Assessment Run C18 2025

Claudin 18.2 (CLDN18.2)

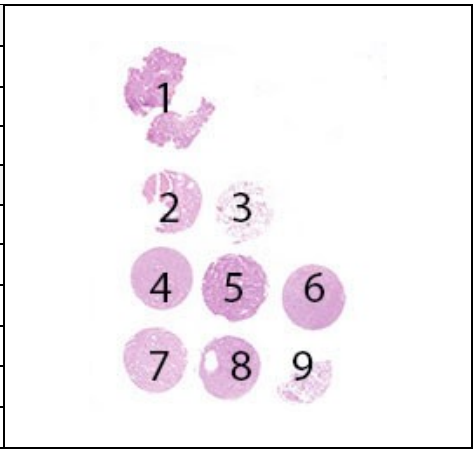
Purpose

This assessment of CLDN18.2 in the NordiQC Companion module focused on the evaluation of the analytical accuracy of the IHC assays performed by the NordiQC participants to identify patients with gastric and gastroesophageal junction (G/GEJ) cancer to be treated with VYLOY™. The CLDN18 (43-14A) RxDx Assay (741-6067, Ventana/Roche) was used as reference standard method. Accuracy was evaluated in six carcinomas with the dynamic and critical relevant expression levels of CLDN18.2 characterized by tumor 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 CLDN18.2 results, is needed.

Material

Table 1. **Content of the TMA used for the NordiQC CLDN18.2 C18 assessment**

Tissue controls	**CLDN18.2 IHC reaction pattern**
1. Gastric Intestinal Metaplasia*	See control section
2. Gastric mucosa	See control section
3. Lung	See control section
Carcinomas	**TCS score****
4. Gastric carcinoma	<75% (0%)
5. Gastric carcinoma	≥75% (90-95%, moderate to strong)
6. Gastric carcinoma	<75% (40-50%, weak to strong)
7. Gastric carcinoma	≥75% (85-90%, moderate to strong)
8. Gastric carcinoma	<75% (1% moderate)
9. Gastric carcinoma	≥75% (80-95%, moderate to strong)



* not present in all sections.
** Tumor Cell Scoring (TCS) determined by CLDN18 (43-14A) RxDx Assay (741-6067, Ventana/Roche) performed in NordiQC reference lab.

All tissues were fixed in 10% neutral buffered formalin.

KEY POINTS FOR CLDN18.2 IMMUNOASSAYS

- The Ventana/Roche RTU CLDN18 assays, based on clone 43-14A, provided a 99% pass rate overall.
- Laboratory developed assays based on concentrated Abs gave an inferior pass rate of 69%.
- Insufficient results were mainly caused by too weak or false negative staining reaction in gastric carcinomas expected to be TCS ≥75%

The participating laboratories were asked to perform their CLDN18.2 IHC assay for treatment decision with VYLOY™, evaluate the CLDN18.2 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 CLDN18.2 IHC assays but also information on the reproducibility and concordance of the CLD18.2 expression read-out results among the laboratories.

CLDN18.2 IHC, Technical assessment

In order to account for heterogeneity of CLDN18.2 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 stained for CLDN18.2 using the CE IVD / FDA approved CLDN18 (43-14A) RxDx Assay (741-6067, 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 of CLDN18.2 (43-14A).

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 Good include:
The result is considered acceptable in all of the included tissues.
The CLDN18.2 expression in one or more tissues varies significantly from the expected scores, but still in right category.
TCS score is concordant to the NordiQC reference data in all neoplasias.

Criteria for assessing an IHC assay as Borderline include:
The result is considered insufficient, e.g., because of a generally too weak staining, a false negative staining or a false positive staining reaction of one of the included tissues.
TCS score is **not** found 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 in more of the included tissues.
TCS score is **not** found 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/in-selective counterstaining or impaired morphology, to the extent where interpretation was compromised.

CLDN18.2 IHC, Read-out
All participating laboratories were asked to submit a scoring sheet with their read-out of the tumor 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.

