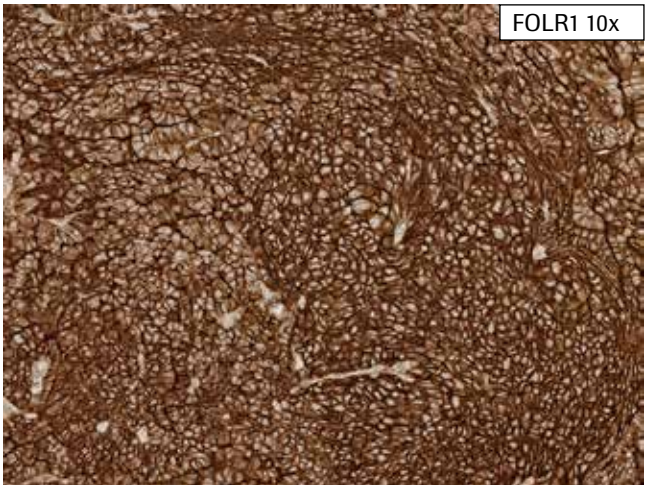
Negative Fallopian Tube Carcinoma Case 5 exhibits 5% moderate and strong fallopian tube staining or < 75% of fallopian tube tumor cells with moderate and/or strong fallopian tube staining relative to the negative control slide. This case is assigned a Negative FOLR1 status.

Positive Cases

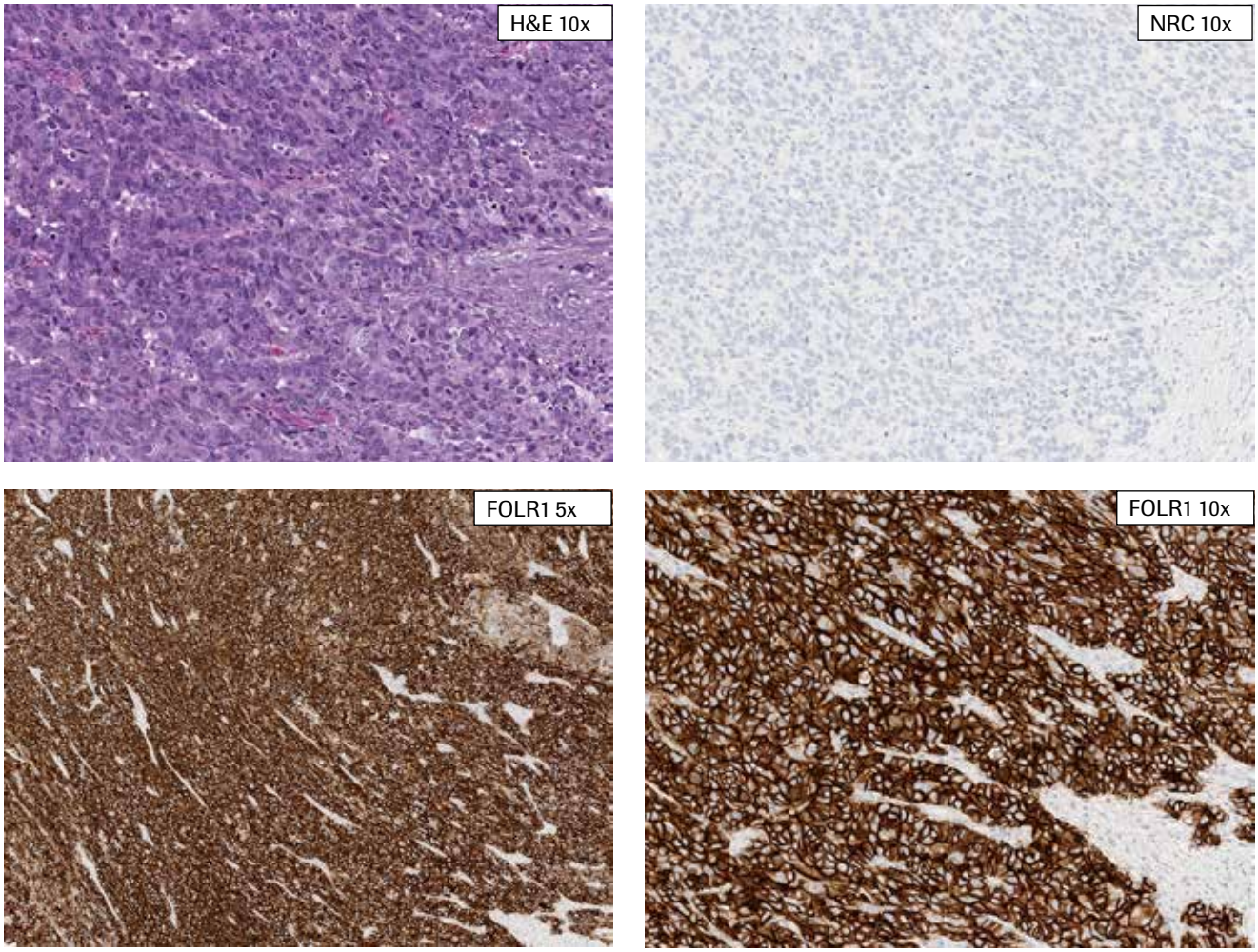
Staining with the VENTANA FOLR1 Assay can be observed in the membrane of tumor cells. A case is deemed positive if 75% or more of the neoplastic cells exhibit staining at a moderate and/or strong intensity. Scores for FOLR1-stained images were determined using the lower magnification image



