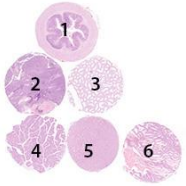
## Assessment Run 64 2022

# Special AT-rich sequence-binding protein 2 (SATB2)

**Purpose**
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for SATB2, identifying and characterizing colorectal carcinomas and neuroendocrine tumours in the diagnostic workup for carcinoma of unknown origin. Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for SATB2 (see below).

**Material**
The slide to be stained for SATB2 comprised:




1. Appendix, 2. Tonsil, 3. Testis, 4. Colon adenocarcinoma, 5. Neuroendocrine tumour (rectosigmoid), 6. Ovarian mucinous adenocarcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a SATB2 staining as optimal included:

- A strong, distinct nuclear staining reaction of virtually all epithelial cells of the appendix, and a weak to moderate but distinct nuclear staining reaction in dispersed ganglion cells of the plexuses of Auerbach (myenteric) and Meissner.
- A weak to moderate, distinct nuclear staining reaction of a subset of interfollicular lymphocytes of the tonsil.
- An at least weak to moderate, distinct nuclear staining reaction of dispersed germ cells (primarily spermatocytes) in seminiferous tubules of the testis.
- An at least weak to moderate, distinct nuclear staining reaction of virtually all neoplastic cells of the rectosigmoid neuroendocrine tumour.
- A moderate to strong, distinct nuclear staining reaction of all neoplastic cells in the colon adenocarcinoma, tissue core 4.
- No staining reaction of neoplastic cells in the ovarian mucinous adenocarcinoma or other cellular structures, including smooth muscle cells of lamina muscularis propria of the appendix and the vast majority of lymphocytes in the tonsil.

**Participation**

| | |
|---|---|
| Number of laboratories registered for SATB2, run 64 | 192 |
| Number of laboratories returning slides | 173 (90%) |

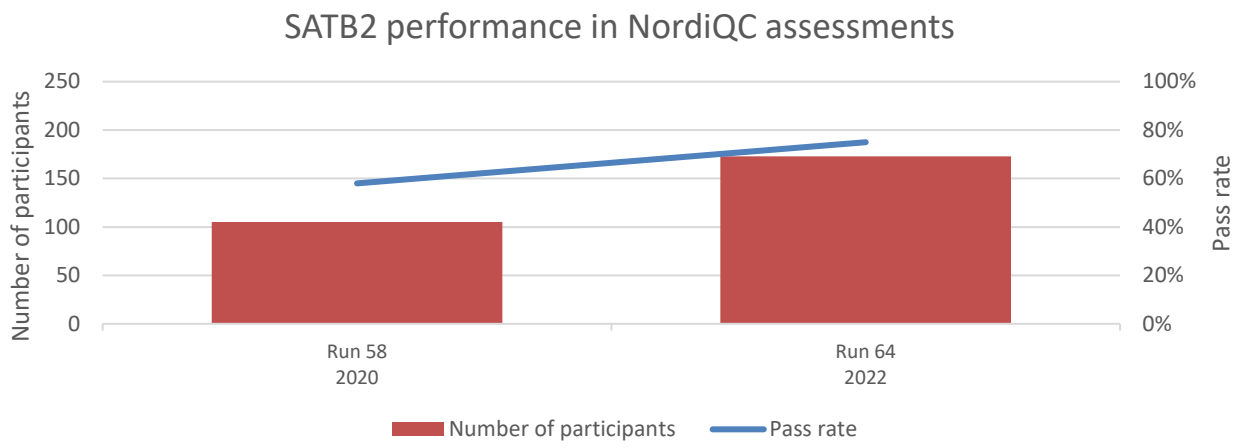
**Results**
At the date of assessment, 90% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

173 laboratories participated in this assessment. 130 (75%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and the assessment marks (see page 3).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Less successful primary antibodies
- Less sensitive detection systems
- Unexplained technical issues

**Performance history**
This was the second NordiQC assessment of SATB2. The pass rate increased significantly from 58% in the first run 58 to 75% in this run 64 (see Graph 1).