Tumor Cell Scoring	What to Include
Cells Included	Greater than 50 Viable Tumor Cells Only
Staining Intensity	Moderate to Strong
Pattern of Staining	Apical, Circumferential (partial and complete), Basolateral/Lateral, Microluminal
Denominator	Total Number of Viable Tumor Cells
Scoring Algorithm for Gastric Adenocarcinoma Including the Gastroesophageal Junction	
Positive	≥75% viable tumor cells demonstrating moderate to strong membrane staining
Negative	<75% viable tumor cells demonstrating moderate to strong membrane staining

VENTANA CLDN18 (43-14A) RxDx Assay Interpretation Guide for Gastric Adenocarcinoma including GEJ, 1016391EN Rev A

Participation

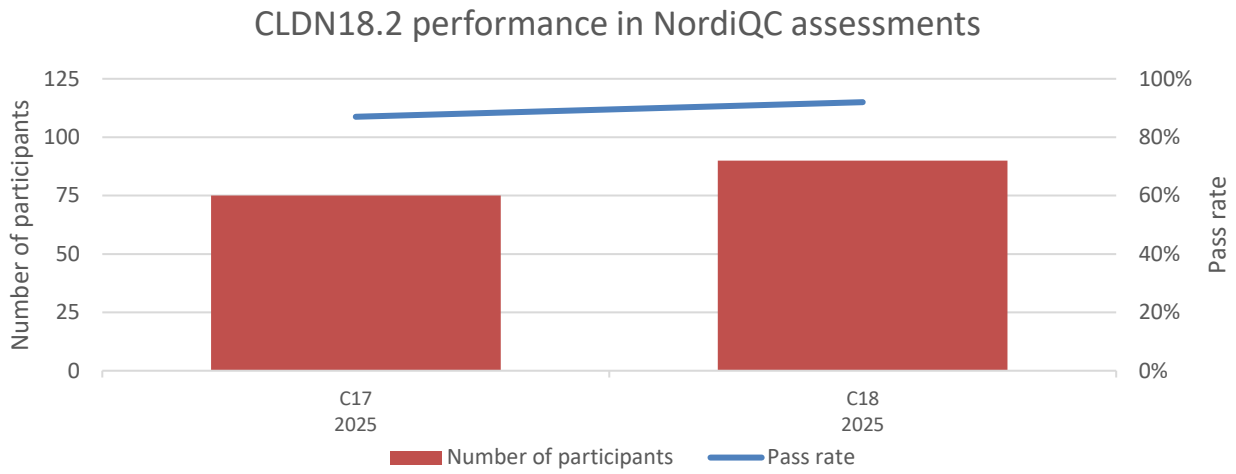
Number of laboratories registered for CLDN18.2 IHC C18	101
Number of laboratories returning CLDN18.2 IHC slides	90 (89%)
Number of laboratories returning CLDN18.2 scoring sheet	84

All slides returned after the assessment were assessed and received advice if the result was insufficient, but data were not included in this report.

Results: 90 laboratories participated in this assessment. 92% (83 of 90) achieved a sufficient mark. Assessment marks for IHC CLDN18.2 assays and CLDN18.2 antibodies are summarized in Table 2a-2d (see page 3-4).

Performance history: This was the second NordiQC assessment of CLDN18.2 in NordiQC. Compared to C17, an improved pass rate was obtained in C18 (see Graph 1).

Graph 1. **Proportion of sufficient results for CLDN18.2 in the NordiQC runs performed.**



Controls

Gastric intestinal metaplasia and gastric mucosa were used as positive and negative tissue controls in concordance with the official scoring guidelines from Ventana/Roche. In gastric intestinal metaplasia, a weak to moderate membranous staining reaction of epithelial cells in the areas of metaplasia should be seen. In gastric mucosa, virtually all normal epithelial cells should show a strong, membranous staining reaction. No staining should be seen in e.g. lymphocytes, smooth muscle cells and nerves.

However, the use of gastric intestinal metaplasia as the critical control is challenging as the staining reaction is very heterogenous and can be present in some levels in the control material and absent in other levels, hereby compromising the utility to monitor the reproducibility of the CLDN18.2 IHC assay.

If the primary Ab also reacts with CLDN18.1 (as observed for the mAb clone 43-14A being used for the approved Ventana/Roche CDx assay for CLDN18.2), pneumocytes in normal lung show a weak membranous staining reaction. This reduced and low level CLDN18 expression in pneumocytes might be more valuable than intestinal metaplasia as critical control for the evaluation of IHC reproducibility. However, more testing is needed to document the potential of lung/pneumocytes to serve as reliable control for CLDN18.2 IHC assays.

