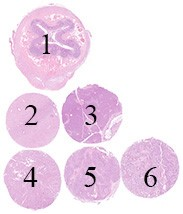
Assessment Run 75 2025

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 to represent 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. Colon neuroendocrine neoplasia, 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.
- 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.
- A strong, distinct nuclear staining reaction of virtually all neoplastic cells of the colon adenocarcinoma tissue core 4.
- An at least moderate, distinct nuclear staining reaction of all neoplastic cells in the colon neuroendocrine neoplasia, tissue core 5.
- 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.

KEY POINTS FOR SATB2 IMMUNOASSAYS
- The number of participants increased with 59% compared to run 64, 2022.
- The rmAb clone **EP281** was used by 87% of all participants.
- The SATB2 **RTU** system from Ventana/Roche applied by vendor recommended protocol settings was most successful giving a pass rate of 89%.
- It was important to select a short primary Ab incubation time for the Ventana/Roche RTU system to avoid excessive background.
- For some of the RTU formats of rmAb clone **EP281** sold by Cell Marque and Ventana/Roche, an unexpected and extensive reaction in smooth muscles was observed most likely being lot related. Submitted slides with this reaction was downgraded.
- For the concentrated format of rmAb clone **EP281** a 3-layer detection system should be used for optimal performance.

Participation

Number of laboratories registered for SATB2, run 75	310
Number of laboratories returning slides	272 (88%)

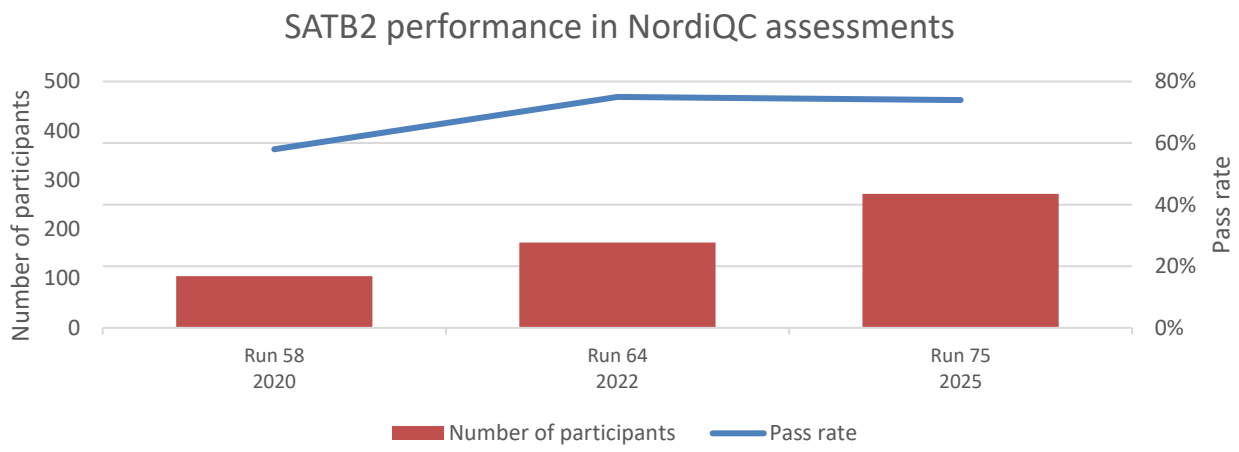
Results
At the date of assessment, 88% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

272 laboratories participated in this assessment and 74% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 3 and 4).

The most frequent causes of insufficient staining were:
- Inefficient HIER – too short time.
- Too low concentration of the primary antibody or too short incubation time.
- Less sensitive detection systems used in combination with other low sensitivity protocol parameters.
- The use of less successful antibodies.
- Lot-to-lot variation of the rmAb clone EP281

Performance history
This was the third NordiQC assessment of SATB2. A significant increase in the number of participants was observed compared to previous runs (see Graph 1), but the pass-rate was maintained at 74% (see Table 1).

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



Control
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.