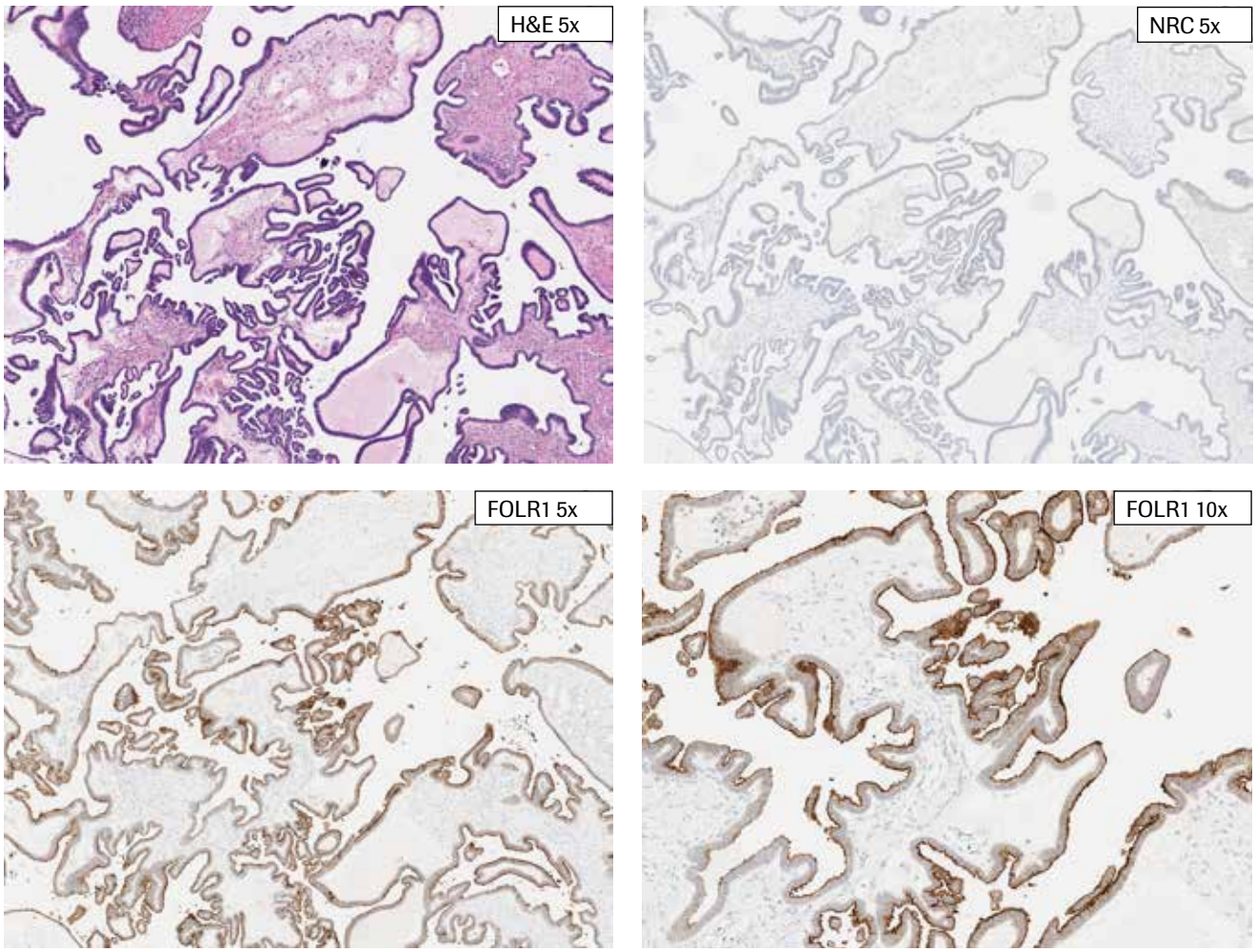




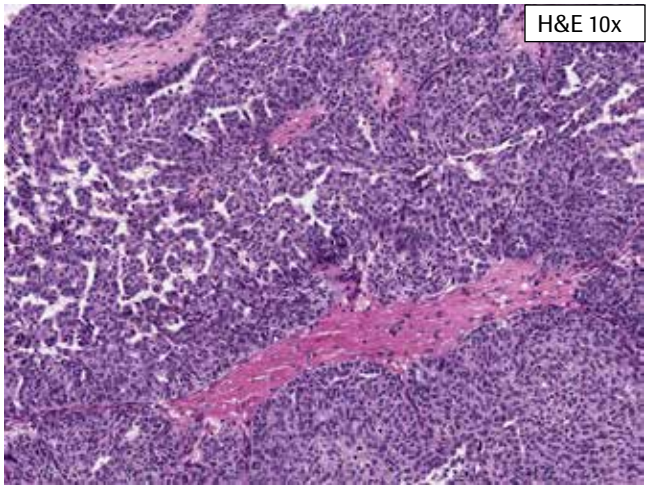
Positive EOC Case 1 exhibits 100% moderate and strong membrane staining with complete circumferential pattern, or ≥ 75% of the tumor cells with moderate and/or strong membranous staining. This case is assigned a Positive FOLR1 status.

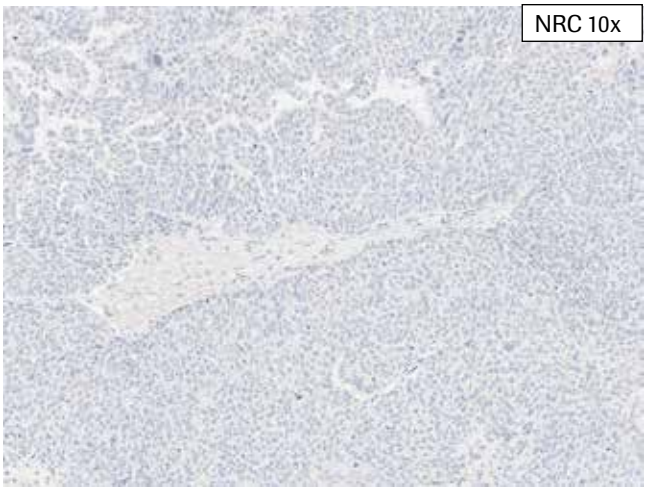


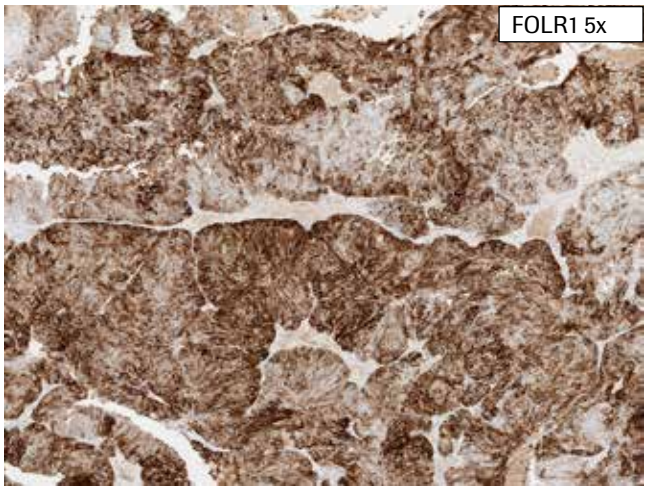
Positive EOC Case 2 exhibits 95% moderate and strong membrane staining with complete circumferential pattern or ≥ 75% of the tumor cells with moderate and/or strong membranous staining. This case is assigned a Positive FOLR1 status.

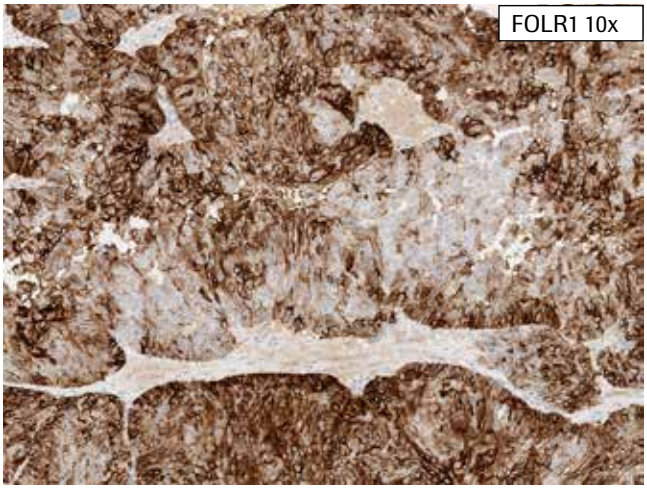


Positive EOC Case 3 exhibits 80% moderate and strong apical membrane staining in tumor cells or ≥ 75% of the tumor cells with moderate and/or strong apical membrane staining. Apical staining is defined as staining in the portion of the cells oriented towards the lumen of the gland/tubule. This case is assigned a positive FOLR1 status.