Conclusion

This was the second NordiQC assessment of CLDN18.2 for TCS in gastric carcinomas in the NordiQC companion module. 90 laboratories participated and an increased pass rate of 92% was observed.

The CLDN18 (43-14A) RxDx Assays 741-6067 and 740-7037 and the RTU IHC assays 790-7027 and 744-7162 all from Ventana/Roche were the most successful assays for the evaluation of CLDN18.2 status in gastric carcinomas with an overall pass rate of 99%. Of the remaining CLDN18 assays, more clones could obtain optimal results, however, with only few observations and limited tissue cohort, the data must be carefully interpreted.

Table 2a. **Overall results for CLDN18.2, run C18**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
CE-IVD / FDA approved CLDN18.2 assays	26	24	2	0	0	100%	92%
Antibodies for laboratory developed CLDN18.2 assays, based on concentrated antibodies	16	4	7	1	4	69%	25%
Ready-To-Use antibodies	48	40	6	2	0	96%	83%
Total	90	68	15	3	4		
Proportion		76%	17%	3%	4%	92%	

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

Table 2b. **Assessment marks for CE-IVD / FDA approved CLDN18.2 assays for CLDN18.2, run C18**

CE-IVD / FDA approved CLDN18.2 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone 43-14A, **741-6067[3]**	25	Ventana/Roche	23	2	0	0	100%	92%
mAb clone 43-14A, **740-7037[3]**	1	Ventana/Roche	1	0	0	0	-	-
Total	26		24	2	0	0		
Proportion			92%	8%	0%	0%	100%	

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 XT/GX/Ultra and cannot be changed.

Table 2c. **Assessment marks for concentrated antibodies for CLDN18.2, run C18**

Antibodies for laboratory developed CLDN18.2 assays, concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **43-14A**	6	Abcam	2	4	0	0	100%	33%
mAb clone **ABT-CLD18**	1	LS Bio	0	0	1	0	-	-
rmAb clone **RBT-CLDN18.2**	1	BioSB	0	0	0	1	-	-
rmAb clone **ZR451**	4	Zeta Corporation	0	2	0	2	-	-
rmAb clone **IHC758**	1	GenomeMe	1	0	0	0	-	-
rmAb clone **3J4G7**	1	Thermo Fisher	0	0	0	1	-	-
rmAb clone **DBRRM1.82**	1	Zytomed Systems	0	1	0	0	-	-
Ab clone **BP6249**	1	Biolynx Biotechnology	1	0	0	0	-	-
Total	16		4	7	1	4		
Proportion			25%	44%	6%	25%	69%	

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[7] for CLDN18.2, run C18**

Ready-To-Use antibodies[8]	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone 43-14A, **744-7162[3,7]**	3	Ventana/Roche	3	0	0	0	-	-
mAb clone 43-14A, **790-7027[7] (VRPS)[5]**	25	Ventana/Roche	23	1	1	0	96%	92%
mAb clone 43-14A, **790-7027[7] (LMPS)[6]**	15	Ventana/Roche	13	2	0	0	100%	87%
rmAb clone RBT-CLDN18.2 **BSB-3835-7-RUO**	1	BioSB	0	1	0	0	-	-
rmAb clone ZR451, **Z2807RP**	2	Zeta Corporation	1	1	0	0	-	-
rmAb clone DBRRM1.82, **RMPD116R**	1	Diagnostic BioSystems	0	1	0	0	-	-
Ab clone **DY49228 4911062**	1	Dakewe	0	0	1	0	-	-
Total	48		40	6	2	0		
Proportion			83%	13%	4%	0%	96%	

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 XT/GX/Ultra/Ultra Plus and cannot be changed.
5) Vendor recommended protocol settings – RTU product used in compliance to protocol settings, platform and package insert.
6) Laboratory modified protocol settings for a RTU product applied either on the vendor recommended platform(s) or other platforms.
7) Ready-To-Use antibodies without predictive claim.