Graph 1. **Proportion of sufficient results for SATB2 in the NordiQC runs performed**




**Conclusion**
The rmAb clones EP281 and QR023 could both provide an optimal result for the demonstration of SATB2. HIER in alkaline buffer, precise calibration of the primary Ab and in particular use of a 3-step polymer or multimer based detection system were the main prerequisites for an optimal result. Protocols based on the mAb SATBA4B10 and the pAbs (HPA001042 and Ab69995) produced inferior results.
In total, 88% (153/173) of all protocols were based on the rmAb clone EP281. The vast majority of laboratories (75%) used 3-step polymer/multimer based detection systems obtaining a superior pass rate of 88%, 59% being optimal compared to 2-step detection systems giving a pass rate of 59% and only 18% being optimal. Laboratory developed (LD) tests and Ready-To-Use formats of EP281 provided similar performance.
Importantly, laboratories should use a robust Ab, calibrate the protocols correctly and stain accordingly to the expected antigen level of the recommended control materials (see below).

**Controls**
Appendix, testis and tonsil are recommendable as positive and negative tissue controls. In appendix, virtually all epithelial cells must show a strong nuclear staining reaction, whereas dispersed ganglion cells of nerve plexuses should display a weak to moderate nuclear staining reaction. No staining reaction should be seen in other cellular structures including smooth muscle cells (lamina muscularis propria) of the appendix. In tonsil, a subset of interfollicular lymphocytes must display a weak to moderate nuclear staining reaction, whereas the vast majority of lymphocytes should be negative. In testis, dispersed germ cells of seminiferous tubules should at least display a weak to moderate, distinct nuclear staining reaction.

Table 1. **Antibodies and assessment marks for SATB2, run 64**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | OR[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **SATBA4B10** | 1<br>3<br>3 | Abcam<br>Santa Cruz<br>Zytomed Systems | 0 | 1 | 6 | 0 | 14% | 0% |
| rmAb clone **EP281** | 13<br>66<br>1<br>3<br>1<br>5<br>3 | Epitomics<br>Cell Marque<br>Diagnostic BioSystems<br>BioSB<br>Biocare Medical<br>Gennova Scientific<br>Zeta Corporation | 48 | 28 | 8 | 8 | 83% | 52% |
| rmAb clone **SP281** | 4<br>1 | Abcam<br>Zytomed Systems | 0 | 4 | 0 | 1 | 80% | 0% |
| rmAb clone **QR023** | 1 | Quartett | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **ZR167** | 1 | Zeta Corporation | 0 | 0 | 0 | 1 | - | - |
| pAb **HPA001042** | 4 | Sigma Aldrich | 0 | 1 | 1 | 2 | - | - |
| pAb **Ab69995** | 1 | Abcam | 0 | 0 | 0 | 1 | - | - |
| Ready-To-Use antibodies | | | | | | | | |
| mAb clone **IHC660** | 1 | GenomeMe | 0 | 0 | 1 | 0 | - | - |
| rmAb clone **EP281 384R-17/18** | 19 | Cell Marque | 9 | 8 | 2 | 0 | 89% | 47% |
| rmAb clone **EP281 760-6075 VRPS[3]** | 4 | Ventana/Roche | 2 | 2 | 0 | 0 | - | - |
| rmAb clone **EP281 760-6075 VRPS[4]** | 23 | Ventana/Roche | 11 | 9 | 1 | 2 | 87% | 48% |
| rmAb clone **EP281 PR/HAR239** | 3 | PathnSitu | 0 | 1 | 2 | 0 | - | - |
| rmAb clone **EP281 API3225** | 3 | Biocare Medical | 0 | 2 | 1 | 0 | - | - |
| rmAb clone **EP281 MAD-000747QD** | 3 | Máster Diagnostica | 0 | 0 | 3 | 0 | - | - |
| rmAb clone **EP281 BSB319 7/8/9** | 2 | BioSB | 0 | 1 | 1 | 0 | - | - |
| rmAb clone **EP281 CSR-0140** | 1 | Celnovte | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **EP281 Z2321RP** | 2 | Zeta Corporation | 1 | 0 | 1 | 0 | - | - |
| rmAb clone **EP281 RMPD112** | 1 | Diagnostic BioSystems | 0 | 0 | 0 | 1 | - | - |
| Total | 173 | | 73 | 57 | 27 | 16 | | |
| Proportion | | | 42% | 33% | 16% | 9% | 75% | |

1) Proportion of sufficient stains (optimal or good) (≥5 asessed protocols).
2) Proportion of Optimal Results (≥5 asessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 asessed protocols).

