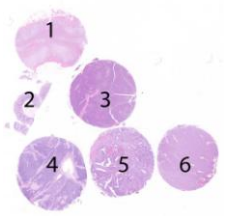
Assessment Run 43 2015

Synaptophysin (SYP)

Material
The slide to be stained for SYP comprised:



1. Adrenal gland, 2. Colon, 3. Pancreas, 4. Small cell lung carcinoma, 5. Colon adenocarcinoma, 6. Intestinal neuroendocrine tumour

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing SYP staining as optimal included:

- A strong, distinct cytoplasmic staining reaction of virtually all endocrine islet cells in the pancreas.
- A moderate to strong, distinct cytoplasmic staining reaction of neuroendocrine cells, ganglion cells and axons of the nerve plexus in the colon
- A moderate to strong, distinct cytoplasmic, dot-like staining reaction of the majority of cortical epithelial cells of the adrenal gland.
- A weak to moderate staining of the majority of goblet cells in the colon mucosa
- An at least moderate, distinct, cytoplasmic staining reaction of the majority of neoplastic cells of the small cell lung carcinoma, and the intestinal neuroendocrine tumour.
- No staining of neoplastic cells in the colon adenocarcinoma.

A weak cytoplasmic staining reaction of the exocrine pancreatic epithelial cells was accepted.

Participation

Number of laboratories registered for SYP, run 43	258
Number of laboratories returning slides	243 (94%)

Results
243 laboratories participated in this assessment. 200 (82%) of these achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:
- HIER in a non-alkaline buffer
- Too low concentration of the primary antibody
- Use of less sensitive and specific detection systems

Performance history
This was the fifth NordiQC assessment of SYP. A major improvement of the pass rate was seen compared to previous runs (see table 2).

Table 2. **Proportion of sufficient results for SYP in the five NordiQC runs performed**

	Run 18 2006	Run 22 2008	Run 29 2010	Run 37 2013	Run 43 2015
Participants, n=	94	112	151	214	243
Sufficient results	68%	58%	55%	58%	82%

Conclusion
The mAb clones **27G12**, **BS15**, **DAK-SYNAP** and **Snp88** and the rmAb clones **MRQ-40** and **SP11** could all be used to obtain an optimal staining reaction for SYP. Irrespective of clone, HIER in an alkaline buffer is mandatory to give an optimal staining reaction, and concentration of the primary Ab must be carefully calibrated.
3-step polymer / multimer based detection systems provided a higher proportion of optimal results compared to 2-step and biotin-based detection systems.
mAb clone **27G12**, was the most commonly used antibody within a laboratory developed assay and provided an optimal result on all three main IHC platforms (Dako, Leica and Ventana).
Colon is at present the most recommendable positive tissue control for SYP. Nerves must show a strong staining reaction, while an at least weak but distinct cytoplasmic staining reaction must be seen in the majority of goblet cells.

Table 1. **Antibodies and assessment marks for SYP, run 43**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **27G12**	69 2 1 1	Leica/Novocastra Biocare Monosan Genetech	28	35	6	4	86%	88%
mAb clone **BS15**	1	Nordic Biosite	1	0	0	0	-	-
mAb clone **DAK-SYNAP**	12	Dako	7	3	2	0	83%	100%
mAb clone **SNP88**	7	Biogenex	2	5	0	0	100%	100%
mAb clone **SY38***	3	Dako	0	2	1	0	-	-
rmAb clone **MRQ-40**	5 1	Cell Marque Monosan	3	2	1	0	83%	100%
rmAb clone **SP11**	10 2 1 1	Thermo/Neomarkers Spring Bioscience Abcam Immunologic	7	4	3	0	79%	83%
pAb **180130**	1	Immuno Diagnostics	0	0	1	0	-	-
pAb **RB-1461**	1	Thermo/Neomarkers	0	0	1	0	-	-
pAb **RBK011**	1	Zytomed	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone **27G12 PA0299**	9	Leica/Novocastra	3	3	2	1	67%	100%
mAb clone **27G12 PM371**	1	Biocare	0	1	0	0	-	-
mAb clone **DAK-SYNAP IR660**	38	Dako	11	23	4	0	89%	90%
mAb clone **SNP88 AM363-5M**	2	Biogenex	0	1	1	0	-	-
mAb clone **SY38 IR/IS776***	5	Dako	0	2	2	1	-	-
rmAb **MRQ-40 760-4595**	31	Ventana/Cell Marque	23	7	1	0	97%	100%
rmAb clone **MRQ-40 336R**	1	Cell Marque	1	0	0	0	-	-
rmAb clone **SP11 790-4407**	33	Ventana	9	14	9	1	70%	81%
rmAb clone **SP11 KIT-0022**	1	Maixin	0	1	0	0	-	-
rmAb clone **SP11 MAD-000313QD**	2	Master Diagnostica	0	1	1	0	-	-
pAb **336A-78**	1	Cell Marque	0	1	0	0	-	-
Total	243		95	105	35	8	-	
Proportion			39%	43%	15%	3%	82%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.
* Product discontinued from vendor

