Assessment Run C19 2026

Folate receptor alpha (FOLR1)

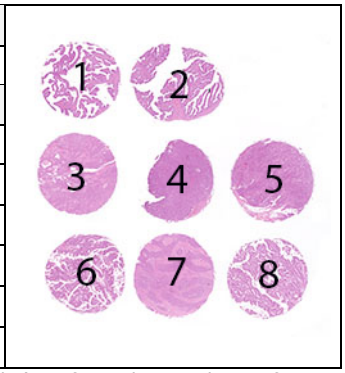
Purpose

This first assessment in the NordiQC Companion module of FOLR1 focused on the evaluation of the analytical accuracy of the IHC assays performed by the NordiQC participants to identify patients with epithelial ovarian, fallopian tube, or primary peritoneal cancer to be treated with ELAHERE®. The technical accuracy was evaluated in six carcinomas with the dynamic and clinically relevant expression levels of FOLR1 characterized by tumour cell scoring (TCS). The assessment mark obtained in NordiQC is indicative of the performance of the IHC tests but due to the limited number and composition of samples, internal validation/verification and extended quality control, e.g. regularly measuring the FOLR1 results, are needed.

Material

Table 1. **Content of the TMA used for the NordiQC FOLR1 C19 assessment**

Tissue controls	**FOLR1 IHC reaction pattern**
1-2. Fallopian tube	See control section
Carcinomas	**TCS score***
3. Ovarian serous adenocarcinoma	≥75% (moderate to strong)
4. Ovarian serous adenocarcinoma	<75%
5. Ovarian serous adenocarcinoma	≥75% (moderate to strong)
6. Ovarian serous adenocarcinoma	<75%
7. Ovarian serous adenocarcinoma	≥75% (moderate to strong)
8. Ovarian endometrioid carcinoma	<75%



*Tumour Cell Scoring (TCS) determined by FOLR1 (FOLR1-2.1) RxDx Assay (741-5076, Ventana/Roche) performed in NordiQC reference laboratory.

All tissues were fixed in 10% neutral buffered formalin.

KEY POINTS FOR FOLR1 IMMUNOASSAYS

- The Ventana/Roche RTU FOLR1 assays, based on clone FOLR1-2.1, provided a 100% pass rate when applied on the intended platform.
- mAb clone BN3.2 was the most used LD assay, with a pass rate of 71%.

The participating laboratories were asked to perform their FOLR1 IHC assay used for treatment decision with ELAHERE®, evaluate the FOLR1 expression level using TCS as read-out method and submit the stained slides and scores to NordiQC. This allowed both an assessment of the technical performance (analytical accuracy) of the FOLR1 IHC assays but also information on the reproducibility and concordance of the FOLR1 expression read-out results among the laboratories.

FOLR1 IHC, Technical assessment

In order to account for heterogeneity of FOLR1 expression in the individual tumour cores included in the tissue micro array (TMA) blocks, reference slides were made throughout the blocks. Every twenty-fifth slide was thus stained for FOLR1 using the CE-IVD / FDA approved FOLR1 (FOLR1-2.1) RxDx Assay (741-5076, Ventana/Roche). During the assessment, TCS categories for each tissue core on the submitted slides were compared to the level in the nearest reference slide.

Criteria for assessing an IHC assay as <u>Optimal</u> include:

The result is considered perfect or close to perfect in all of the included tissues.
<u>TCS score is concordant to the NordiQC reference data in all neoplasias.</u>

Criteria for assessing an IHC assay as <u>Good</u> include:

The result is considered acceptable in all of the included tissues.
The FOLR1 expression in one or more tissues varies significantly from the expected scores, but still in right category.
<u>TCS score is concordant to the NordiQC reference data in all neoplasias.</u>

Criteria for assessing an IHC assay as Borderline include:
The result is considered insufficient, e.g., because of generally too weak staining, a false negative staining or a false positive staining reaction of one of the included tissues.
TCS score is **not** concordant to the NordiQC reference data in all neoplasias.

Criteria for assessing an IHC assay as Poor include:
The result is considered very insufficient e.g., because of a false negative or a false positive staining reaction of more than one of the included tissues.
TCS score is **not** concordant to the NordiQC reference data in all neoplasias.