Conclusion
The rmAb clones **EP281**, **SP281** and **QR023** could provide optimal results for the demonstration of SATB2. In total, 87% (236/272) of all protocols were based on the rmAb clone EP281. 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**, **CL0276** and the rmAb clone **ZR169** produced inferior results.
The Ventana/Roche RTU system **760-6075** (based on the rmAb clone **EP281**) using vendor recommended protocol settings provided the highest pass-rate of 89%, 68% being optimal. The Ventana/Roche RTU system on the intended platform provided optimal results using both 2- and 3-layer detection systems. Prolonging antibody incubation time increased the risk of producing results with poor signal-to-noise ratio or excessive background. An unexpected positive staining reaction of the smooth muscles was observed by some protocols performed by reported optimal protocol settings using RTU formats of the rmAb clone EP281 indicating lot-to-lot variations of this product.

Table 1a. **Overall results for SATB2, run 75**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	112	41	35	35	1	68%	37%
Ready-To-Use antibodies	160	54	71	35	-	78%	34%
Total	272	95	106	70	1		
Proportion		35%	39%	26%	0,4%	74%	

1) Proportion of sufficient results (optimal or good).
2) Proportion of optimal results.

Table 1b. **Concentrated antibodies and assessment marks for SATB2, run 75**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **SATBA4B10**	2 2 1	Zytomed Santa Cruz Abcam	-	-	5	-	-	-
mAb clone **CL0276**	1	Novus	-	-	1	-	-	-
mAb clone **OTI5H7**	1	ZSGB-Bio	-	1	-	-	-	-
rmAb clone **EP281**	70 5 3 2 1 1 1	Cell Marque Bio SB BioCare Medical Nordic Biosite Master Diagnostica PathNSitu Zeta Corporations	39	28	16	-	81%	47%
rmAb clone **SP281**	3	Abcam	1	1	1	-	-	-
rmAb clone **QR023**	5	Quartett	1	3	1	-	80%	20%
rmAb clone **ZR167**	9	Zeta Corporations	-	-	9	-	-	-
rmAb clone **EPNCIR130A**	2	Abcam	-	1	1	-	-	-
pAb clone **HPA001042**	2	Sigma-Aldrich	-	1	1	-	-	-
Clone **GR19**	1	GeneBioSolutions	-	-	-	1	-	-
Total	112		41	35	35	1		
Proportion			37%	31%	31%	1%	68%	

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

Table 1c. **Ready-To-Use antibodies and assessment marks for SATB2, run 75**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone **EP281 760-6075 (VRPS)[3]**	28	Ventana/Roche	19	6	3	-	89%	68%
mAb clone **EP281 760-6075 (LMPS)[4]**	53	Ventana/Roche	17	25	11	-	79%	32%
rmAb clone **EP281 384R-17/18**	47	Cell Marque	14	27	6	-	87%	30%
rmAb clone **EP281 API3225**	5	Biocare Medical	-	3	2	-	60%	-
rmAb clone **EP281 BSB319 7/8/9**	7	BioSB	2	1	4	-	43%	29%
rmAb clone **EP281 CSR-0140**	1	Celnovte	-	1	-	-	-	-
mAb clone **SATBA4B10 MSK101-05**	1	Zytomed systems	-	-	1	-	-	-
rmAb clone **EP281 Z2321RP**	1	Zeta Corporation	-	-	1	-	-	-
rmAb clone **ZR167**	1	Zeta Corporation	-	1	-	-	-	-
rmAb clone **EP281 PR239**	1	PathNSitu	1	-	-	-	-	-
rmAb clone **EP281 RMPD112**	1	Diagnostic Biosystems	-	1	-	-	-	-
rmAb clone **EP281 MAD-000747QD**	9	Master Diagnostica	1	3	5	-	44%	11%
rmAb clone **EP281 RMA-0750**	1	Fuzhou Maixin	-	1	-	-	-	-
rmAb clone **EP281 GT229402**	1	GeneTech	-	-	1	-	-	-
rmAb clone **IHC095 IHC095**	1	GenomeMe	-	1	-	-	-	-
rmAb **QR023 P-S002-30**	1	Quartett	-	1	-	-	-	-
Clone **DY49971 4912112**	1	Dakewe	-	-	1	-	-	-
Total	160		54	71	35	-		
Proportion			34%	44%	22%	0%	78%	

1) Proportion of sufficient results (optimal or good). (≥5 assessed protocols).
2) Proportion of optimal results (OR).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 assessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 assessed protocols)