Detailed analysis of SYP, Run 43

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **27G12**: Protocols with optimal results were based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (5/12)*, TRS pH 9 (Dako) (2/10), Cell Conditioning 1 (CC1; Ventana) (15/30), Bond Epitope Retrieval Solution 2 (BERS2; Leica) (4/13) or Tris-EDTA/EGTA pH 9 (2/4) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 57 of 65 (88%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **BS15**: One protocol with an optimal result was based on HIER in PT module for 20 min. using Tris-EDTA/EGTA pH 9 as retrieval buffer. The mAb was diluted 1:250 using a 2-step polymer based detection system and performed on the Autostainer, LabVision.

mAb clone **DAK-SYNAP**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (3/4), TRS pH 9 (Dako) (1/2) or CC1 (Ventana) (3/3) as retrieval buffer. The mAb was diluted in the range of 1:50-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 9 of 9 (100%) laboratories produced a sufficient staining result.

mAb clone **SNP88**: Two protocols with optimal results were both performed on the Bond platform (Leica) using HIER in BERS2 (Leica) buffer for 20 or 30 min. The mAb was diluted 1:50 or 1:100 using Bond Refine as detection system. Using these protocol settings 2 of 2 (100%) laboratories produced an optimal staining result.

rmAb clone **MRQ-40**: Protocols with optimal results were all based on HIER for 48-64 min. in CC1 (Ventana) (3/3) as retrieval buffer and performed on the BenchMark platform (Ventana). The rmAb was diluted in the range of 1:50-1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 of 3 (100%) laboratories produced an optimal staining result.

rmAb clone **SP11**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (1/2), CC1 (Ventana) (4/7) or Tris-EDTA/EGTA pH 9 (2/3) as retrieval buffer. The mAb was diluted in the range of 1:50-1:150 depending on the total sensitivity of the protocol employed. Using these protocol settings 10 of 12 (83%) laboratories produced a sufficient staining result.

Table 3. **Proportion of optimal results for SYP for the most commonly used antibody as concentrate on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **27G12**	6/13** (46%)	0/2	15/28 (54%)	-	4/11 (36%)	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)

Ready-To-Use antibodies and corresponding systems
mAb clone **27G12**, product no. **PA0299**, Leica, Bond-III:
Protocols with optimal results were all based on HIER using BERS2 pH 9 (Bond, Leica) (efficient heating time 20 min. at 99-100°C), 20-30 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings 4 of 4 (100%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **DAK-SYNAP**, product no. **IR660**, Dako, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (efficient heating time 10-20 min. at 97°C), 20 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 19 of 21 (90%) laboratories produced a sufficient staining result.

rmAb clone **MRQ-40**, product no. **760-4595**, Ventana, BenchMark XT, GX, ULTRA:
Protocols with optimal result typically based on HIER using Cell Conditioning 1 (efficient heating time 24-76 min.), 16-60 min. incubation of the primary Ab and UltraView (760-500) +/- amplification kit or OptiView (760-700) as detection system. Using these protocol settings 26 of 26 (100%) laboratories produced a sufficient staining result.