## Detailed analysis of SATB2, Run 64

The following protocol parameters were central to obtain optimal staining:

### Concentrated antibodies

rmAb **EP281**: Protocols with optimal results were all based on Heat Induced Epitope Retrieval (HIER) using an alkaline buffer as Cell Conditioning 1 (CC1, Ventana/Roche) (26/45)*, Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (3/7) or Target Retrieval Solution (TRS) High pH (Dako/Agilent) (19/26) as retrieval buffer. The rmAb was typically diluted in the range of 1:50–1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings, 69 of 77 (90%) laboratories produced a sufficient staining result (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

Table 2. **Proportion of optimal results for SATB2 for the most commonly used antibody as concentrate on the 4 main IHC systems***

| Concentrated antibodies | **Dako Autostainer Link/Classic** | | **Dako Omnis** | | **Ventana BenchMark GX /XT/ Ultra** | | **Leica Bond III / Max** | |
|---|---|---|---|---|---|---|---|---|
| | TRS pH 9.0 | TRS pH 6.1 | TRS pH 9.0 | TRS pH 6.1 | CC1 pH 8.5 | CC2 pH 6.0 | BERS2 pH 9.0 | BERS1 pH 6.0 |
| rmAb clone **EP281** | 0/8** (0%) | - | 19/26 (73%) | - | 26/45 (58%) | | 3/7 (43%) | - |

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** (number of optimal results/number of laboratories using this buffer).

**Ready-To-Use antibodies and corresponding systems**
rmAb clone **EP281**, product no. **760-6075**, Ventana/Roche, BenchMark XT/Ultra:
Protocols with optimal results were typically based on HIER using CC1, efficient heating time 24-48 min. and 16-28 min. incubation of the primary Ab. OptiView (760-700) was used as detection system. Using these protocol settings, 17 of 18 (94%) laboratories produced a sufficient staining result (optimal or good).

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. 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. **Proportion of sufficient and optimal results for SATB2 for the most commonly used RTU IHC systems**

| RTU systems | **Vendor recommended protocol settings*** | | **Laboratory modified protocol settings**** | |
|---|---|---|---|---|
| | Sufficient | Optimal | Sufficient | Optimal |
| Ventana Benchmark rmAb clone **EP281, 760-6075** | 4/4 | 2/4 | 20/23 (87%) | 11/23 (48%) |

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

**Comments**
In this assessment and in concordance with the previous NordiQC assessment of SATB2, the prevalent feature of an insufficient result was generally too weak/false negative staining reaction of cells expected to be demonstrated seen in 86% of the insufficient results (37 of 43). The majority of all laboratories were able to stain SATB2 in epithelial cells of the appendix and neoplastic cells of the colon adenocarcinoma, whereas demonstration of SATB2 in germ cells (primarily spermatocytes) in seminiferous tubules of the testis and neoplastic cells of the rectosigmoid neuroendocrine tumour was more challenging and required a carefully calibrated protocol. In 14% (6 of 43) of the insufficient results, a poor signal-to-noise ratio and/or false positive staining reaction was observed.

64% (111 of 173) of the laboratories used Abs as concentrated format within laboratory developed (LD) tests for SATB2. The rmAb clone EP281 was the most widely used clone, being applied by 83% (92 of 111) with a pass rate of 83%, 52% optimal (see Table 1). Optimal results could be obtained on the fully automated systems on the three main platforms from Dako/Agilent, Ventana/Roche and Leica Biosystems (see Table 2). No optimal staining results were obtained on the Autostainer (Dako/Agilent), despite using "optimal protocol settings", and a relatively low pass rate of 50% (4 of 8) was seen. The main prerequisites for a sufficient staining with rmAb clone EP281 were efficient HIER in an alkaline buffer, careful calibration of the titre of the primary Ab and the use of a sensitive detection system, preferably a 3-step polymer/multimer based detection system. The proportion of sufficient staining results for the use of 3-step polymer/multimer based detection systems and 2-step polymer/multimer based systems was 87% and 70%, respectively. Two laboratories used dual linker (4-step detection) on the Dako Omnis, both with optimal results.

