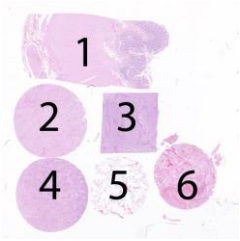
Assessment Run 44 2015
Napsin A

Recommended Napsin A protocols Recommended Napsin A control tissue

Material
The slide to be stained for **Napsin A** comprised:



1. Colon, 2. Kidney, 3-4. Lung adenocarcinomas, 5. Lung, 6. Renal clear cell carcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing Napsin A staining as optimal included:

- An at least moderate, granular cytoplasmic staining reaction of virtually all type II pneumocytes and alveolar macrophages in the lung
- An at least moderate, granular cytoplasmic staining reaction of the majority of the epithelial cells of the proximal tubules in the kidney
- A moderate to strong, granular cytoplasmic staining reaction of the majority of the neoplastic cells in the renal clear cell carcinoma and lung adenocarcinomas
- Negative staining reaction of normal columnar epithelial cells and macrophages in lamina propria in the colon

Participation

Number of laboratories registered for Napsin A, run 44	174
Number of laboratories returning slides	162 (93%)

Results
162 laboratories participated in this assessment. 126 laboratories (78%) achieved a sufficient mark (optimal or good). Abs used and assessment marks are summarized in table 1 (see page 2)

The most frequent causes of insufficient staining were:
- Less successful performance of polyclonal Napsin A antibodies
- Too low concentration of the primary Ab

Performance history
This was the second NordiQC assessment of Napsin A. A significant increase of the pass rate was seen compared to run 39 in 2013 (see table 2).

Table 2. **Proportion of sufficient results for Napsin A in the two NordiQC runs performed**

	Run 39 2013	Run 44 2015
Participants, n=	104	162
Sufficient results	58%	78%

Conclusion
The mAbs clones **IP64**, **MRQ-60**, **TMU-Ad02**, **BS10** and the rmAbs **KCG1.1**, **EP205**, **EPR6252**, **BC15** are all recommendable Abs for demonstrating Napsin A. In concordance with the previous assessments for Napsin A in NordiQC (run 39, 2013), the mAb clone IP64 was the most robust and specific Ab for the demonstration of Napsin A. With mAb clone IP64 optimal results could be obtained on all the 3 main IHC systems (Dako, Ventana and Leica). HIER and an appropriate calibration of the concentration of the primary Ab are mandatory for optimal performance. The mAb clone MRQ-60 and the rmAb KCG1.1 also showed an overall high pass rate. Optimal MRQ-60 and KCG1.1 protocols were all based on HIER in alkaline buffer. The pAb 352A-X (Cell Marque) and pAb760-4446 (Ventana) gave a very high proportion of false positive staining reactions and should be replaced. Kidney is recommendable as positive tissue control; virtually all the epithelial cells of the proximal tubules must show an at least moderate and distinct granular cytoplasmic staining reaction. Colon can be used as negative tissue control as no staining should be seen in columnar epithelial cells or stromal macrophages.

Table 1. **Antibodies and assessment marks for Napsin A, run 44**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **IP64**	86	Leica/Novocastra	39	39	6	2	91%	92%
mAb clone **MRQ-60**	8	Cell Marque	3	4	1	0	88%	100%
mAb, clone **TMU-Ad02**	4 3	Biocare IBL	1	2	4	0	43%	-
rmAb clone **KCG1.1**	2 2 1 1	Zytomed Diagnostic Biosystems Abcam Acris	1	5	0	0	100%	-
rmAb clone **BC15**	1	Zytomed	1	0	0	0	-	-
mAb, clone **BS10**	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone **EPR6252**	1	Abcam	1	0	0	0	-	-
pAb **352A-7x**	8	Cell Marque	0	1	1	6	13%	-
Ready-To-Use antibodies								
mAb clone **MRQ-60 760-4867**	18	Ventana/Cell Marque	1	16	1	0	84%	-
mAb clone **MRQ-60 352M-98**	3	Cell Marque	0	3	0	0	-	-
mAb clone **MRQ-60 MAD-000633QD**	3	Master Diagnostica	0	3	0	0	-	-
rmAb clone **BC15 API 3043**	1	Biocare	0	0	1	0	-	-
mAb clone **IP64 AM701-5M**	1	BioGenex	0	0	1	0	-	-
mAb clone **IP64 ZM-0473**	1	ZSGB-BIO	0	1	0	0	-	-
rmAb clone **EP205 352R-18**	1	Cell Marque	1	0	0	0	-	-
mAb clone **MX015 MAB-0704**	1	Maixin	0	1	0	0	-	-
pAb **760-4446**	12	Ventana/Cell Marque	0	1	0	11	8%	-
pAb **PPM428DS**	1	Biocare	0	0	0	1	-	-
pAb **MP-394-DS6**	1	Menapath	0	0	0	1	-	-
pAb **RAB-0639**	1	Maxim	0	1	0	0	-	-
Total	162		49	77	15	21	-	
Proportion			30%	48%	9%	13%	78%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Detailed analysis of Napsin A, Run 44
The following protocol parameters were central to obtain an optimal staining:

Concentrated Antibodies
mAb clone **IP64**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using either Cell Conditioning 1 (Ventana) (17/35)*, Target Retrieval Solution pH 9 (Dako) (6/9), Target Retrieval Solution pH 9 (3-in-1) (Dako) (4/6), Bond Epitope Retrieval Solution 1 (Leica) (4/12), Tris-EDTA/EGTA pH 9 (3/5), Bond Epitope Retrieval Solution 2 (Leica) (2/8), Cell Conditioning 2 (Ventana) (1/1), Borg Decloaker pH 9,5 (BioCare) (1/1) or Target Retrieval Solution pH 6.1 (Dako) (1/5) as the retrieval buffer. The mAb was typically diluted in the range of 1:20-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 77 of 84 (92%) laboratories produced a sufficient staining result (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

mAb clone **MRQ-60**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using Target Retrieval Solution pH 9 (3-in-1) (Dako) (3/4). The mAb was diluted in the range of 1:200-

1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 of 5 (100%) laboratories produced a sufficient staining result.

mAb, clone **TMU-Ad02**: One protocol with an optimal result was based on HIER using Cell Conditioning 1 (Ventana) (efficient heating time 64 min. at 95°C) and 32 min. incubation of the primary Ab at dilution 1:50 and UltraView-RED (Ventana 760-501) as detection system.

mAb, clone **BS10:** One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 (efficient heating time 20 min. at 98°C) and 30 min. incubation of the primary Ab at dilution 1:200 and BioSite Histo Plus-HRP Polymer (Nordic Biosite KDB-10007) as detection system.

rmAb clone **KCG1.1:** One protocol with an optimal result was based on HIER using Target Retrieval Solution pH 9 (3-in-1) (Dako) (efficient heating time 10 min. at 97°C) and 20 min. incubation of the primary Ab at dilution 1:50 and EnVision FLEX (Dako K8000) as detection system.

rmAb clone **BC15:** One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 (efficient heating time 40 min. at 95°C) and 60 min. incubation of the primary Ab at dilution 1:100 and ZytoChemPlus-HRP Polymer (Zytomed Systems POLHRP-100) as detection system.

rmAb clone **EPR6252:** One protocol with an optimal result was based on HIER using Citrate pH 6 (efficient heating time 20 min. at 97°C) and 30 min. incubation of the primary Ab at dilution 1:600 and EnVision FLEX (Dako SM802) as detection system.

Table 3 summarizes the overall proportion of optimal staining results for the most frequently used concentrated antibodies on the three most commonly used IHC stainer platforms.

Table 3. **Proportion of optimal results for Napsin A using concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	Dako Autost. Link / Classic, Omnis		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 **IP64**	10/16 (63%)**	1/5 (20%)	17/35 (49%)	1/1	2/8 (25%)	4/12 (33%)
mAb clone **MRQ-60**	3/4	-	0/1	-	-	-

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

Ready-To-Use (RTU) Antibodies and corresponding systems
mAb clone **MRQ-60** product no. 760-4867, Ventana/Cell Marque, Ventana Benchmark XT/Ultra:
One protocol with optimal results was based on 64 min. HIER using Cell Conditioning 1 (Ventana), 32 min. incubation of the primary Ab and OptiView (Ventana 760-700) with amplification (4 min. + 4 min.) as detection system.

Comments
In concordance with the previous NordiQC assessments for Napsin A (run 39, 2013), the prevalent feature of the insufficient results were either a false positive staining reaction and/or a generally too weak staining reaction. False positive staining reaction was seen in 44% of the insufficient results (16 of 36), typically in the columnar epithelial cells of the colon and in macrophages in lamina propria. The concentrated format of the pAb 352A-X (Cell Marque) and the pAb 760-4446 as Ready-To-Use format (Ventana) both gave a very high proportion of insufficient results as 6 of 8 and 11 of 12 protocols, respectively, gave a false positive staining reaction of columnar epithelial cells of the colon (Fig. 6a and Fig. 6b). Furthermore, the false positive staining reaction was often seen in combination with a too weak specific staining reaction (Fig. 4a – Fig. 5b). The mAb clone TMU-Ad02 often gave a false positive granular staining reaction in macrophages in lamina propria of the colon. The aberrant staining pattern in non-alveolar macrophages was assessed as insufficient as a highly specific staining reaction is needed for the identification of neoplastic cells e.g. in lymph nodes and other macrophage rich tissues. A weak non-granular false positive staining reaction in plasma cells in lamina propria of the colon was seen in some cases with the mAb clone MRQ-60. Since this staining pattern did not interfere with the specific reaction in cells expected to be positive for Napsin A, it was fully accepted.

Biotin based detection systems cannot be recommended as granular cytoplasmic staining reaction for Napsin A is similar to unspecific staining pattern of endogenous biotin.