rmAb clone **SP11**, product no. **790-4407**, Ventana, BenchMark GX, ULTRA:
Protocols with optimal result typically based on HIER using Cell Conditioning 1 (efficient heating time 36-72 min.), 30-72 min. incubation of the primary Ab and UltraView (760-500) +/- amplification kit or OptiView (760-700) as detection system. Using these protocol settings 22 of 27 (81%) laboratories produced a sufficient staining result.

Comments
In concordance with the previous NordiQC assessments for SYP, the prevalent feature of an insufficient staining result was a too weak or completely false negative staining reaction of cells and structures expected to be demonstrated. Virtually all participating laboratories were able to stain SYP in cells with high-level expression as peripheral nerves, pancreatic endocrine cells and neoplastic cells of the intestinal

neuroendocrine tumour, whereas demonstration of SYP in neoplastic cells of the SCLC was more challenging and only seen when antibodies with a high sensitivity and otherwise appropriate protocol settings were applied. A too weak or false negative staining result was seen in 84% of the insufficient results (36 of 43). Too low concentration of the primary Ab and/or usage of detection systems with low sensitivity typically were the main causes for a too weak staining result. In the remaining 17% of the insufficient results poor signal-to-noise ratio or false positive staining reaction was seen. This pattern compromised interpretation and was typically caused by use of biotin based detection systems or too high concentration of the primary Ab.

Optimal staining results could be obtained with the mAbs clones 27G12, BS15, DAK-SYNAP and SNP88 and the rmAb clones MRQ-40 and SP11 (see Table 1). Irrespective of the clone applied HIER in an alkaline buffer was mandatory for optimal performance.
The mAb clone 27G12 was the most widely used antibody for demonstration of SYP. Used as a concentrate in a laboratory developed (LD) assay, mAb clone 27G12 gave an overall pass rate of 86% (63 of 73). As shown in table 3, optimal results could be obtained on all three main IHC platforms from Dako, Leica and Ventana. Previously the mAb clone 27G12 showed a less successful performance on the Ventana BenchMark platform as observed in run 37, where only 4% (1 of 26) of LD assays using this clone on the BenchMark platform provided an optimal result. In this run 54% (15 of 28) of protocols based on mAb clone 27G12 and performed on BenchMark gave an optimal result. The availability and extended use of the 3-step multimer based detection system OptiView (Ventana) seemed to be one of the central parameters for the improved performance. In this run, 13 of 30 laboratories used the mAb clone 27G12 in combination with OptiView in a LD assay. All protocols gave a sufficient result and 62% (8 of 13) were marked as optimal. Using another detection system as iView or UltraView 77% of the protocols gave a sufficient result of which 41% were evaluated as optimal. Only 4 of 38 laboratories in run 37 used OptiView as detection system by a LD assay for mAb clone 27G12 on BenchMark.
Additionally the average dilution factor for mAb clone 27G12 was increased in this run to 1:55 (range 1:25 to 100) compared to 1:85 (range 1:20-400) in run 37.
Virtually all Abs capable of providing an optimal staining performance occasionally gave an aberrant staining reaction. In some protocols, especially performed on the Dako Autostainer system, the mAb clone 27G12 provided an aberrant granular cytoplasmic staining reaction in the neoplastic cells of the colon adenocarcinoma. No single specific explanation (e.g., lot-to-lot variation, Ab titre, detection system) could be identified as the cause.
The mAb clone SNP88 occasionally gave an aberrant granular cytoplasmic staining reaction in the epithelial cells of colon, pancreatic exocrine cells and neoplastic cells of the colon adenocarcinoma. This was caused by the Mouse Ascites Golgi (MAG) reaction in blood type A tissue.
Finally the mAb clone DAK-SYNAP and the rmAb clone MRQ-40 gave a staining reaction of the colon glycocalyx and smooth muscle cells in some protocols.
The different aberrant staining reactions were accepted providing less than 30% of the cells were labelled (in accordance with the WHO guidelines for carcinoma classification).