Detailed Analysis

CE IVD / FDA approved assays

43-14A (741-6067, Ventana/Roche): In total, 23 of 25 (92%) protocols were assessed as optimal. This product has a locked protocol on all BenchMark platforms and cannot be changed. The protocol is based on Heat Induced Epitope Retrieval (HIER) in Cell Conditioning 1 (CC1) for 48 min., 16 min. incubation of primary Ab and OptiView as detection system. Using these protocols settings and applied on BenchMark XT/GX/Ultra, 25 of 25 (100%) laboratories produced a sufficient staining result (optimal or good).

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used CDx assays with a predictive claim. The performance was evaluated both as "true" plug-and-play systems performed strictly accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 3. **Comparison of pass rates for vendor recommended and laboratory modified protocols**

CDx assays	**Vendor recommended protocol settings[1]**		**Laboratory modified protocol settings[2]**	
	Sufficient	Optimal	Sufficient	Optimal
Ventana BenchMark XT, GX, Ultra rmAb 43-14A, **741-6067**	25/25 (100%)	23/25 (92%)	-	-
Ventana BenchMark XT, GT, Ultra rmAb 43-14A, **740-7037**	1/1	1/1	-	-

1) Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
2) Modifications in one or more of parameters mentioned above. Only protocols performed on the specified vendor IHC stainer are included.

Ready-To-Use antibodies for laboratory developed (LD) assays
43-14A (790-7027, Ventana/Roche): In total, 29 of 32 (91%) protocols performed on the intended BenchMark XT/GX/Ultra provided an optimal result. Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 32-64 min.), 16 min. incubation of primary Ab and OptiView as detection system. Using these settings, 31 of 32 (97%) produced a sufficient staining result.
8 laboratories used the assay on another platform than intended. These data are not included here.

Block construction and assessment reference standards
The tissue micro array (TMA) blocks constructed for this CLDN18.2 run consisted of six gastric carcinomas, one gastric mucosa, one normal lung and one gastric intestinal metaplasia. The six gastric carcinomas were selected to comprise three carcinomas with an TCS ≥75% and three with TCS score <75%.
Reference slides throughout the individual TMA blocks (interval at each twenty-fifth slide) were stained using the companion diagnostic assay CLDN18 (43-14A) RxDx Assay (741-6067, Ventana/Roche).
In total, three 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 CLDN18.2 IHC using TCS scoring to guide treatment with VYLOY™
In this NordiQC run C18 for CLDN18.2 in the companion module, a pass rate of 92% was observed for the participants performing CLDN18.2 IHC assays to identify patients with gastric carcinomas VYLOY™ using the TCS method.

It was observed that insufficient results were most frequently characterized by a reduced proportion of cells demonstrated or a completely false negative staining reaction of neoplastic cells in one or more of the tissue cores and was seen in 86% (6 of 7) of the insufficient results. This was especially observed in tissue cores no. 5 and 7. In the remaining 14% (1 of 7), the insufficient staining result was caused by a false positive staining reaction seen in tissue core no. 6.

The Ventana/Roche CLDN18 (43-14A) RxDx Assays **741-6067** and **740-7037** (BenchMark Ultra/XT/GX) with predictive claim for VYLOY™ was used by 29% (26 of 90) of the participants on the intended platform and provided a pass rate of 100%. The assay is locked for central protocol settings and based on HIER in CC1 for 64 min., incubation in primary Ab for 16 min. and use of OptiView as detection system for BenchMark XT/GX/Ultra.