Detailed analysis of SATB2, Run 75

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

rmAb clone **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) (27/45)* or Target Retrieval Solution (TRS) High pH (Dako/Agilent) (11/20) 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, 52 of 61 (85%) 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 four main IHC systems***

Concentrated antibody	**Dako/Agilent Autostainer[1]**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark[2]**		**Leica Biosystems Bond[3]**	
	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**	0/2**	-	12/21 (57%)	-	27/45 (60%)	-	0/11	0/1

* 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)
1) Autostainer Classical, Link 48.
2) BenchMark GX, XT, Ultra, Ultra Plus
3) Bond III, Prime, Max

Ready-To-Use antibodies and corresponding systems

rmAb clone **EP281**, product no. **760-6075**, Ventana/Roche, BenchMark GX/XT/Ultra/Ultra Plus: Protocols with optimal results were typically based on HIER using CC1, efficient heating time 32-64 min. and 8-32 min. incubation of the primary Ab. UltraView (760-500) with amplification or OptiView (760-700) was used as detection system. Using these protocol settings, 47 of 50 (94%) laboratories produced a sufficient staining result (optimal or good).
**2 laboratories used product no. 760-6075 for staining on another platform. Data was not included in the description above*

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 according 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	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Ventana BenchMark[1] rmAb **EP281 760-6075**	89% (25/28)	68% (19/28)	78% (40/51)	33% (17/51)

* 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, detection kit – only protocols performed on the specified vendor IHC stainer are integrated.
1) BenchMark GX, XT, Ultra, Ultra Plus

Comments

In this assessment and in concordance with the previous NordiQC assessment of SATB2, the prevalent feature of an insufficient result was a generally too weak/false negative staining reaction of cells expected to be demonstrated and seen in 79% of the insufficient results (56 of 71). In addition, poor signal-to-noise ratio was seen in 7 insufficient cases and an unspecific aberrant cytoplasmic reaction was observed in 11 cases in the ovarian mucinous carcinoma which was expected to be negative both in the cytoplasmic and nuclear compartment of the neoplastic cells. The majority of all laboratories were able to stain SATB2 in epithelial cells of the appendix and neoplastic cells of both the colon adenocarcinoma and neuroendocrine neoplasia, whereas demonstration of SATB2 in germ cells (primarily spermatocytes) in seminiferous tubules of the testis was more challenging and required a carefully calibrated protocol. In this assessment, no tumours with low level SATB2 expression were included. The assessment marks were thus based on the general performance observed in all tissue cores and with special attention to the demonstration of the critical and low-level expression of SATB2 in the testicular gem cells. Testis has in the two previous assessment runs shown to be valuable as iCAPC (immunohistochemical critical assay performance control) to evaluate the level of analytical sensitivity of the IHC assay for SATB2.
41% (112 of 272) 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 74% (83 of 112) with a pass rate of 81%, 47% optimal (see Table 1b). Optimal results were obtained on two of the main IHC platforms from Dako/Agilent and Ventana/Roche (see Table 2). On the Ventana Benchmark systems a pass-rate of 92% (34 of 37), 70% optimal was observed when OptiView was used as detection system in combination with EP281 as concentrate. Similar performance was observed when the concentrate was applied on the Dako Omnis with Envision FLEX+ or FLEX++ as detection system providing a pass rate of 95% (19 of 20), 60% optimal. Optimal results were mainly achieved using Ab incubation time for 30-32 minutes and HIER in an alkaline buffer for 30-32 minutes on both platforms. The mean dilution of the primary Ab was around 1:130 depending on the protocol applied. If the rmAb clone EP281 as concentrate was applied with a 2-layer detection system on these two platforms a pass-rate of 46% (6 of 13) was provided, indicating that a 3-layer detection system was preferable.

No optimal staining results were obtained on the Autostainer (Dako/Agilent) or the Bond systems (Leica Biosystem). Only two participants stained SATB2 on the Autostainer system, one without using linker and one with a too high concentration of the primary antibody compared with other optimal protocols. On the Leica Biosystem platforms a pass-rate of 67% was observed which is significantly lower compared to the Dako Omnis or Ventana Benchmark systems. For Bond platforms it should be taken into account that the Bond Refine detection system will only act as a 2-layer detection system, when used with rabbit primary antibodies as the post primary linker is a reagent only amplifying the signal for mouse primary antibodies. Analyzing the data provided by participants using the rmAb clone EP281 on the Bond platforms, it seems to be a very difficult task to produce optimal results even with very sensitive protocol settings.