In concordance with the previous NordiQC SATB2 assessment, less successful results were obtained when using either polyclonal Abs or mAb clone SATBA4B10, providing a total pass rate of 18% (2 of 11) with none optimal.
Although the number of protocols submitted by the participants was low, it seems unlikely that extensive protocol optimization can improve the staining reaction to an acceptable level comparable to the result obtained by the rmAb EP281. The combination of both a reduced analytical sensitivity providing false

negative results as seen in this run, and supported by the results from run 58 also giving a coexisting aberrant false positive reaction patterns, complicates the optimization process. Thus, laboratories are recommended to substitute these unsuccessful antibodies with more robust antibody as the rmAb EP281. Ready-To-Use (RTU) antibodies were used by 36% (62 of 173) of the laboratories. The Ventana/Roche RTU system based on rmAb clone EP281, 760-6075, was the most widely used RTU system with a total pass rate of 89% (24 of 27). Four laboratories used the recommended protocol settings, all with sufficient staining results (see Table 3). The majority of participants modified the protocol settings (typically minor adjustments of HIER time and/or incubation time of the primary Ab), with a pass rate of 87%, 48% optimal (see Table 3).

The rmAb clone EP281 based RTU Ab from Cell Marque, 384R-17/18 provided sufficient staining results on both BenchMark (Ventana/Roche), Omnis (Dako/Agilent) and Bond (Leica Biosystems) platforms with a total pass rate of 89%, 47% optimal (see Table 1). The main prerequisites for a sufficient staining were efficient HIER in an alkaline buffer and use of a 3-step polymer/multimer based detection system.

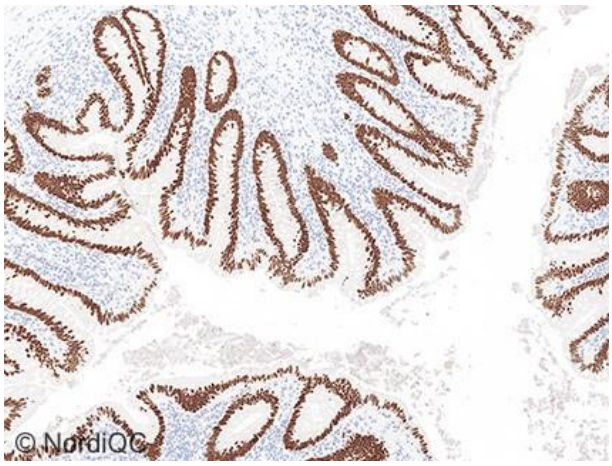



Fig. 1a
Optimal staining result for SATB2 of the appendix using the Ventana/Roche RTU system based on the rmAb clone EP281.
The protocol was performed in compliance with the protocol settings recommend by Ventana using HIER in CC1 for 32 min., 16 min. incubation in primary Ab and the 3-step OptiView as detection system and applied on BenchMark Ultra - same protocol used in Figs. 2a – 6a.
Virtually all epithelial cells show a distinct and strong nuclear staining reaction.

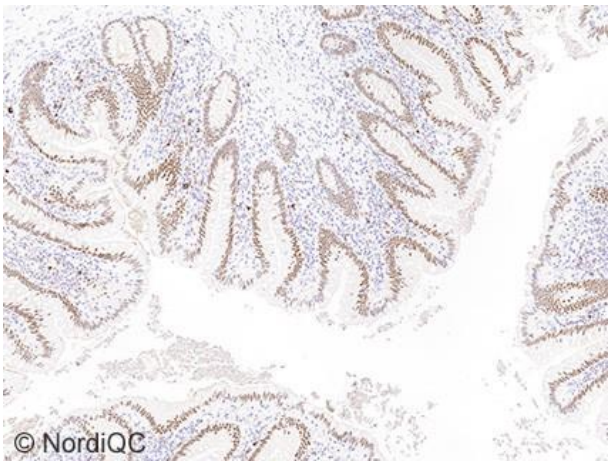



Fig. 1b
Insufficient staining for SATB2 of the appendix using the rmAb EP281 as a concentrate (1:50), HIER TRS pH 9 (3-in-1) and EnVision Flex (Dako/Agilent) as detection system on Autostainer (Dako/Agilent) - same protocol used in Figs. 2b – 5b.
The protocol provided too low analytical sensitivity due to the use of a less sensitive 2-step detection system - compare with Fig. 1a.