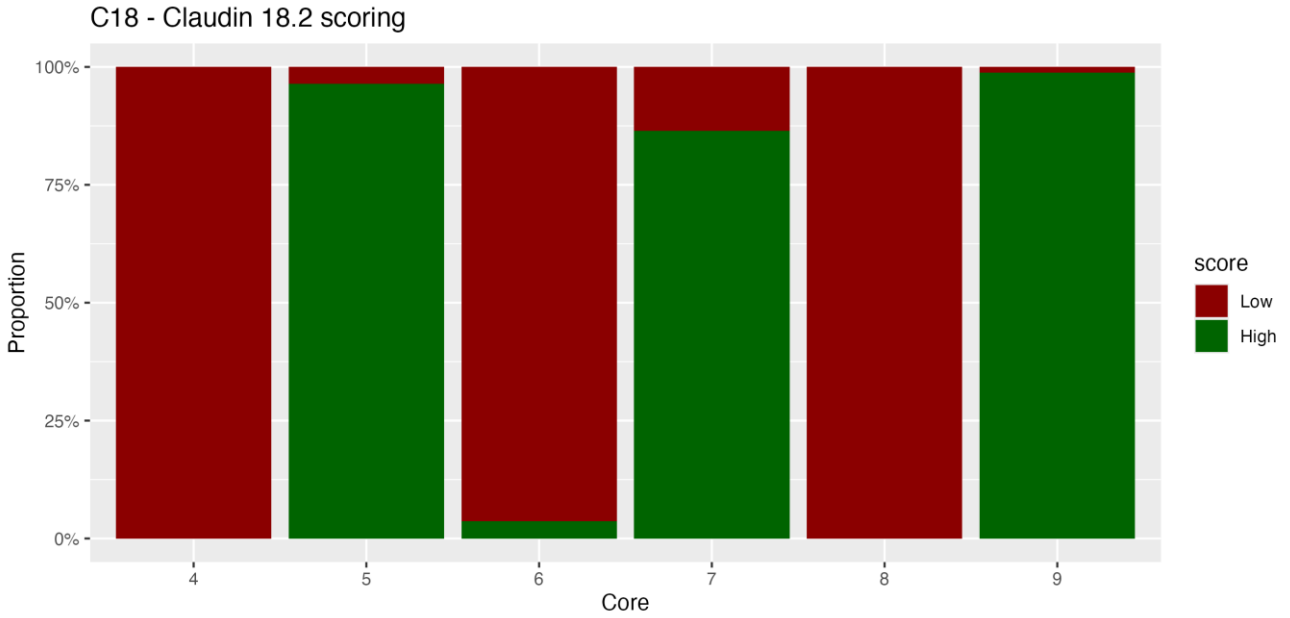
The Ventana/Roche CLDN18.2 43-14A assay **790-7027** (BenchMark Ultra/XT/GX) without predictive claim and available as an analytical or generic CLDN18.2 assay was used by 36% (32 of 90) of the participants on the intended platform. This assay is based on same recommended protocol settings as the corresponding CDx products 741-6067/740-7037, but with ordinary options for the laboratories to modify the protocol settings in their optimization and validation process for the implementation of the test. Overall, the CLDN18.2 790-7027 format also gave a high pass rate of 97% as the locked assay. A similar pass rate and proportion of optimal results was obtained, when using the vendor recommended protocol settings, compared to the CDx formats of the same clone as seen in Table 2b + 2d (see page 3 and 4). Eight laboratories used the 790-7027 on another platform than developed for, all with a sufficient result.

Laboratory developed (LD) assays based on concentrated primary Abs gave an overall significantly inferior performance and reduced pass rate of 69% (11 of 16), 25% optimal (n=4). Within LD-assays, and no matter which Ab clone is used, meticulous calibration and validation of the assay is required. Internal NordiQC studies have e.g. shown that different Ab clones for CLDN18.2 give different staining patterns in

normal tissues, which must be taken into account when evaluating the reaction pattern and to verify if the result is as expected in clinical tissues.

Six laboratories used the concentrated format of **mAb clone 43-14A (Abcam)**, all with a sufficient result. The submitted protocol settings were all based on HIER in an alkaline buffer and a 3- or 4-step detection system. The titre ranged from 1:200-8,000, and with only few observations, it is not possible to identify an optimal and robust, recommendable protocol for the different commercially available IHC platforms.

CLDN18.2 scoring
Participants were asked to evaluate the TCS in each of the six gastric carcinomas (TCS with 75% cut-off) included in the assessment material. The overall read-out of the CLDN18.2 expression among the participants is shown in Graph 2.



Graph 2. **NordiQC CLDN18.2 run C18: Read-out of TCS in six gastric carcinomas.**

As seen in Graph 2, a relatively high consensus rates were observed in general. Tissue cores no 4 and 8, expressing ≤1% positive tumour cells were scored as negative by all participants. The slightly reduced consensus rate in tissue core no 6 might be caused by the level of 40-50% positive tumour cells, and thus closer to the ≥75% cut-off – see Fig. 3a. The reduced consensus rate in cores 5, 7 and 9 was directly related to the proportion of false negative results and thus calling these cores TCS low (<75%) and not TCS high as expected – see Figs. 2 and 5.