The rmAb clone **ZR167** was used by 9 laboratories compared to the latest assessment run 64 only one laboratory submitted a protocol based on this Ab. The clone was used on three of the fully automated main IHC instruments but none provided a sufficient result. Protocols were used within otherwise optimal conditions with highly sensitive protocol settings but without reaching the level of analytical sensitivity expected. In general, a weak staining intensity was observed in all structures expected to be moderately to strongly labelled and of central importance none of the protocols were able to stain the dispersed germ cells of the testis.

The mAb clone **SATBA4B10** was used by 5 laboratories in this assessment. In concordance with previous runs, this Ab did not produce any sufficient protocols and provided a high number of false positive results as the resting B-cells of the mantle zone and interfollicular lymphocytes displayed a distinct positive nuclear reaction and were therefore downgraded to insufficient, even though the clone was able to stain other cores sufficiently (see fig. 5b). This staining pattern was also observed in mAb **CL0276** clone. The low number of protocols submitted by the participants using these two clones provided limited data, however 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. In both previous NordiQC runs 58 and 64 for SATB2, the mAb clone SATBA4B10 and CL0276 have been less successful giving both false positive and false negative results especially in the neuroendocrine tumours, which supports the observations generated in this run. Thus, laboratories are recommended to substitute these unsuccessful antibodies with a more robust antibody as the rmAb clone EP281.

Ready-To-Use (RTU) antibodies were used by 59% (160 of 272) of the laboratories which is a big increase compared to the latest assessment run 64 where only 36% were using RTU systems.

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 83% (67 of 81). 28 laboratories used the recommended protocol settings, with a pass rate of 89%, 68% optimal (see Table 3). Recommended protocols were available for both UltraView and OptiView with similar settings using CC1 for 32 min. and an antibody incubation time for 16 min. Using the RTU system both 2- and 3-layer systems provided a high pass-rate contrary to the concentrated format of EP281, where 3-layer systems were preferable. The RTU product in general seemed to be based on a high concentration of the primary Ab and for laboratory developed assays increasing the Ab incubation time for more than 24 min. could cause problems with an excessive background reaction, whereas increasing HIER time did not seem to have the same impact on the staining result. There is reason to believe that some lot numbers were more problematic than others as identical protocol settings could provide very different results. It was observed that some protocols (n=8) gave a strong background reaction along with a moderate cytoplasmic staining reaction of the smooth muscle cells lining the tubules of the testis (see figs. 6a and 6b) and in the lamina muscularis propria of the appendix. During the data analysis a specific lot number was not identified as the cause of the problem, but the lack of inter-protocol reproducibility among the laboratories using same settings do indicate lot-to-lot variation. If the aberrant staining reaction was extensive, the assessment mark was downgraded according to the severeness of the reaction, see Figs. 6a and 6b.

The rmAb clone **EP281** based RTU Ab from Cell Marque, **384R-17/18** provided sufficient staining results on BenchMark (Ventana/Roche), Omnis (Dako/Agilent) and Bond (Leica Biosystems) platforms with a total pass rate of 87% (41 of 47), 30% optimal (see Table 1c). The performance of the Cell Marque product was on the Ventana platform very similar to the EP281, 760-6075 sold by Ventana/Roche and same protocol settings could be applied for a sufficient result, however optimal results were only obtained using OptiView as detection systems. 15 laboratories applied the RTU product on the Dako Omnis with a pass rate of 93% (14 of 15), but only 20% optimal. Optimal results were achieved using the 3-layer polymer EnVision Flex+ system, both TRS high and Ab incubation for 30 minutes. On the Bond (Leica Biosystems) platform no optimal results were obtained, however using BERS2 as retrieval buffer all 3 protocols produced a sufficient result.

On all three fully automated platforms, staining of the smooth muscle of the testis that was mentioned for the Ventana RTU product was observed in 5 cases using different lot numbers and different detection systems. The cause of this reaction was therefore more likely to be related to the RTU product than a reaction caused by other factors related to a specific instrument.