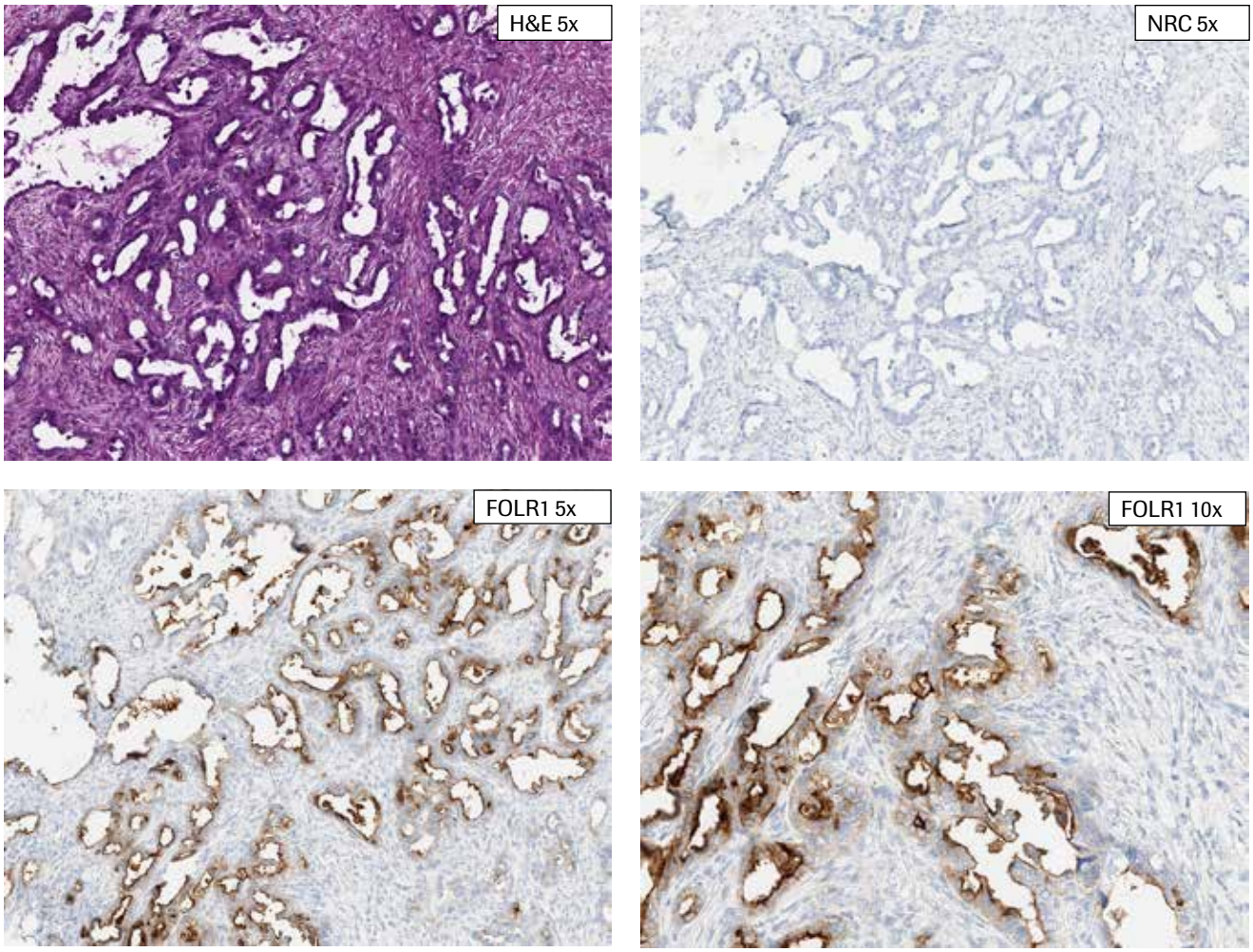








Positive EOC Case 4 exhibits 85% moderate and strong membrane staining with complete circumferential pattern or ≥ 75% of the tumor cells with moderate and/or strong apical membrane staining. This case is assigned a Positive FOLR1 status.



Positive EOC Case 5 exhibits 85% moderate and strong apical membranous staining or ≥ 75% of the tumor cells with moderate and/or strong apical membrane staining. Apical staining is defined as staining in the portion of the cells oriented towards the lumen of the gland/tubule. This case is assigned a Positive FOLR1 status.

Challenging Cases

While the vast majority of cases stained with the VENTANA FOLR1 Assay are clearly positive or negative in their staining results, a few cases have been observed that present a challenge in interpretation. Cases are placed into FOLR1 clinical categories according to the percentage of cells staining. The staining can be heterogeneous. Percent cell staining is determined by noting the number of tumor cells showing membranous staining. Viewing a case at multiple levels of magnification may be useful in visually estimating the relative percentages of tumor cells staining and differentiating tumor cells from normal stained cells.

Some cases may be particularly challenging due to the following issues:

- **Heterogeneous Expression**

Heterogeneity presents a challenge in case interpretation because it contributes to variable membrane staining. Variable staining requires closer examination to determine percentage of staining.

- **Cytoplasmic Staining**

Some cases may exhibit cytoplasmic staining. This may present a challenge in interpretation of presence or absence of membrane staining. Evaluation of the FOLR1 slide must include examination at higher magnification. Cytoplasmic staining is not included in scoring.

- **Dot-Like Staining**

Some cases may exhibit dark brown secretions filling the gland lumens. This pattern should be included in scoring.

- **Tissue or Staining Artifact**

Histologic artifacts originating from the sample processing and microtomy processes can also complicate the determination of FOLR1 Clinical Score. These artifacts may include, but are not limited to, fixation gradients and edge effects, DAB trapping, nuclear bubbling, lack of staining in some regions of the tissue, tearing or folding of the tissue, and loss of the tissue section. In some instances, repeat staining of new sections or acquisition of a new specimen may be required.

- **Borderline Category**

Some cases near the cut off are at the border between a positive and negative FOLR1 status (± 10% of cutoff). These cases are particularly challenging to estimate the number of tumor cells staining. For these borderline cases it may be helpful to view the case at a magnification that enables the entire tumor area to be assessed.