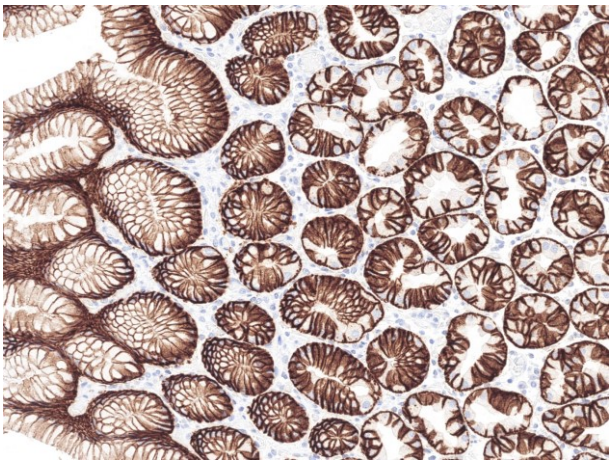


Fig. 1a
Optimal staining result of gastric mucosa using the CLDN18.2 RxDx IHC assay 741-6067 from Ventana/Roche, based on the mAb clone 43-14A following the recommended protocol settings.
Same protocol used in Figs. 2a-5a.
Virtually all epithelial cells show a moderate to strong membranous staining reaction.

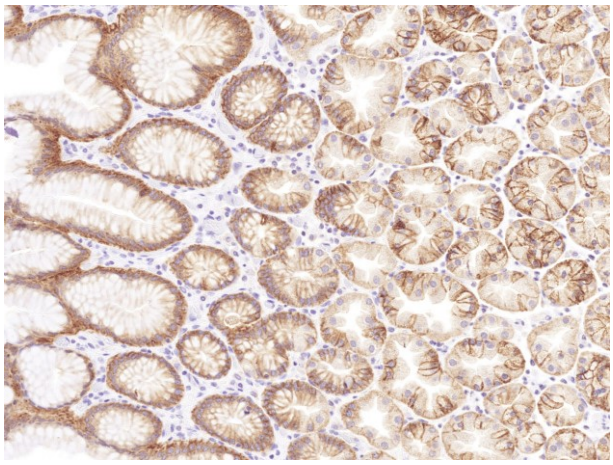


Fig. 1b
Staining result of gastric mucosa using the rmAb clone ZR451 as an LD assay. The Ab was diluted 1:50, HIER was performed in an alkaline buffer, and a 3-step detection system was used.
Same protocol used in Figs. 2b-5b.
The staining intensity of epithelial cells is significantly reduced compared to the optimal result in Fig. 1a.
Overall, in all tissues/neoplasias evaluated, both a reduced and increased analytical and diagnostic sensitivity was observed, making it difficult to optimize the protocol based on this clone.

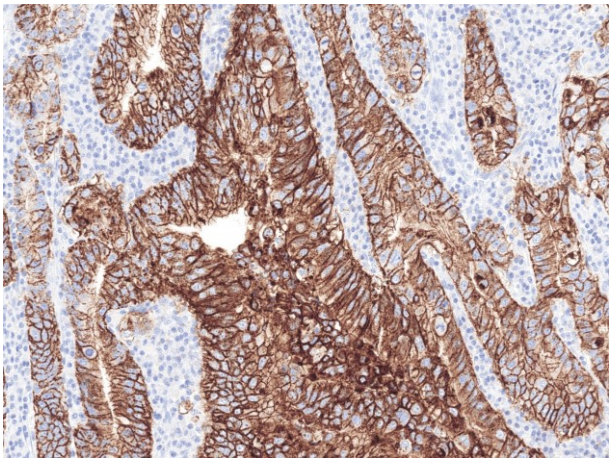


Fig. 2a
Optimal staining result of gastric carcinoma, tissue core no. 5, using the same protocol as in Fig. 1a and providing the expected results in all the included tissues/neoplasias.
A moderate to strong, membranous staining reaction is seen in ≥75% of the neoplastic cells and thus tumour being eligible for treatment with VYLOY™.