An IHC result could also be assessed as **Borderline/Poor**, if the signal-to-noise ratio was low, e.g., because of moderate cytoplasmic reaction, excessive/less-selective counterstaining or impaired morphology, to the extent where interpretation was compromised.

FOLR1 IHC, Read-out
All participating laboratories were asked to submit a scoring sheet with their read-out of the tumour cell scoring (TCS) in the six carcinomas using a ≥75% cut-off. Results were compared to NordiQC data from the reference laboratory to analyse scoring consensus.

Tumour Cell Scoring	**What to Include**
Cells Included	Approximately 100 or more viable tumour cells
Staining Intensity	Moderate to Strong
Pattern of Staining	Apical, Circumferential (partial and complete), Basolateral/Lateral, Microluminal
Denominator	Total Number of Viable Tumour Cells
Scoring Algorithm	
Positive	≥75% viable tumour cells demonstrating moderate to strong membrane staining
Negative	<75% viable tumour cells demonstrating moderate to strong membrane staining

VENTANA FOLR1 (FOLR1-2.1) RxDx Assay Interpretation Guide for EOC, 1015222EN Rev B

Participation

Number of laboratories registered for FOLR1 IHC C19	98
Number of laboratories returning FOLR1 IHC slides	83 (85%)
Number of laboratories returning FOLR1 scoring sheet	72

Results: 83 laboratories participated in this assessment. 90% (75 of 83) achieved a sufficient mark. Assessment marks for IHC FOLR1 assays and FOLR1 antibodies are summarized in Table 2a-2d (see page 3-4). All slides returned after the assessment were assessed and received advice if the result was insufficient, but data were not included in this report.

Controls
Normal fallopian tube serves as both positive and negative on-slide tissue control: Normal epithelium exhibits moderate to high expression levels of the folate receptor alpha protein, characterized by predominantly moderate circumferential membranous staining reaction and strong apical membranous staining reaction. Normal fallopian tube stroma should not show any staining reaction. This staining pattern was seen in both fallopian tubes included in this FOLR1 assessment, however, in most screened fallopian tubes, a generally weaker staining reaction was observed.

Conclusion
The FOLR1 (FOLR1-2.1) RxDx Assays **741-5076** and **740-5065** from Ventana/Roche were the most successful assays for the evaluation of FOLR1 status in ovarian carcinomas with pass rates of 100%, when used on the intended platform. Of the remaining FOLR1 assays, the mAb clone **BN3.2** used as a concentrate within a LD assay was most successful and obtained a pass rate of 71%.

Table 2a. **Overall results for FOLR1, run C19**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
CE-IVD / FDA approved FOLR1 assays*	67	59	7	1	-	99%	88%
Antibodies for laboratory developed FOLR1 assays, based on concentrated antibodies	10	1	6	2	1	70%	10%
Ready-To-Use antibodies	6	0	2	4	0	33%	0%
Total	83	60	15	7	1		
Proportion		72%	18%	9%	1%	90%	

1) Proportion of sufficient results (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).
* Including all uses of product on non-intended platforms.

Table 2b. **Assessment marks for CE-IVD / FDA approved FOLR1 assays, run C19**

CE-IVD / FDA approved FOLR1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone FOLR1-2.1, **741-5076**[3]	42	Ventana/Roche	38	4	-	-	100%	90%
mAb clone FOLR1-2.1, **741-5076**[4]	14	Ventana/Roche	11	3	-	-	100%	79%
mAb clone FOLR1-2.1, **740-5065**[3]	7	Ventana/Roche	7	-	-	-	100%	100%
mAb clone FOLR1-2.1, **740-5065**[4]	4	Ventana/Roche	3	-	1	-	-	-
Total	67		59	7	1	-		
Proportion			88%	10%	2%	0%	98%	

1) Proportion of sufficient results (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).
3) This product has a locked protocol on BenchMark Ultra and cannot be changed.
4) RTU products applied on another platform than developed for.