- **Apical Staining**

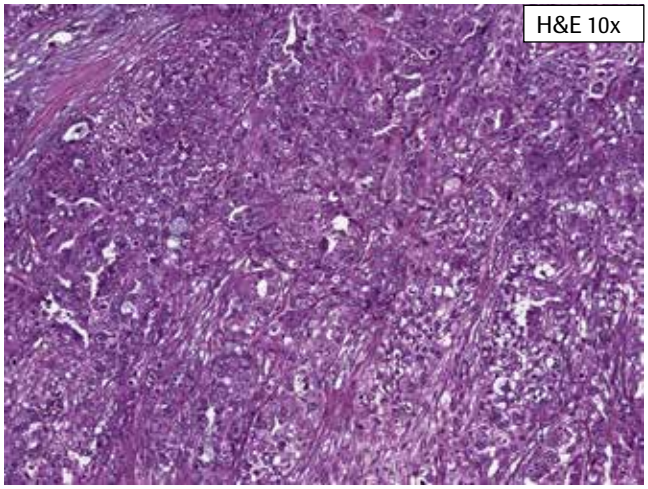
Some cases may exhibit apical, or luminal, staining. In tissues with glandular or tubular morphology, apical or luminal staining is defined as staining in the portion of the cells oriented towards the lumen of a gland or tubule, in contrast to basolateral staining, which describes staining in the portion of cells oriented away from the lumen (i.e., at the base). This staining pattern should be included in scoring.

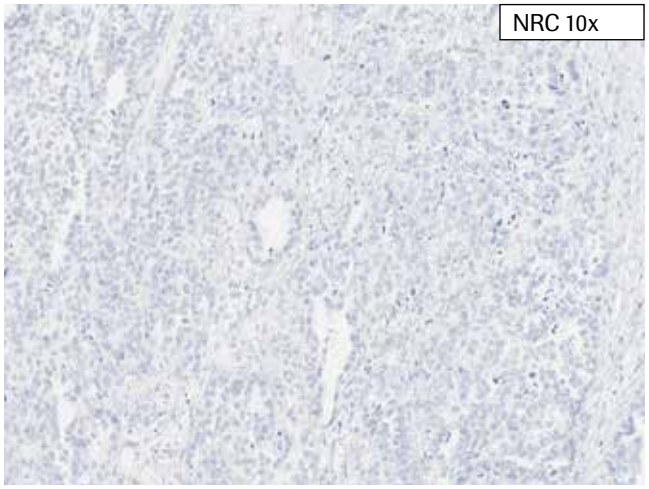
- **Non-Specific Background**

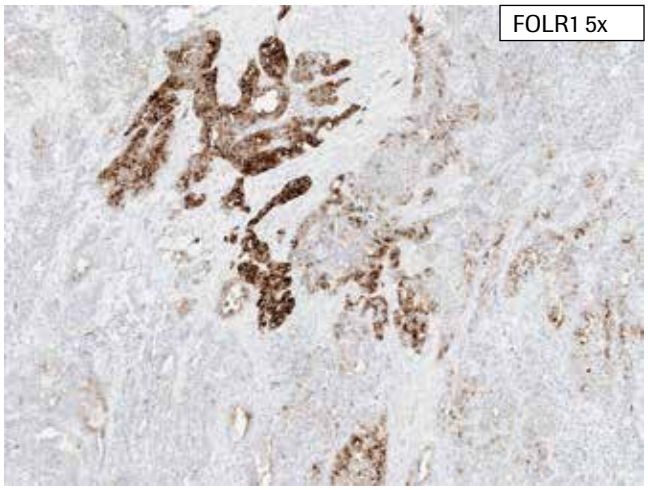
Some specimens may exhibit non-specific background staining for reasons that are not well understood. For this reason, evaluation of the FOLR1 IHC slide must include a comparison of the slide to the negative control slide to determine the level of non-specific background staining.

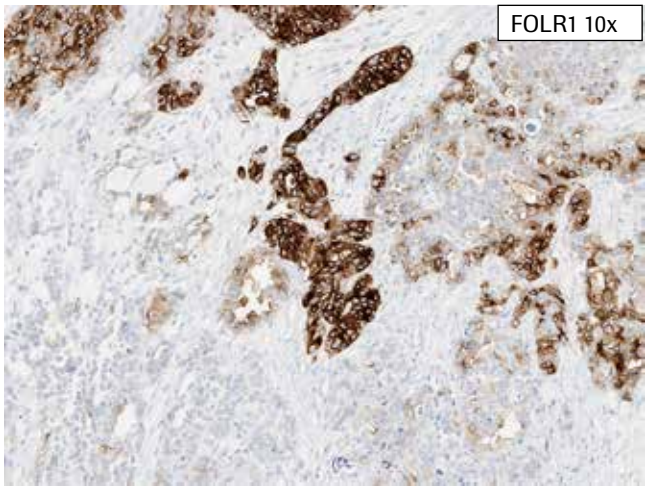
Examples of challenging cases are shown on the following pages. Scores for FOLR1-stained images were determined using the lower magnification image.