This was the third assessment for SATB2 in NordiQC (see Graph 1). The pass rate in this assessment was very similar to the previous run 64 even although there was a big increase in the number of new participants (59% increase). Most of the new participants were using RTU systems, however within the four main IHC systems only Ventana/Roche provided a Ready-to-use product for the intended platforms. The rmAb clone EP281 both as a concentrate and as an RTU-product was the most popular clone used by a total of 87% (236/272) laboratories. The clone seemed to be a robust choice and could provide optimal results on 2 of the fully automated platforms (Omnis, Dako/Agilent and Benchmark, Ventana/Roche). For laboratories using the Dako Autostainer (Dako/Agilent) or the Bond platforms (Leica Biosystem) no optimal results were observed in this assessment challenging the option to identify recommended protocol settings for these platforms. However, for the Bond platforms optimal results were produced in earlier runs using the EP281 clone as a concentrate and careful calibration and validation of the protocols with the use of appropriate controls and neoplastic tissues should be a good fundament to establish a well working IHC assay for SATB2.

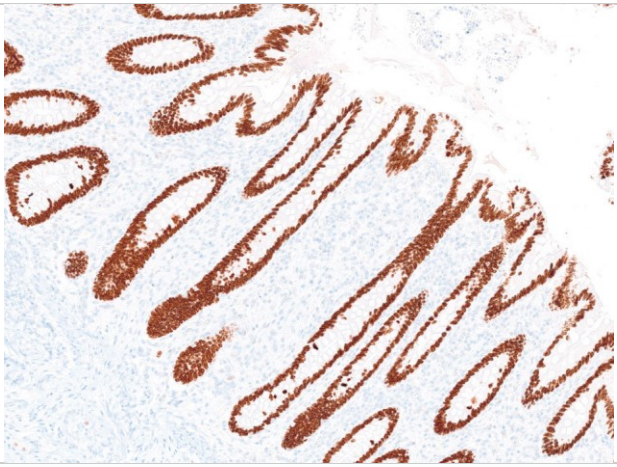


Fig. 1a (x100)
Optimal staining result for SATB2 of the appendix using the rmAb clone EP281 as a concentrate (1:100), using HIER in CC1 for 48 min., 32 min. incubation in primary Ab and the 3-step OptiView as detection system and applied on BenchMark Ultra - same protocol used in Figs. 2a–5a. Virtually all epithelial cells show a distinct and strong nuclear staining reaction.

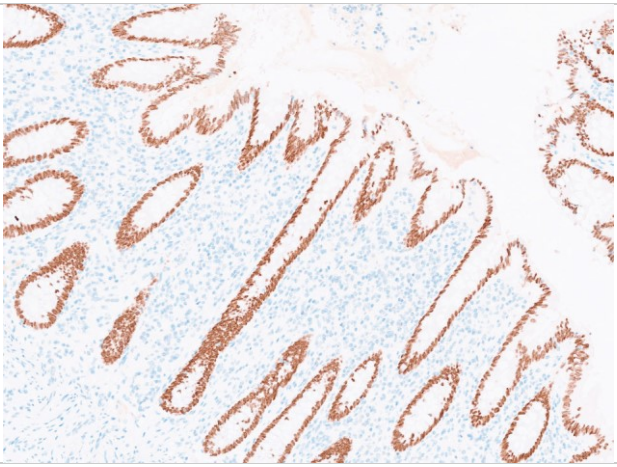


Fig. 1b (x100)
Staining result for SATB2 of the appendix using the rmAb clone EP281 as a concentrate (1:100), using HIER in CC1 for 36 min., 32 min. incubation in primary Ab and the 2-step UltraView as detection system and applied on BenchMark Ultra - same protocol used in Figs. 2b–4b.
The protocol provided an overall too low analytical sensitivity due to the use of a less sensitive 2-step detection system - compare with Fig. 1a – same field and most importantly Fig. 2b with a too weak staining reaction in germ cells of testis being identified as critical control in previous assessment runs for SATB2.