Table 2c. **Assessment marks for concentrated antibodies for FOLR1, run C19**

Antibodies for laboratory developed FOLR1 assays, concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **34265T**	1	Cell Signaling	-	1	-	-	-	-
mAb clone **BN3.2**	7	Leica Biosystems	1	4	1	1	71%	14%
rmAb clone **EPR20277**	1	Abcam	-	-	1	-	-	-
Ab clone **BP6286**	1	Biolynx Biotechnology	-	1	-	-	-	-
Total	10		1	6	2	1		
Proportion			10%	60%	20%	10%	70%	

1) Proportion of sufficient results (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).

Table 2d. **Assessment marks for Ready-To-Use antibodies[5] for FOLR1, run C19**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **26B3.F2 APR3005AA**	2	BioCare Medical	-	-	2	-	-	-
rmAb clone **QR145 P-F005-70-RUO**	1	Quartett	-	-	1	-	-	-
rmAb clone MXR066 **RMA-1147**	1	Fuzhou Maixin	-	1	-	-	-	-
Ab clone 436I6G8, **PA589**	1	Abcarta	-	-	1	-	-	-
Ab clone C12A21 **CFR-0120**	1	Celnovte	-	1	-	-	-	-
Total	6		0	2	4	0		
Proportion			0%	33%	67%	0%	33%	

1) Proportion of sufficient results (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).
5) Ready-To-Use antibodies without predictive claim.

Detailed Analysis
CE-IVD / FDA approved assays
mAb clone **FOLR1-2.1** (741-5076/740-5065, Ventana/Roche) on BenchMark Ultra: In total, 45 of 49 (92%) protocols were assessed as optimal. This product has a locked protocol and cannot be changed. The protocol is based on Heat Induced Epitope Retrieval (HIER) in Cell Conditioning 1 (CC1) for 64 min., 32 min. incubation of primary Ab and OptiView as detection system. Using these protocols settings and applied on BenchMark Ultra, 49 of 49 (100%) laboratories produced a sufficient staining result (optimal or good).
The products were used by 18 laboratories on a non-intended platform. This data is not included.

Block construction and assessment reference standards
The tissue micro array (TMA) blocks constructed for this FOLR1 run consisted of six ovarian carcinomas and two fallopian tubes. The six ovarian carcinomas were selected to comprise three carcinomas with a TCS ≥75% and three with TCS score of <75%.
Reference slides throughout the individual TMA blocks (at interval of 25 slides) were stained using the companion diagnostic assay FOLR1 (FOLR1-2.1) RxDx Assay (741-5076, Ventana/Roche).
In total, two identical TMA blocks were constructed and used for this assessment.
During the assessment, TCS for each tissue core on the submitted slides were compared to the level in the nearest reference slides.

Comments – accuracy of FOLR1 IHC using TCS scoring to guide treatment with ELAHERE®
In this first NordiQC run C19 for FOLR1 in the companion module, a pass rate of 90% was observed for the participants performing FOLR1 IHC assays to identify patients with ovarian carcinomas eligible for treatment with ELAHERE® using the TCS method.

In 29% (2 of 7) of the insufficient results, one or more tissue cores showed either a reduced proportion of stained neoplastic cells or a completely false negative staining reaction. This was mainly observed in tissue core 5. Among the remaining insufficient results, 57% (4 of 7) were mainly characterized by excessive cytoplasmic staining that complicated the read-out, most notably in tissue core 6. In tissue core 6 a false positive reaction was in addition observed for one insufficient result.