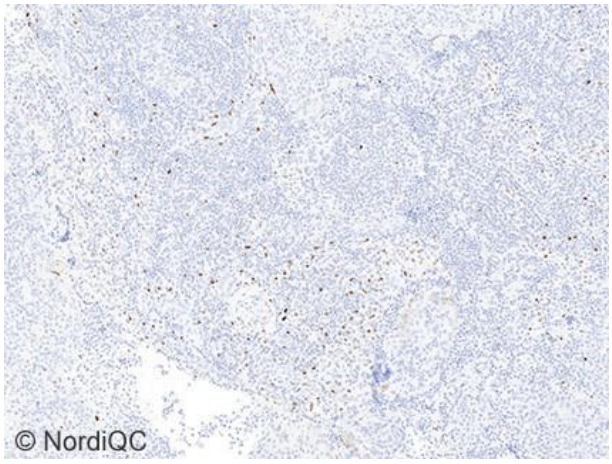



Fig. 2a
Optimal staining for SATB2 of the tonsil using same protocol as in Fig. 1a. A subset of lymphocytes, primarily situated in the T-zones, display a weak to moderate but distinct nuclear staining reaction.

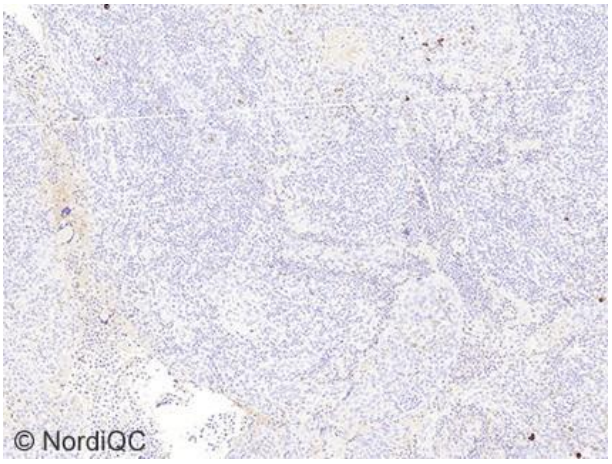



Fig. 2b
Insufficient staining for SATB2 of the tonsil using same protocol as in Fig. 1b. The staining intensity is too weak, and proportion of positive lymphocytes is significantly reduced - compare with Fig. 2a.

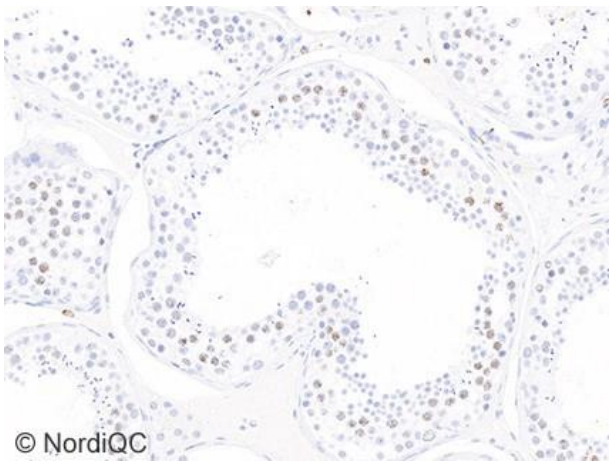



Fig. 3a
Optimal staining for SATB2 of testis using same protocol as in Figs. 1a and 2a. Dispersed germ cells, primarily spermatocytes, in seminiferous tubules display a weak to moderate nuclear staining reaction, whereas the majority of spermatogonia (basal compartment) are negative or only faintly demonstrated.