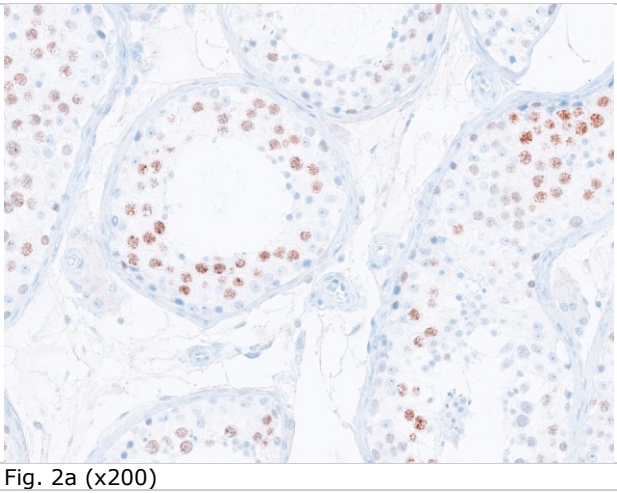


Fig. 2a (x200)

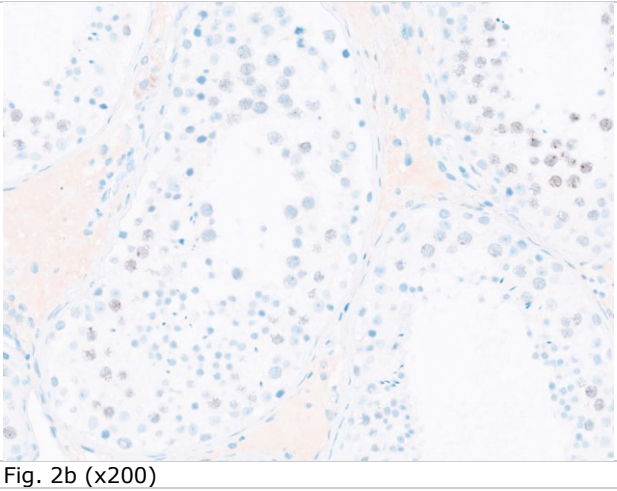


Fig. 2b (x200)

Optimal staining reaction for SATB2 of testis using same protocol as in Fig. 1a. 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 oronly faintly demonstrated.	Insufficient staining reaction for SATB2 of testis using same protocol as in Fig. 1b. The germ cells are false negative or only show faint nuclear staining reaction - compare with Fig. 2a. Testis has in the two previous assessment runs shown to be valuable as iCAPC (immunohistochemical critical assay performance control) to evaluate the level of analytical sensitivity of the IHC assay for SATB2.

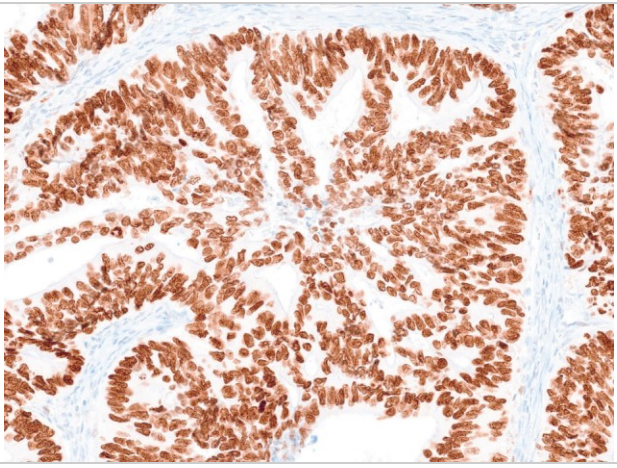
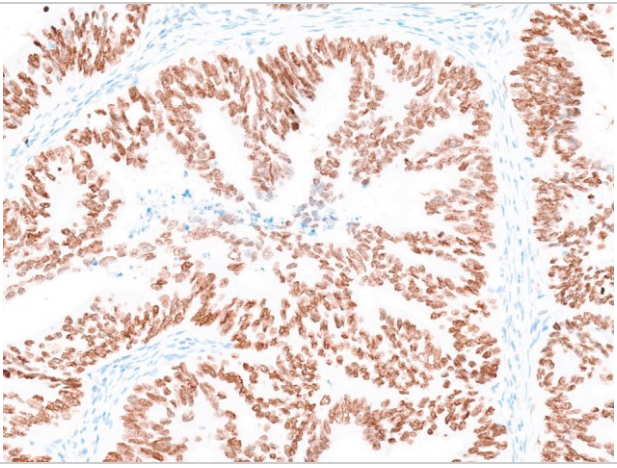

	

Fig. 3a (x200) Optimal staining reaction for SATB2 of the colon adenocarcinoma, tissue core no. 4, using same protocol as in Figs. 1a-2a. The vast majority of neoplastic cells display a moderate to strong nuclear staining reaction.	Fig. 3b (x200) Staining reaction for SATB2 of the colon adenocarcinoma, tissue core no. 4, using same insufficient protocol as in Figs. 1b-2b. The intensity of positive neoplastic cells is reduced - compare with Fig. 3a – same field.