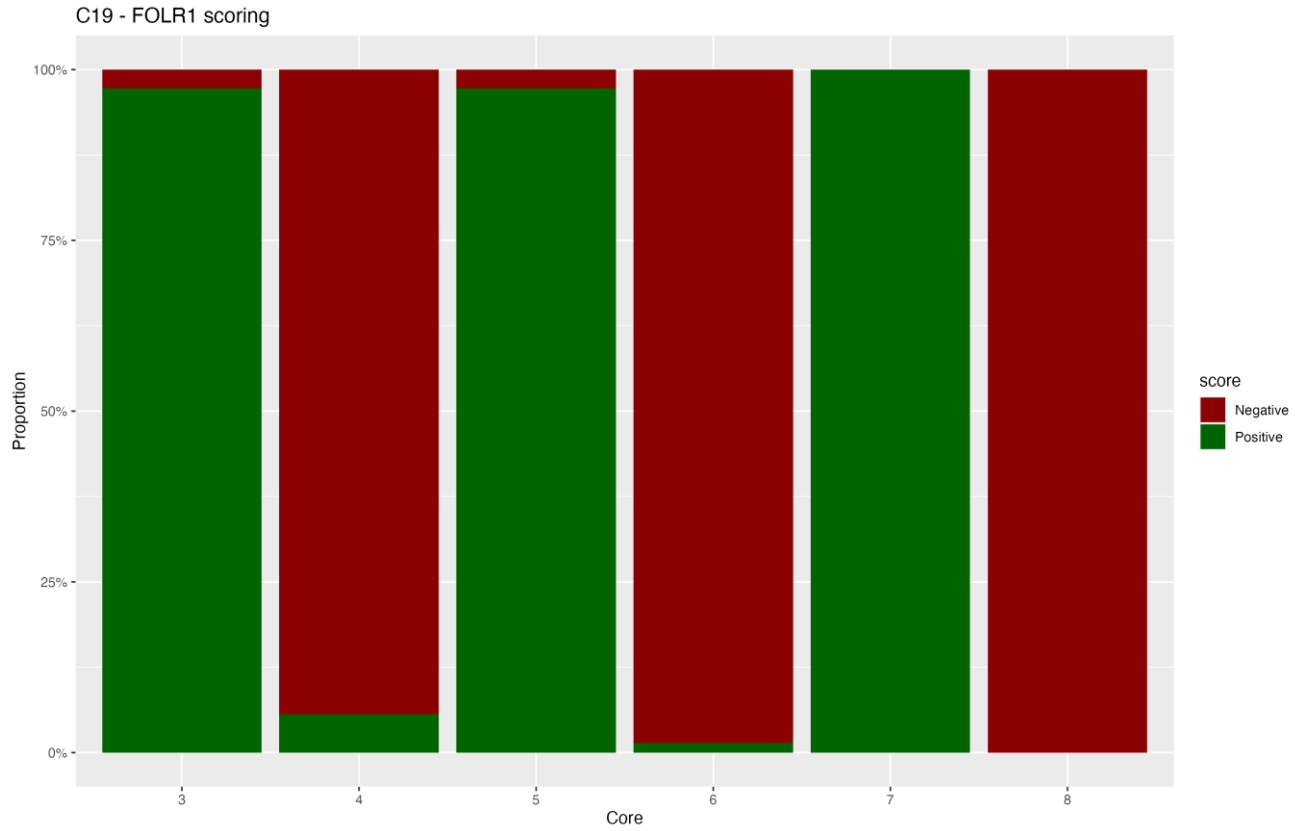
The Ventana/Roche FOLR1 (FOLR1-2.1) RxDx Assays **741-5076** and **740-5065** (BenchMark Ultra) with predictive claim for ELAHERE® was used by 59% (49 of 83) of the participants on the intended platform and provided a pass rate of 100% - see Table 2b. The assays are locked for central protocol settings and based on HIER in CC1 for 64 min., incubation in primary Ab for 32 min. and use of OptiView as detection system for BenchMark Ultra. 17 laboratories applied the products on BenchMark GX or Ultra Plus, with a pass rate of 94%, 83% optimal, however, the assay is not recommended by vendor for these platforms.

Laboratory developed (LD) assays based on concentrated primary Abs gave an overall inferior performance and reduced pass rate of 70% (7 of 10), 10% optimal (n=1) – see Table 2c.

Seven laboratories used the mAb clone **BN3.2**, but only one obtained an optimal result. This was performed on BenchMark Ultra (Ventana/Roche). Sufficient results were also seen on Omnis (Dako/Agilent) and Bond III (Leica Biosystems). The sufficient results were based on HIER in an alkaline buffer and a 3- or 4-step detection system. The titre ranged from 1:25-100, but with only few observations on three different platforms, it is not possible to identify an optimal and robust, recommendable protocols for the different platforms.