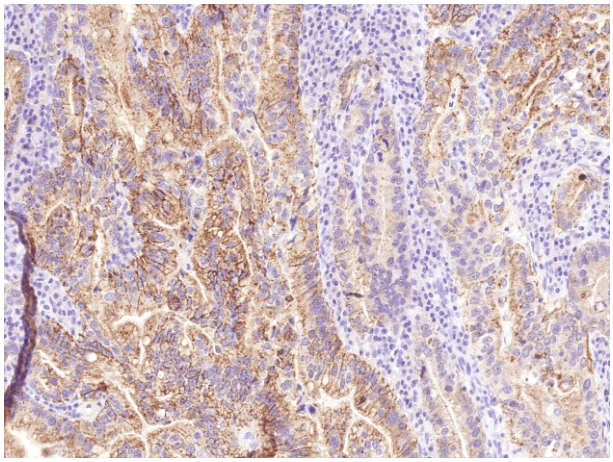


Fig. 2b
Insufficient staining result of gastric carcinoma, tissue core no. 5, using same protocol as in Fig. 1b. The neoplastic cells show a too weak staining reaction, changing the TCS from positive to negative and tumour not being eligible for treatment with VYLOY™.
Compare with Fig. 2a – same area.

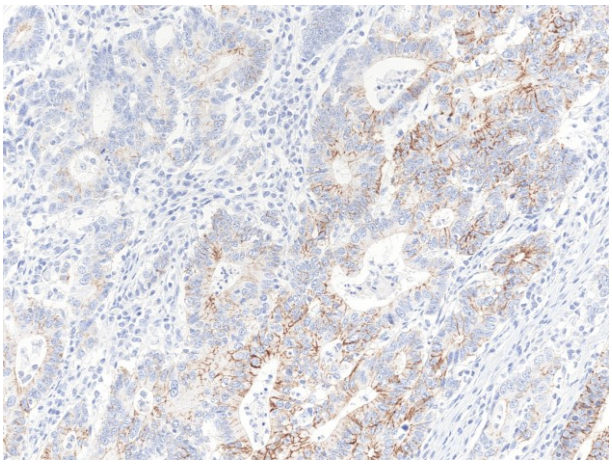


Fig. 3a
Optimal staining result of the gastric carcinoma, tissue core no. 6, using same protocol as in Figs. 1a and 2a. A weak to moderate membranous staining reaction is seen in about 40% of the neoplastic cells and tumour being TCS negative.

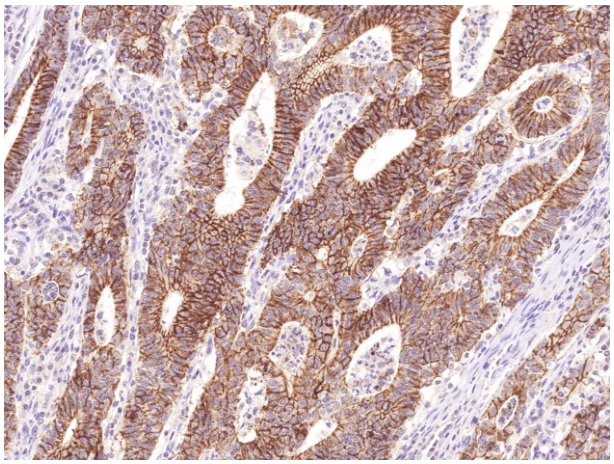


Fig. 3b
Insufficient staining result of the gastric carcinoma, tissue core no. 6, using same protocol as in Figs. 1b and 2b. A significantly increased intensity and proportion of positive cells are seen, changing the TCS in the tumour from negative to positive. Compare with Fig. 3a – same area.

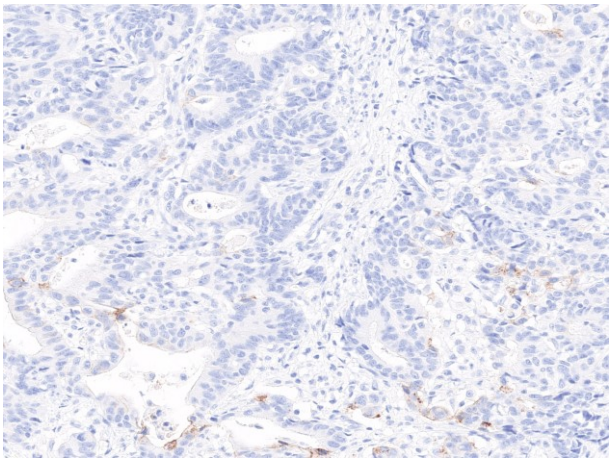


Fig. 4a
Optimal staining result of the gastric carcinoma, tissue core no. 8, using same protocol as in Figs. 1a-3a. Only scattered neoplastic cells are positive, and tumour being TCS negative.