Heterogenous FOLR1 expression



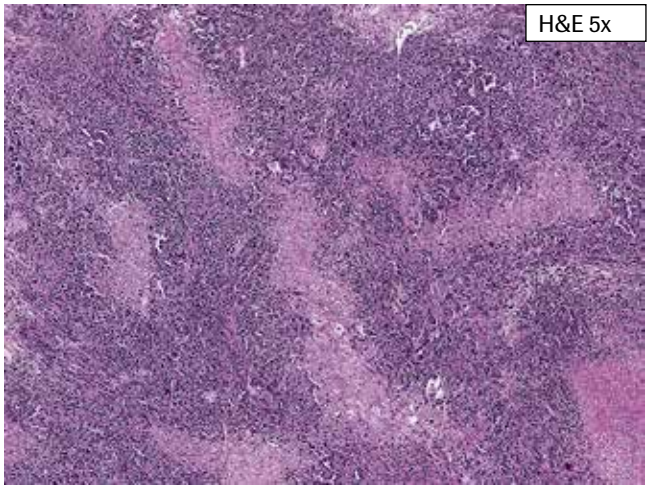


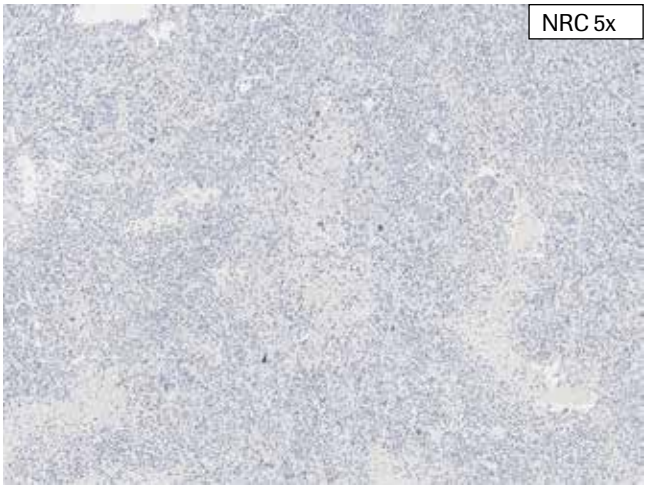


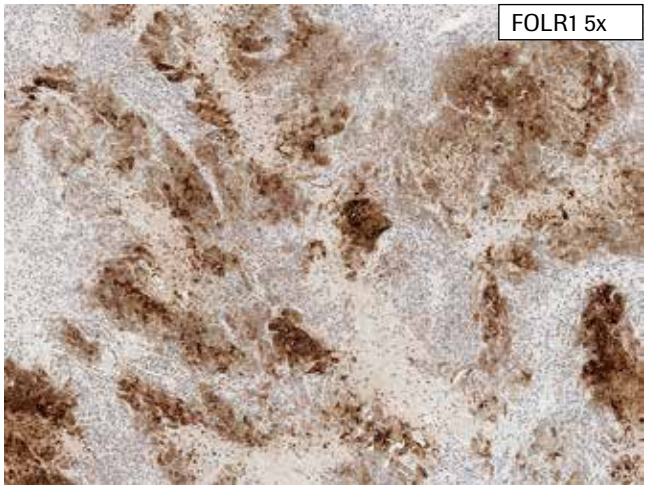


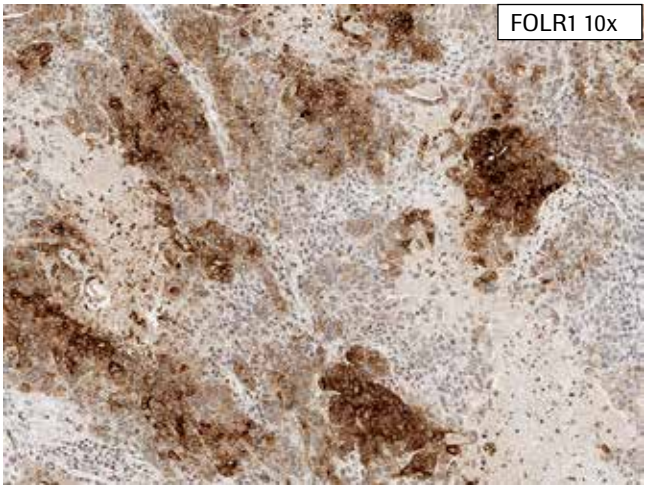
Challenging EOC Case 1 exhibits 40% moderate and strong membrane staining in tumor cells or < 75% of tumor cells with moderate and/or strong tumor cell membrane relative to the negative control slide. This case is assigned a Negative FOLR1 status.

Cytoplasmic FOLR1 expression



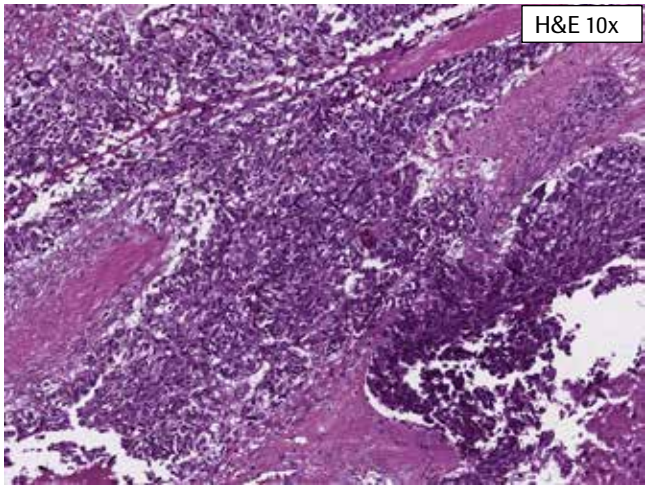


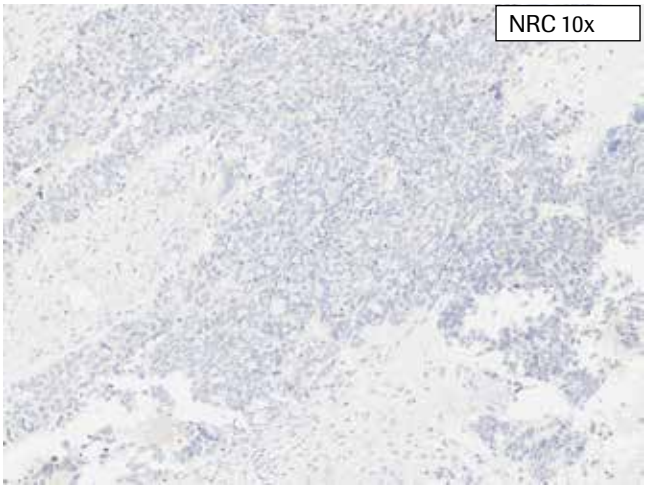


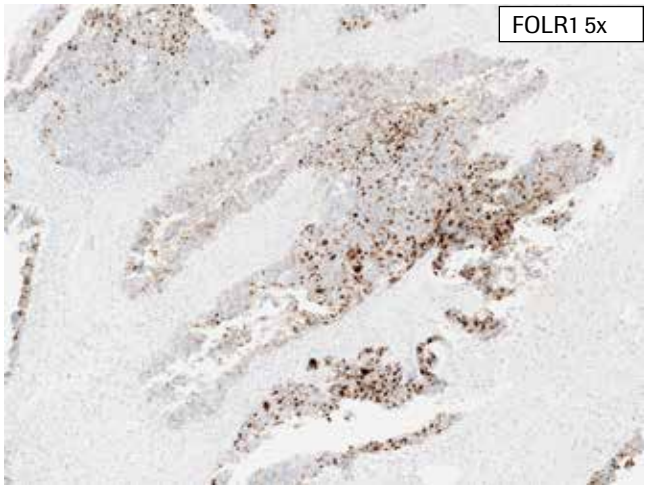


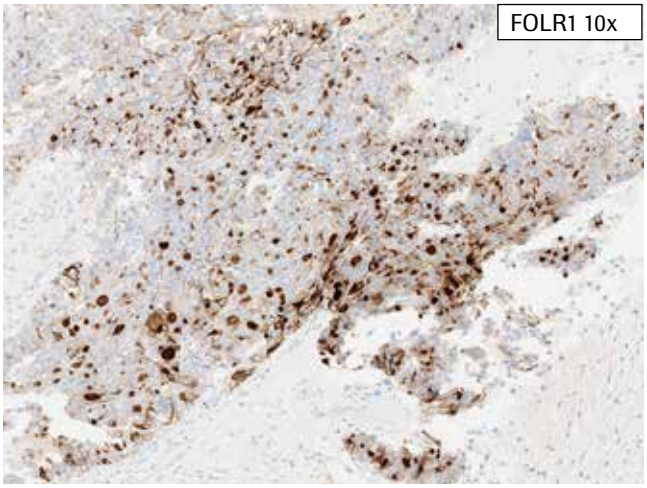
Challenging EOC Case 2 exhibits 55% moderate and strong membrane staining in tumor cells but also exhibits cytoplasmic staining which is not included as part of the FOLR1 scoring method. Cytoplasmic staining can interfere with membrane assessment. In this case, although cytoplasmic staining is present, moderate and/or strong membrane staining is not present in more than 75% of tumor cells. This case is assigned a Negative FOLR1 status.