FOLR1 scoring
Participants were asked to evaluate the TCS in each of the six ovarian carcinomas (TCS with 75% cut-off) included in the assessment material. The overall read-out of the FOLR1 expression among the participants is shown in Graph 1.

Graph 1. **NordiQC FOLR1 run C19: Read-out of TCS in six ovarian carcinomas.**



As seen in Graph 1, relatively high consensus rates were observed in general. Tissue cores no 3, 5, 7 and 6, 8 were scored as high and low, respectively, by the vast majority of participants. The slightly reduced consensus rate in tissue core 4 might be caused by a TCS of about 30-50% positive tumour cells – rather close to the ≥75% cut-off.

Summary

This was the first NordiQC assessment of FOLR1 for TCS in ovarian carcinomas in the NordiQC companion module. 83 laboratories participated and a pass rate of 90% was observed.

The FOLR1 (FOLR1-2.1) RxDx Assays 741-5076 and 740-5065 were the most successful assays for the evaluation of FOLR1 status in ovarian carcinomas with pass rates of 100%, when applied on the intended platform. 17 laboratories applied the products on BenchMark GX or Ultra Plus, with a pass rate of 94%, 83% optimal. Of the remaining FOLR1 assays, the mAb clone BN3.2 used as a concentrate within a LD assay was most successful and obtained a pass rate of 71%, but due to the limited number and composition of samples, internal validation/verification and extended quality control, e.g. regularly measuring the FOLR1 results, is needed.

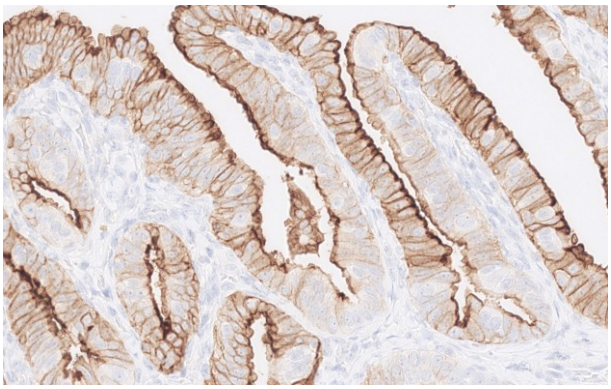


Fig. 1a
Optimal staining result of fallopian tube using the FOLR1 IHC assay 741-5076 from Ventana/Roche, based on the mAb clone FOLR1-2.1 following the recommended protocol settings. Same protocol used in Figs. 2a-5a.
Virtually all epithelial cells show an at least weak membranous staining reaction.

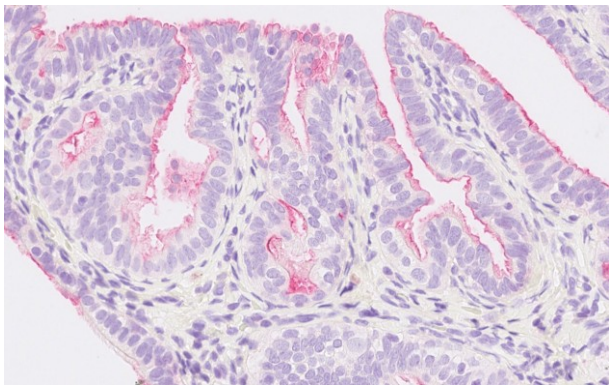


Fig. 1b
Staining result of fallopian tube using the mAb clone BN3.2 as an LD assay. The Ab was diluted 1:100, HIER was performed in a low pH buffer and a 3-layer detection system was used.
Overall, a reduced analytical and diagnostic sensitivity was observed.
Same protocol used in Figs. 2b-3b.
The staining intensity and proportion of positive epithelial cells are significantly reduced as only a luminal staining reaction is seen. Compare to optimal result in Fig. 1a.