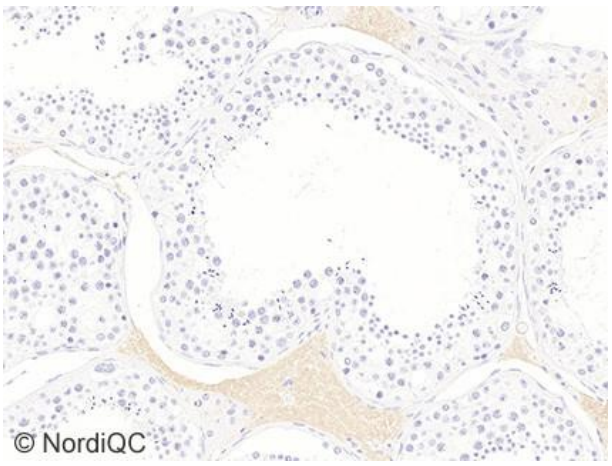



Fig. 3b
Insufficient staining for SATB2 of testis using same protocol as in Figs. 1b and 2b. The germ cells are false negative or only show faint nuclear staining reaction - compare with Fig. 3a.

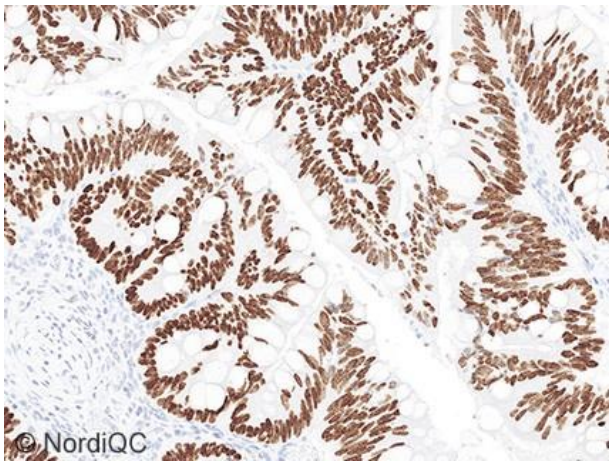



Fig. 4a
Optimal staining for SATB2 of the colon adenocarcinoma, tissue core 4, using same protocol as in Figs. 1a - 3a. The vast majority of neoplastic cells display a moderate to strong nuclear staining reaction.

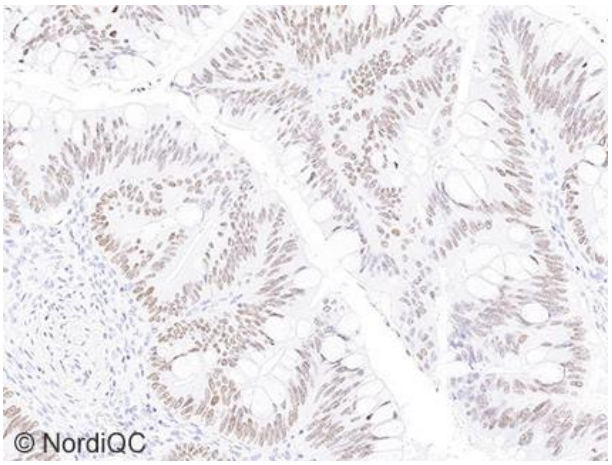
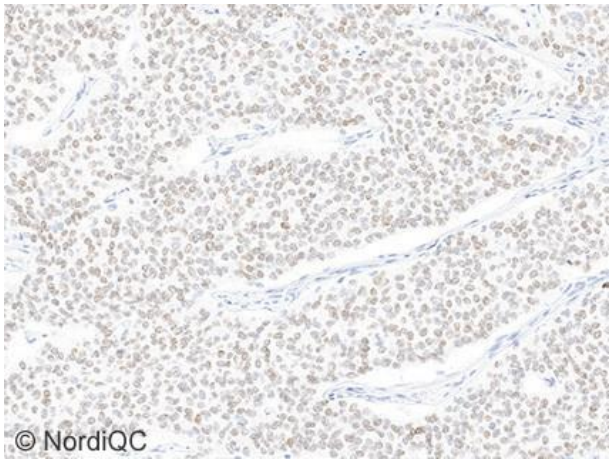



Fig. 4b
Staining for SATB2 of the colon adenocarcinoma, tissue core 4, using same insufficient protocol as in Figs. 1b -3b. The intensity of positive neoplastic cells is significantly reduced - compare with Fig. 4a.