The corresponding Ready-To-Use (RTU) systems from Dako, Leica and Ventana provided similar pass rates and proportion of optimal results compared to LD assays.
The Ventana RTU system based on the rmAb clone MRQ-40 was the most successful RTU system giving the highest overall pass rate and proportion of optimal results.
Optimal results for the RTU formats were typically obtained by usage of official protocol recommendations given by the respective companies. Laboratory modified protocol settings (typically adjusting HIER, incubation time of the primary Ab and/or choice of detection system) could also provide sufficient and optimal results.

In this run a pass rate of 82% was seen, which was an improvement compared to 58% in run 37, 2013. It is difficult to conclude on the specific causes for the improvement of the pass rate obtained in this run, as many laboratories participated for the first time. However extended use of high sensitive 3-step polymer / multimer detection systems and reduced use of the less successful mAb clone SY38 seems to be the central issues. In run 37 15% of the participants used the mAb clone SY38 compared to 3% in this run. No optimal results were obtained by mAb clone SY38 in the two runs.

Controls
It is difficult to identify a reliable and robust positive tissue control for SYP. At present the best recommendation is still to use colon or appendix as control and to calibrate the protocol to give an intense staining reaction of the axons of the Auerbach's and Meissner's plexus with a high-level expression of SYP. The endocrine cells of the mucosa must show an at least moderate staining reaction and importantly the majority of goblet cells in the mucosa must show an at least weak to moderate cytoplasmic staining reaction. The reason for the staining reaction of goblet cells in the colon mucosa is still not known.

However, as all Abs could give this staining pattern and these cells only show a weak to moderate staining reaction, the capability to obtain a positive SYP reaction in the goblet cells is thus superior to nerves as critical staining quality indicator for SYP. No staining must be seen in the vast majority of smooth muscle cells.

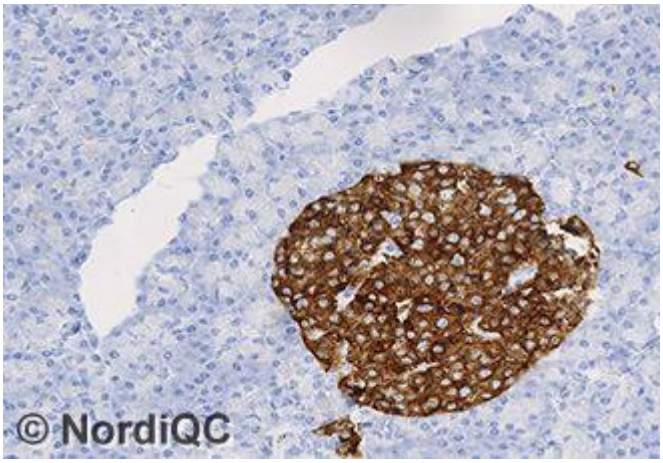


Fig. 1a
Optimal SYP staining of the pancreas using the mAb clone 27G12, optimally calibrated, HIER in an alkaline buffer and a 3-step multimer based detection system. Virtually all endocrine islet cells show a strong and distinct cytoplasmic staining reaction and a high signal-to-noise ratio is observed. Also compare with Figs. 2a - 5a – same protocol.