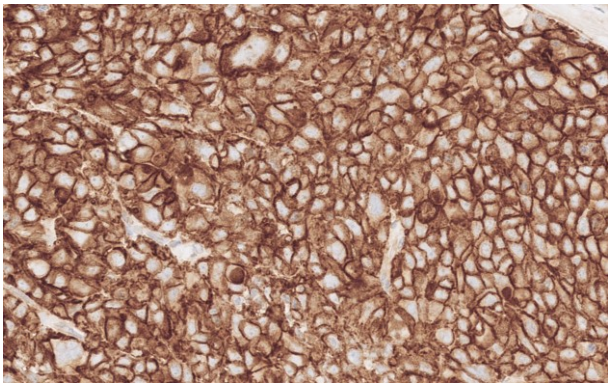


Fig. 2a
Optimal staining result of ovarian carcinoma, tissue core 3, using the same protocol as in Fig. 1a and providing the expected results in all the included tissues/neoplasms.
A moderate to strong, membranous staining reaction is seen in ≥75% of the neoplastic cells and thus eligible for treatment with ELAHERE®.

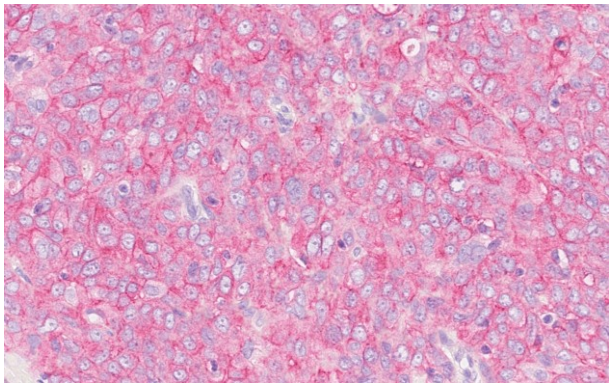


Fig. 2b
Staining result of ovarian carcinoma, tissue core 3, using same protocol as in Fig. 1b. The neoplastic cells show a less distinct membranous staining reaction, however, the TCS score is still ≥75%.
Compare with Fig. 2a – same area.

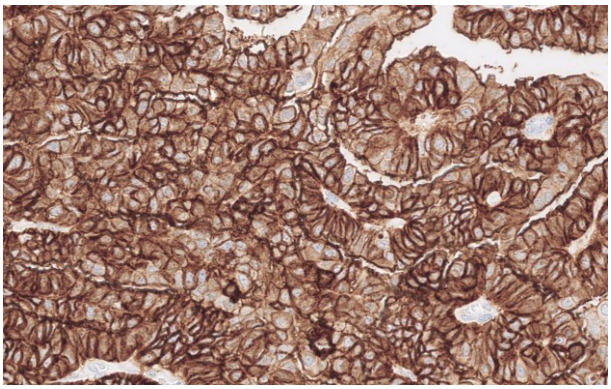


Fig. 3a
Optimal staining result of the ovarian carcinoma, tissue core 5, using same protocol as in Figs. 1a and 2a. A moderate to strong, membranous staining reaction is seen in ≥75% of the neoplastic cells and thus eligible for treatment with ELAHERE®.

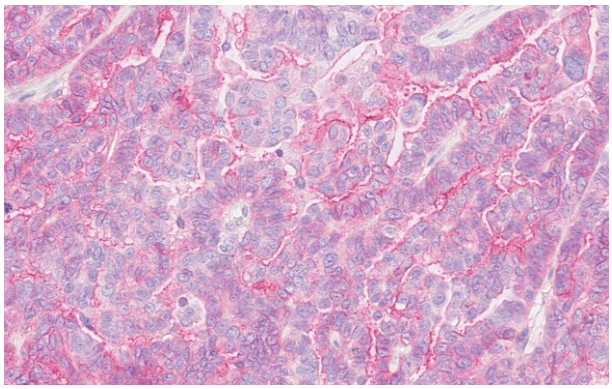


Fig. 3b
Insufficient staining result of the ovarian carcinoma, tissue core 5, using same protocol as in Figs. 1b and 2b. Reduced proportion and intensity of positive neoplastic cells are seen, changing the TCS from positive to negative, not being eligible for treatment with ELAHERE® based on this result.