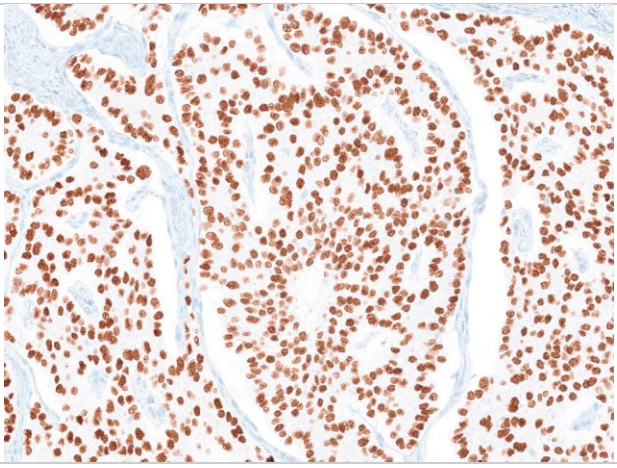
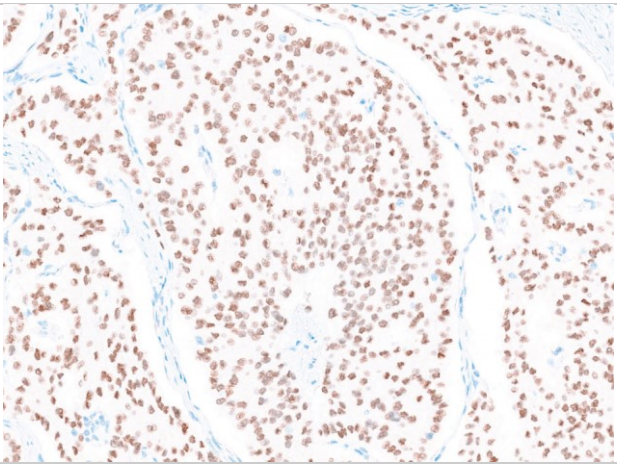

	

Fig. 4a (x200) Optimal staining reaction for SATB2 of the colon neuroendocrine neoplasia, tissue core no. 5, using same protocol as in Figs. 1a-3a. Virtually all neoplastic cells show a weak to moderate, but distinct nuclear staining reaction.	Fig. 4b (x200) Staining reaction for SATB2 of the colon neuroendocrine neoplasia, tissue core no. 5, using same protocol as in Figs.1b - 3b. A significant proportion of neoplastic cells express a reduced positivity - compare with Fig. 4a – same field.

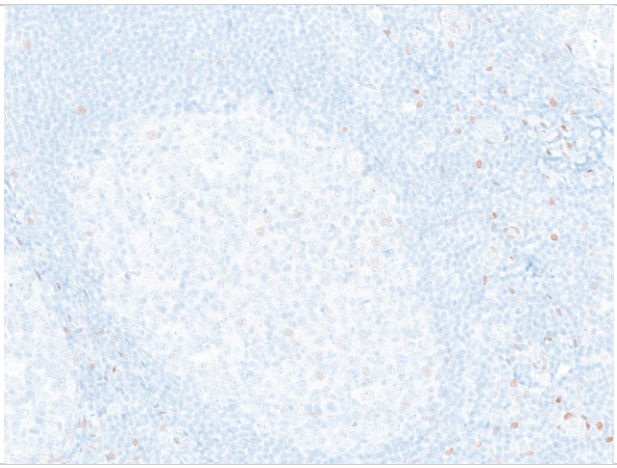


Fig. 5a (x200)
Optimal staining reaction for SATB2 of the tonsil using same protocol as in Figs. 1a-4a.
A subset of lymphocytes, primarily situated in the T-zones, display a weak to moderate but distinct nuclear staining reaction.