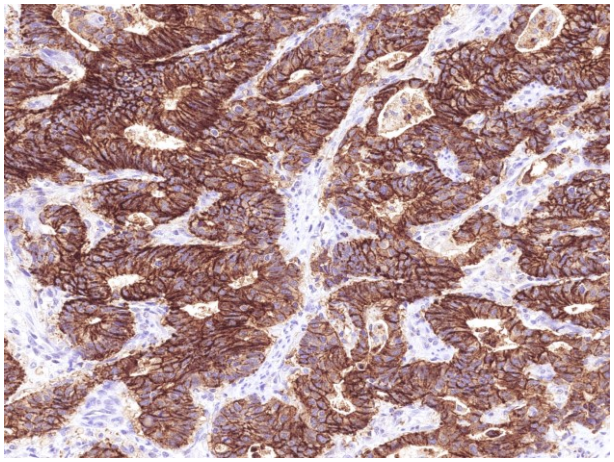


Fig. 4b
Insufficient staining result of the gastric carcinoma, tissue core no. 8, using same protocol as in Figs. 1b-3b. A significantly increased intensity and proportion of positive cells are seen, changing the TCS in the tumour from negative to positive. Compare with Fig. 4a – same area.

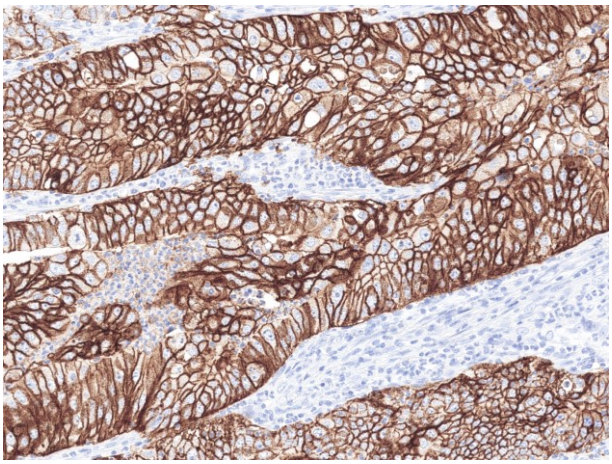


Fig. 5a
Optimal staining result of the gastric carcinoma, tissue core no. 9, using same protocol as in Figs. 1a-4a. A moderate to strong, membranous staining reaction is seen in ≥75% of the neoplastic cells and thus tumour being eligible for treatment with VYLOY™.

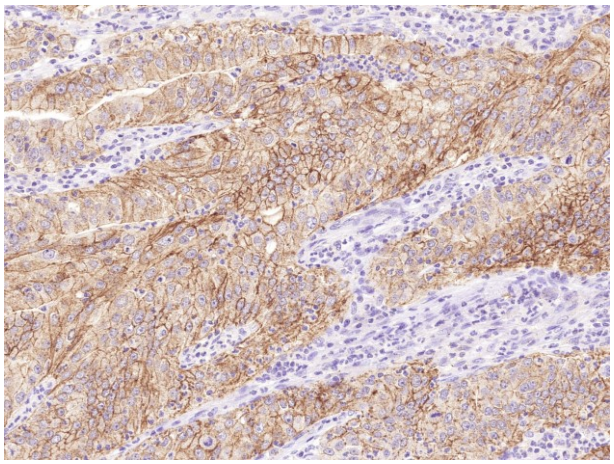


Fig. 5b
Insufficient staining result of the gastric carcinoma, tissue core no. 9, using same protocol as in Figs. 1b-4b. The neoplastic cells show a weaker membranous staining reaction than expected and in addition a diffuse cytoplasmic staining reaction is seen complicating the interpretation. Overall the result in the tumour was scored as TCS low and not high as expected. Compare with Fig. 5a – same area.

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