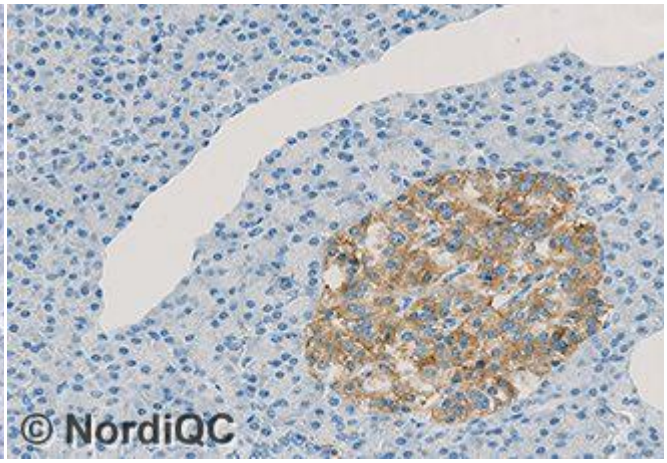


Fig. 1b
SYP staining of the pancreas using an insufficient protocol giving a too low sensitivity – same field as in Fig. 1a. The protocol was based on the rmAb clone SP11, HIER in an alkaline buffer. However the combination of a too low concentration of the primary Ab and use of a less sensitive 2-step multimer based detection system was less successful. The intensity of the endocrine cells is significantly reduced compared to the level expected and obtained in Fig. 1a. Also compare with Figs. 2b - 4b – same protocol.

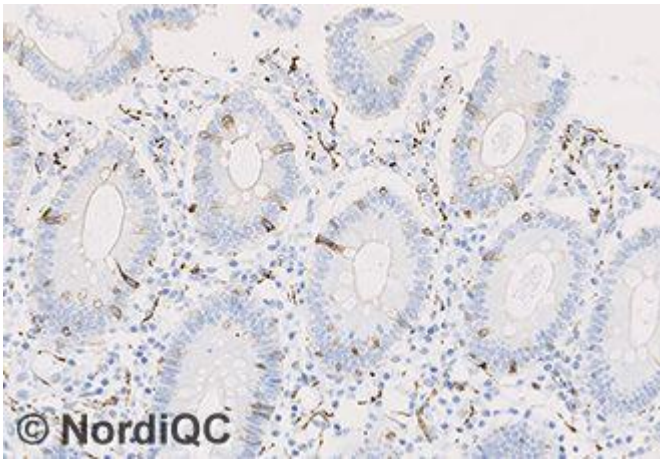


Fig. 2a
Optimal SYP staining of the colon using same protocol as in Fig. 1a. The peripheral nerves in lamina propria and neuroendocrine cells in the mucosa show a strong and distinct cytoplasmic staining reaction. Scattered goblet cells show a weak to moderate cytoplasmic staining reaction. Also compare with Figs. 3a and 4a – same protocol.

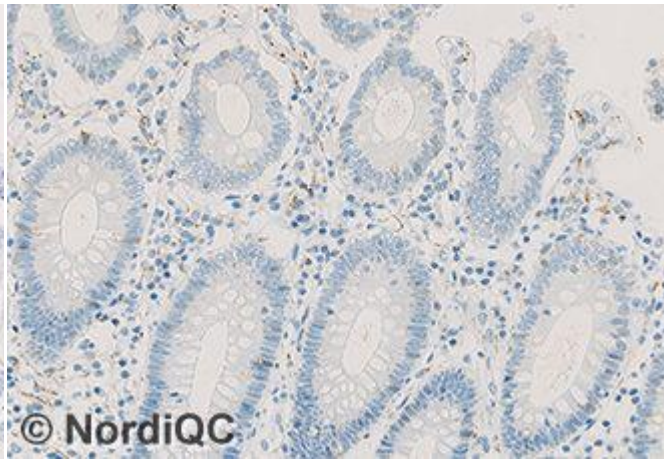


Fig. 2b
Insufficient SYP staining of the colon using same protocol as in Fig. 1b – same field as in Fig. 2a. The peripheral nerves are demonstrated, while the proportion and intensity of the staining reaction in the neuroendocrine cells is reduced. No staining reaction is seen in the goblet cells. Also compare with Figs. 3b and 4b – same protocol.