Dot-like staining



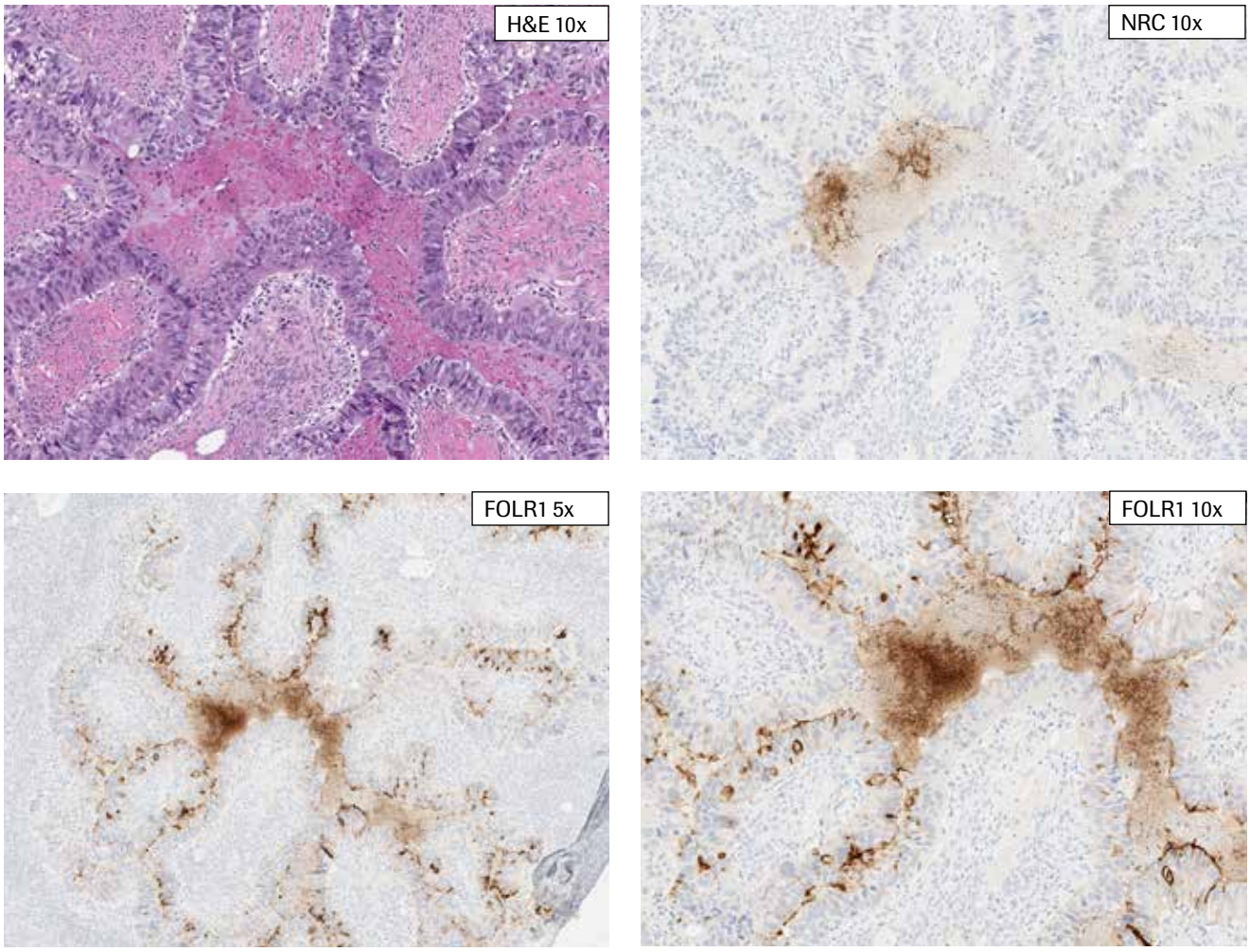






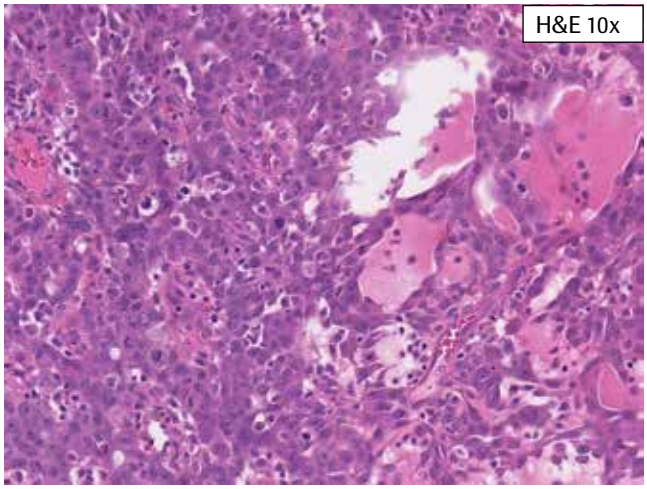
Challenging EOC Case 3 exhibits mostly dot-like staining, which is included as part of the FOLR1 scoring method. This image exhibits 70% moderate and strong membranous tumor cell staining or < 75% of the tumor cells with moderate and/or strong apical membrane staining. This case is assigned a Negative FOLR1 status.