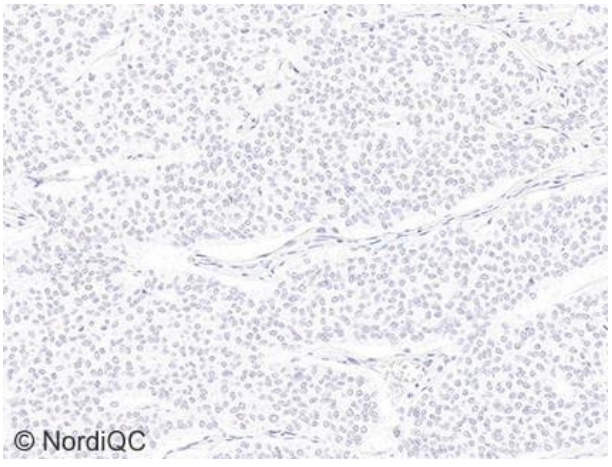



Fig. 5a
Optimal staining for SATB2 of the rectosigmoid neuroendocrine tumour using same protocol as in Figs. 1a - 4a. Virtually all neoplastic cells show a weak to moderate, but distinct nuclear staining reaction.

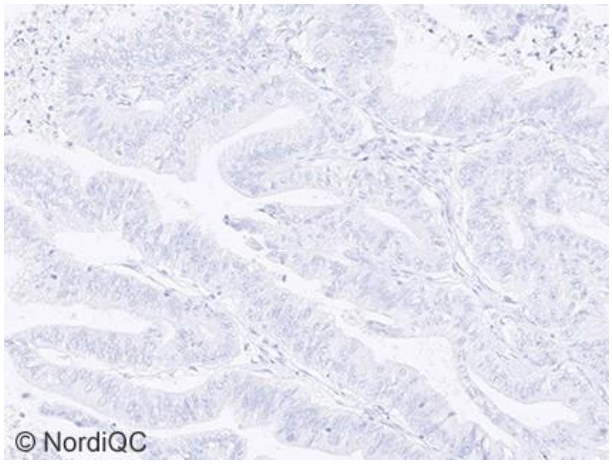



Fig. 5b
Insufficient staining for SATB2 of the rectosigmoid neuroendocrine tumour using same protocol as in Figs.1b - 4b. A significant proportion of neoplastic cells are completely negative or only faintly demonstrated - compare with Fig. 5a.

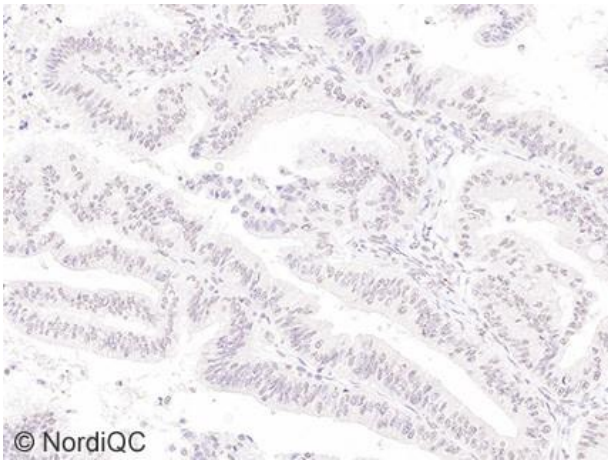



Fig. 6a
Optimal staining for SATB2 of the ovarian mucinous adenocarcinoma using same protocol as in Figs. 1a - 5a. No staining reaction is observed.

Fig. 6b
Insufficient staining for SATB2 of the ovarian mucinous adenocarcinoma using the rmAb EP281 as a concentrate (1:25), HIER TRS pH 9 (3-in-1) and EnVision Flex+ (Dako/Agilent) as detection system. A weak nuclear staining reaction is seen in neoplastic cells, expected to be negative - compare with Fig. 6a. In general, excessive background staining was seen in various tissue cores when using this protocol.

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