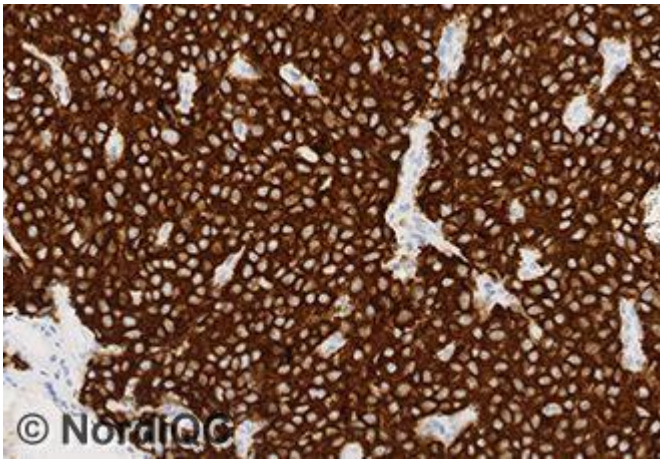


Fig. 3a
Optimal SYP staining of the intestinal neuroendocrine carcinoma using same protocol as in Figs. 1a. and 2a. Virtually all the neoplastic cells show a strong and distinct staining reaction. No background staining is seen.

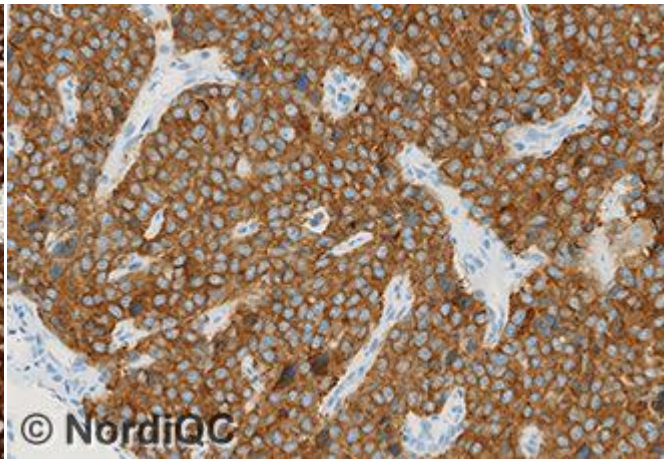


Fig. 3b
Staining for SYP of the intestinal neuroendocrine carcinoma using same insufficient protocol as in Figs. 1b and 2b - same field as in Fig. 3a. The vast majority of the neoplastic cells are demonstrated. However also compare with Fig. 4b – same protocol.

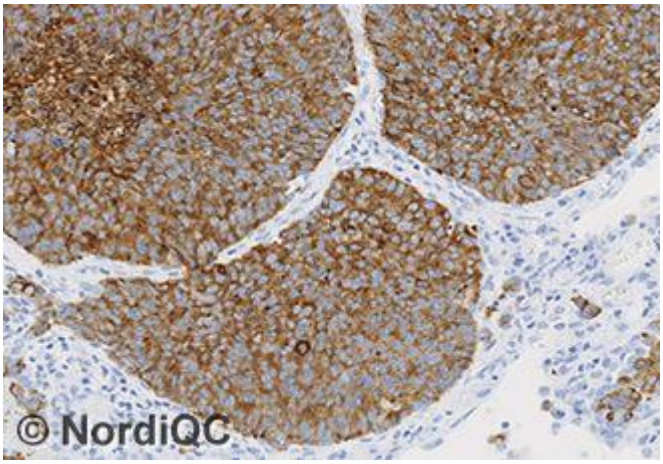


Fig. 4a
Optimal SYP staining of the SCLC using same protocol as in Figs. 1a - 3. Virtually all the neoplastic cells show a strong and distinct cytoplasmic staining reaction with a dot-like accentuation. No background staining is seen.