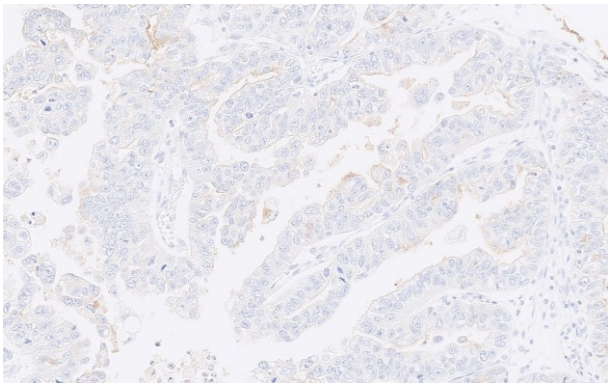


Fig. 4a
Optimal staining result of the ovarian carcinoma, tissue core 6, using same protocol as in Figs. 1a-3a. Only scattered neoplastic cells are positive, and tumour is TCS negative.

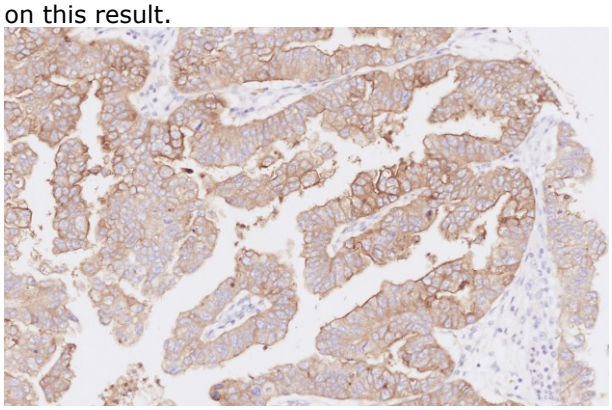


Fig. 4b
Insufficient staining result of the ovarian carcinoma, tissue core 6, using the Ab clone 436I6G8 as an RTU, with HIER in an alkaline buffer and a sensitive detection system. A diffuse cytoplasmic staining reaction is observed, and increased intensity and proportion of positive cells are seen, changing the TCS from negative to positive. Compare with the optimal result shown in Fig. 4a – same area.

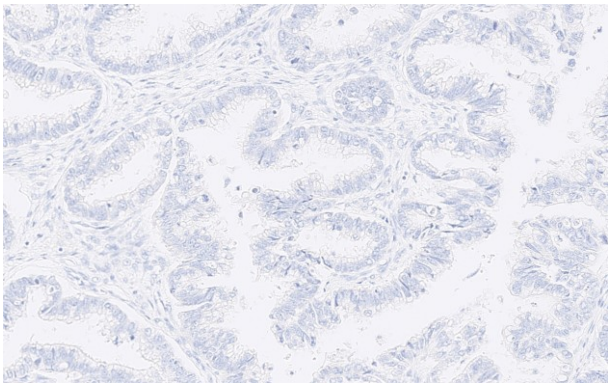


Fig. 5a
Optimal staining result of the ovarian carcinoma, tissue core 8, using same protocol as in Figs. 1a-4a. Virtually all neoplastic cells are negative, and tumour is TCS negative.

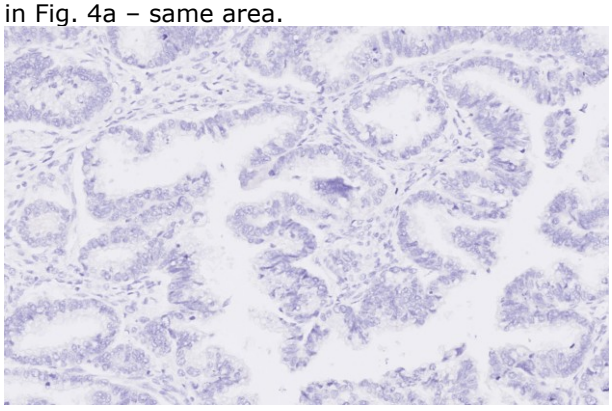


Fig. 5b
Staining result of the ovarian carcinoma, tissue core no. 8, using same protocol as in Fig. 4b. No staining reaction is observed, and tumour is TCS negative as expected. Compare with Fig. 5a.

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