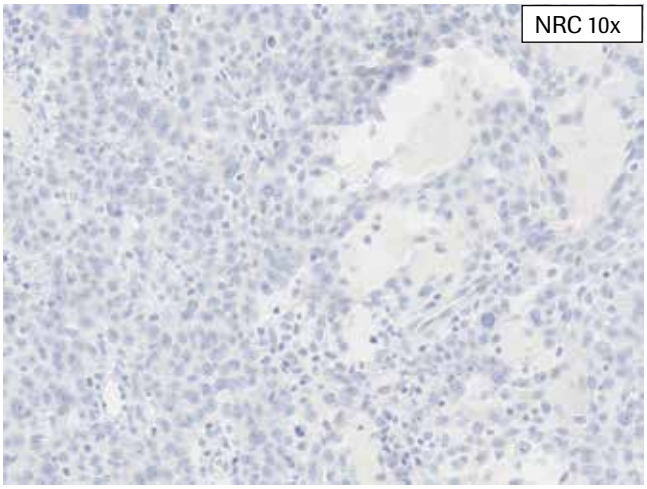
Staining artifact

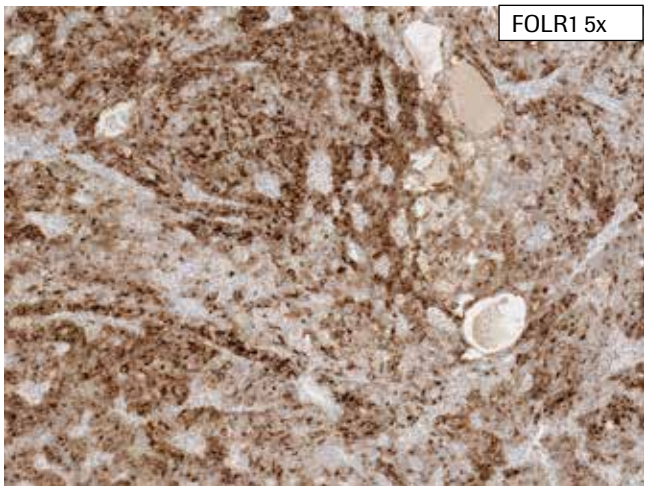


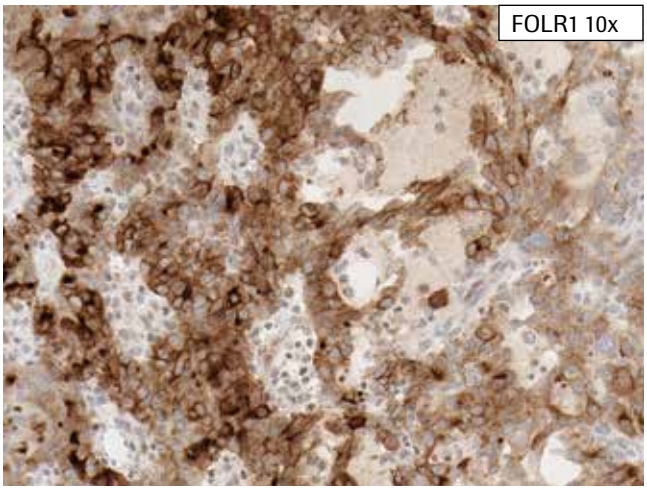
Challenging EOC Case 4 exhibits a staining artifact: DAB trapping. This is not included as part of the FOLR1 scoring method. Moderate and/or strong membrane staining, primarily apical, is present in < 75% of tumor cells. This image exhibits 35% moderate and strong primarily apical membrane staining or < 75% of tumor cells with moderate and/or strong tumor cell membrane relative to the negative control slide. This case is assigned a Negative FOLR1 status.

Borderline category



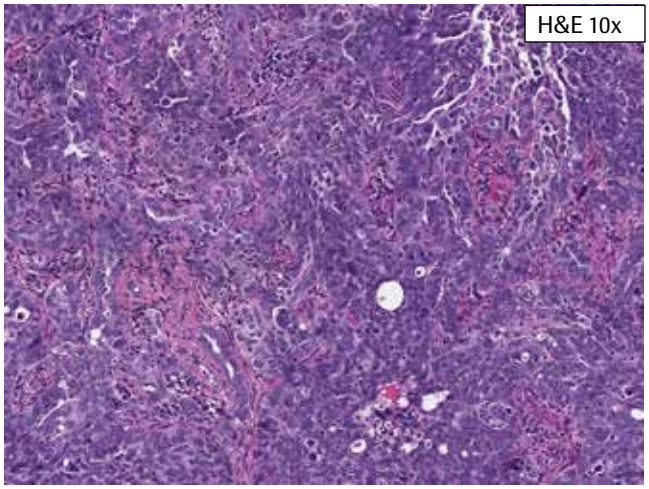


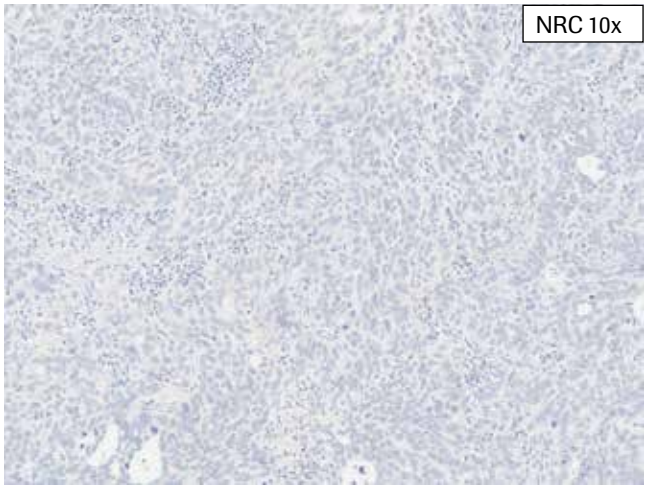


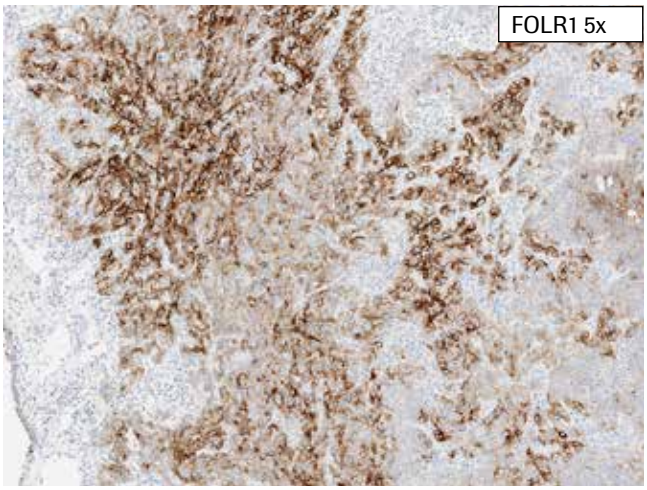


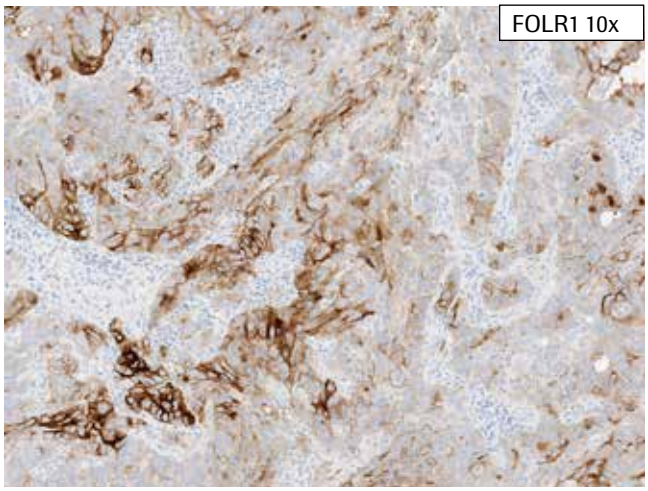
Challenging EOC Case 5 exhibits moderate and strong membrane staining that is at the borderline for the 75% cut off. This image exhibits 75% moderate and strong membrane staining in tumor cells. This borderline case is assigned Positive FOLR1 status when the 75% cut off is used.