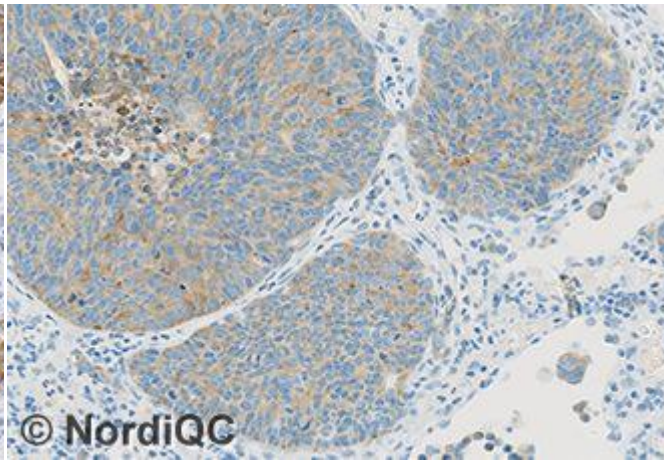


Fig. 4b
Insufficient SYP staining of the SCLC using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a.
Only scattered neoplastic cells show a weak and diffuse cytoplasmic staining reaction.

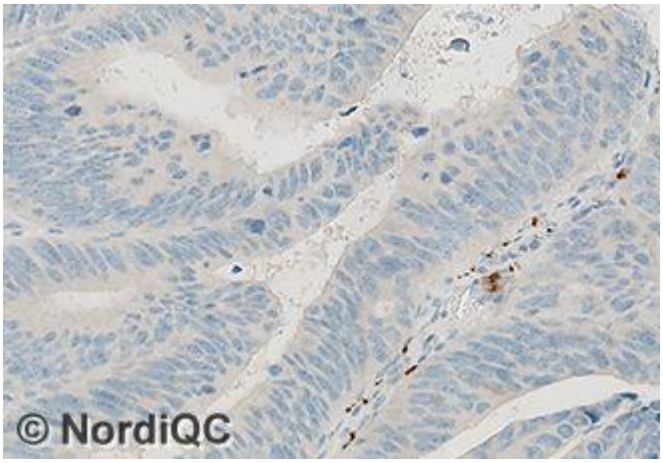


Fig. 5a
Optimal SYP staining of the colon adenocarcinoma using same protocol as in Figs. 1a – 4a. All neoplastic cells are negative and only peripheral nerves in the stromal compartment are positive. The protocol was based on the mAb clone 27G12, HIER in an alkaline buffer, a sensitive 3-step multimer based detection system and performed on the BenchMark ULTRA, Ventana. Virtually no background staining is seen.

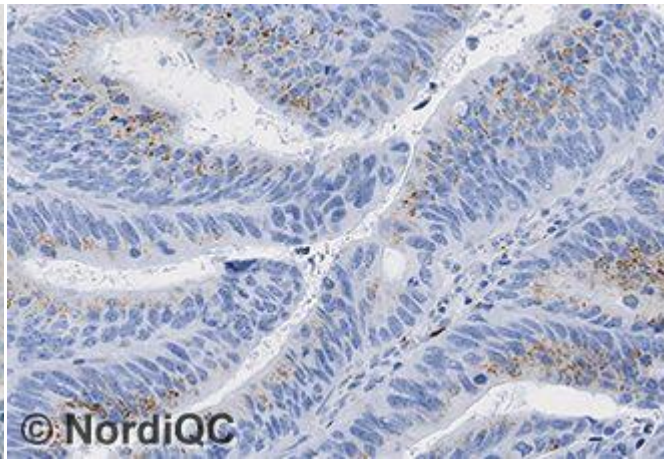


Fig. 5b
Staining for SYP of the colon adenocarcinoma – same field as in Fig. 5a. An aberrant granular cytoplasmic staining reaction is seen diffusely in the neoplastic cells. This pattern was occasionally observed, when the mAb clone 27G12 was applied on the Dako Autostainer and Biocare Intellipath platform with otherwise optimal settings based on HIER in an alkaline buffer and a 3-step polymer based detection system. The aberrant staining pattern for clone 27G12 was not observed on the BenchMark platform and different washing conditions and efficiency might cause the different staining pattern. As the staining reaction was not consistent on e.g. the Dako platform, a lot-to-lot variation might be a cofactor. If the aberrant cytoplasmic staining reaction was observed in > 30% of the neoplastic cells, the result was evaluated as borderline.

SN/RR/LE/MV 25-3-2015