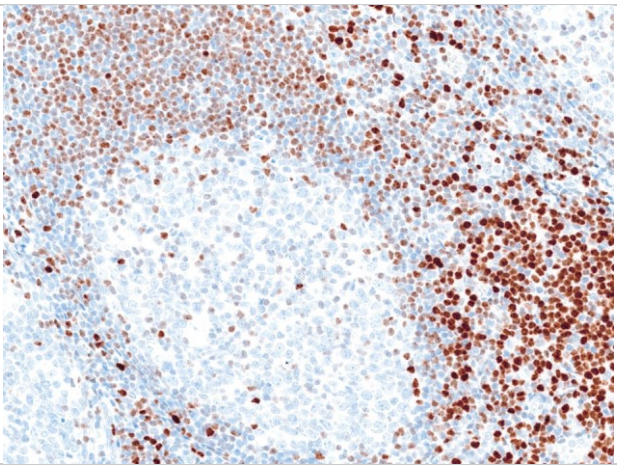


Fig. 5b (x200)
Insufficient staining result for SATB2 of the appendix using the mAb SATBA4B10 as a concentrate (1:250), using HIER in CC1 for 48 min., 16 min. incubation in primary Ab and the 3-step OptiView as detection system and applied on BenchMark Ultra.
A false positive staining reaction of the vast majority of mantle zone B-cells and of interfollicular lymphocytes is seen - compare with optimal result in Fig. 5a. – same field.

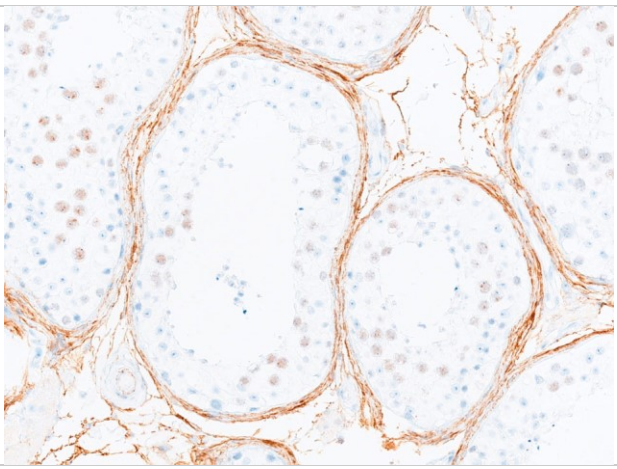


Fig. 6a (x200)
Insufficient staining result for SATB2 of the testis using the Ventana/Roche RTU system based on the rmAb clone EP281. The protocol was performed using HIER in CC1 for 48 min., 36 min. incubation in primary Ab and the 2-step UltraView as detection system and applied on BenchMark Ultra - same protocol used in Fig. 6b.
The germ cells only show a faint nuclear staining reaction whereas the smooth muscles lining the seminiferous tubules show a moderate cytoplasmic staining reaction – compare with Figs. 2a+2b.

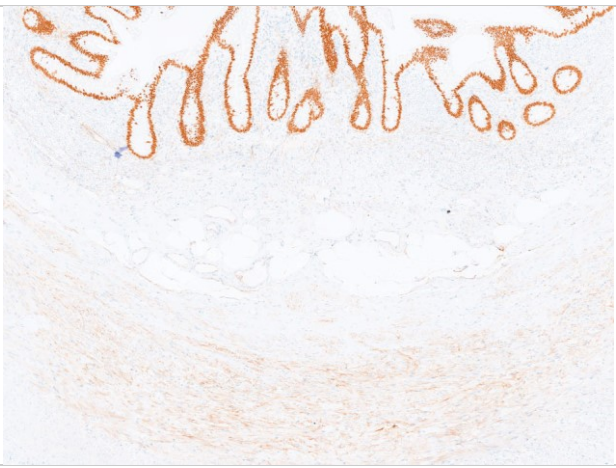


Fig. 6b (x50)
Insufficient staining result for SATB2 of the appendix using same protocol as in Fig. 6a.
Virtually all epithelial cells show a distinct but only moderate nuclear staining reaction and at the same time, the smooth muscle cells in muscularis propria is displaying a weak but distinct aberrant cytoplasmic staining reaction.

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