Borderline category



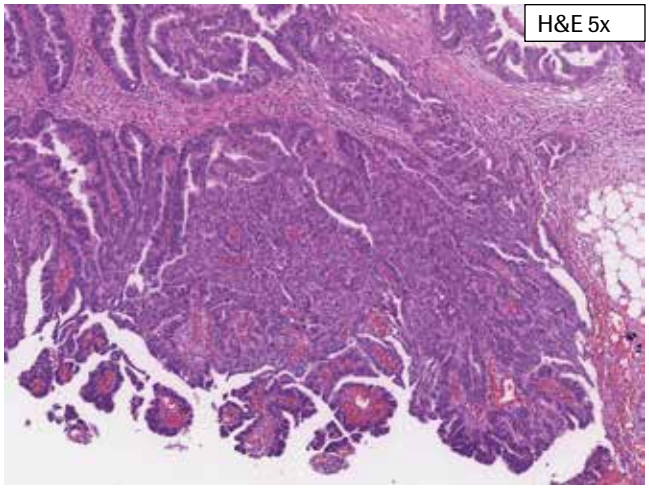


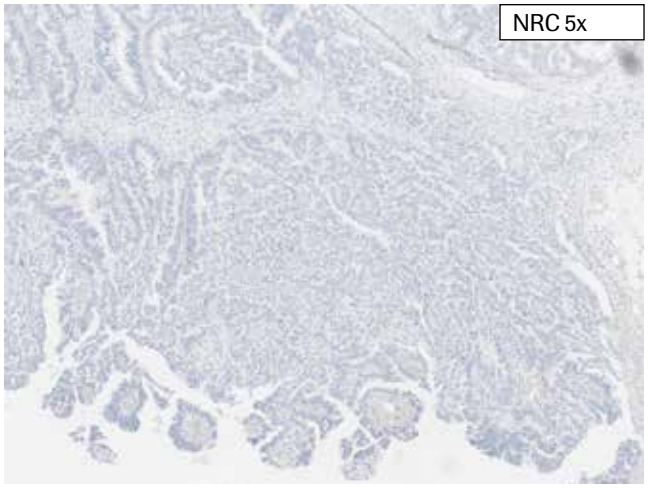


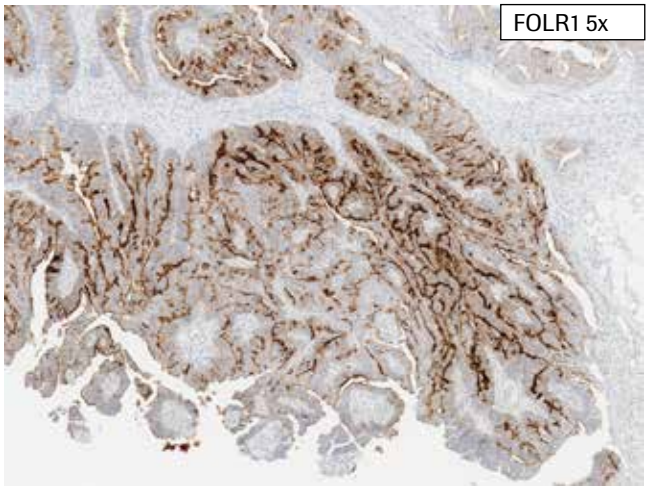


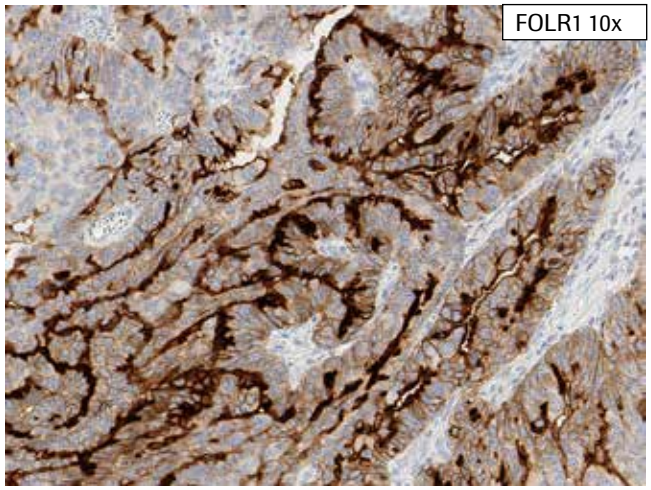
Challenging EOC Case 6 exhibits moderate and strong membrane staining that is at the borderline for the 75% cut off. This image exhibits 70% moderate and strong tumor cell membrane staining. This borderline case is assigned a Negative FOLR1 status when the 75% cut off is used.

Apical staining









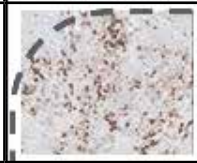
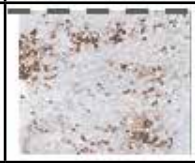
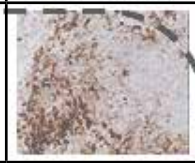
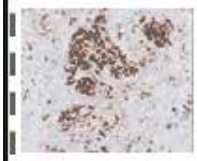
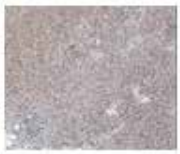
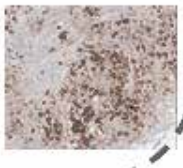
Challenging EOC Case 7 exhibits 80% moderate and strong primarily apical membrane staining or ≥ 75% of the tumor cells with moderate and/or strong apical membrane staining. Apical staining is defined as staining in the portion of the cells oriented towards the lumen of the gland/tubule. This case is assigned a Positive FOLR1 status.

Impact of Pre-analytical Conditions on VENTANA FOLR1 (FOLR1-2.1) RxDx Assay

Acceptable Fixation Conditions to Achieve Optimal Staining Results

- **Ventana recommends fixation in 10% NBF for 12-72 hours.** See acceptable fixatives and fixation times in rectangular box below.

Table 5: VENTANA FOLR1 (FOLR1-2.1) RxDx Assay Staining of Prostate Cancer Xenografts Across Fixatives and Fixation Time

Fixation Time (hrs)	Fixative					
	10% NBF	Zinc Formalin	Z-5	Prefer**	AFA**	95% Ethanol**
1*						
6*						
12						
24						
48						
72						

The following fixatives and fixation times are **not recommended**:

*Less than 12 hour fixation is not recommended.

**Use of Prefer or AFA or alcoholic fixatives (weaker staining) is not recommended. Use of Zinc Formalin or Z-5 are not recommended due to variability in percent tumor cell staining and potential for change in FOLR1 status.

Cut Slide Stability

Ventana has determined that the VENTANA FOLR1 Assay should not be performed on cut slides that have been stored longer than 45 days. Ventana has not tested the impact of cut slide stability combined with different fixatives, and 45 days may not be the optimal stability for fixatives other than NBF.

References

1. Sudimack J, Lee RJ. Targeted drug delivery via the folate receptor. Adv Drug Deliv Rev. 2000, 41:147-62.
2. Kelemen LE. The role of folate receptor α in cancer development, progression and treatment: cause, consequence or innocent bystander? Int J Cancer. 2006, 119:243-50.
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