A too weak staining result was typically characterized by a reduced staining reaction both in regard to the intensity and proportion of the structures expected to be demonstrated. This was in particular observed in the neoplastic cells of the renal clear cell carcinoma, the normal epithelial cells of the renal proximal tubules and the lung adenocarcinoma in core no. 3. A too weak staining was most frequently caused by a too low titre of the primary Ab often in combination with HIER in non-alkaline buffer (Fig. 1a – Fig. 3b), or use of less successful polyclonal Abs.

The mAb clone IP64 was the most widely used antibody for demonstration of Napsin A and provided optimal results on all three main IHC platforms from Dako, Leica and Ventana, respectively (see table 3). Used as a concentrate within a laboratory developed (LD) assay, mAb clone IP64 gave an overall pass rate of 91% (78 of 86) out of which 45% were optimal (see table 1). Both HIER in alkaline and non-alkaline buffers could be used to obtain optimal results. A prerequisite for obtaining optimal results after HIER in non-alkaline buffers was the use of a sensitive 3-step polymer/multimer system, whereas optimal results could be achieved with both 2 and 3-step polymer/multimer systems when using HIER in alkaline buffers. Within a LD assay, both the mAb clone MRQ-60 and the rmAb KCG1.1 were equally successful giving an overall pass rate of 88% (7 of 8) and 100% (5 of 5), respectively. In both cases optimal protocols (4 of 12) were all based on HIER in alkaline buffer.

In this assessment, 27% (44 of 162) of the labs used RTU antibodies. Optimal results could only be obtained with the mAb clone MRQ-60 based RTU system from Ventana (760-4867). The pass rate for the MRQ-60 RTU system (84%) was comparable to LD assays using the same clone (88%), but the proportion of optimal results were surprisingly low. Only 6% (1 of 18) using the RTU system obtained optimal results, compared to 38% (3 of 8) for the LD assays. The reason for this is uncertain, but optimal results could not be obtained with the RTU system using the official Ventana recommendations. Only one lab, using modified protocol settings, achieved optimal staining results with 760-4867. The modified protocol was based on OptiView with amplification, utilizing prolonged incubation time for both the primary Ab (32 min. compared to the recommended 8-16 min) and the HIER pretreatment (64 min in CC1 compared to the recommended 32 min).

This was the second NordiQC assessment of Napsin A and despite of many new participants a significantly increase in pass rate was seen compared to run 39 in 2013 (see table 2). The pass rate improved from 58% in 2013 to 78% in the current run. The primary reason for this improvement seems to be closely related to, an increased use of both mAb clone IP64 and mAb clone MRQ-60 at the expense of less successful polyclonal Abs. In Run 39 (2013) mAb clone IP64 was used by 42% (44 of 104) and mAb clone MRQ-60 by 8% (8 of 104) of the labs. In the current run 53% (86 of 162) used mAb clone IP64 and 20% (32 of 162) used mAb clone MRQ-60. In the same period the proportion of labs using polyclonal Abs decreased from 32% (33 of 104 in Run 39) to 14% (23 of 162 in Run 44).

Controls

Both kidney and lung can be used as positive tissue control for Napsin A. However, in concordance with the previous assessments for Napsin A in NordiQC (run 39, 2013), kidney was found more informative for an appropriate calibration of the protocol. In kidney virtually all epithelial cells of the proximal tubules must show an at least moderate, distinct granular cytoplasmic staining reaction. Type II pneumocytes and alveolar macrophages of the lung showed a high staining intensity independent of protocol parameters applied and lung tissue is thus less useful to calibrate the protocol for the demonstration of Napsin A in low-level antigen expressing cells and neoplasias. Colon is useful as negative control; no staining should be seen in the columnar epithelial cells and macrophages.

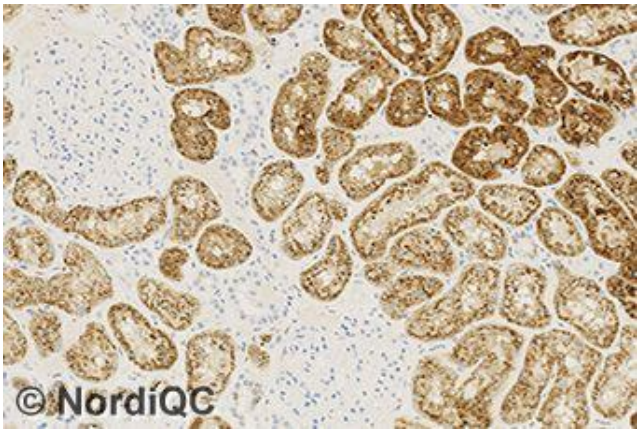


Fig. 1a (x200)
Optimal Napsin A staining of the kidney using the mAb clone IP64 (Leica) diluted 1:100 and with an incubation time of 25 min. after HIER in an alkaline buffer (pH 9.0) (BERS2, Leica). Staining was performed on the Bond III using a 3-step polymer system (Refine, Leica). A moderate to strong, granular cytoplasmic staining reaction of the majority of the epithelial cells of the the proximal tubules is seen. (same protocol used in Figs. 1a - 3a). Compare with Fig. 1b.

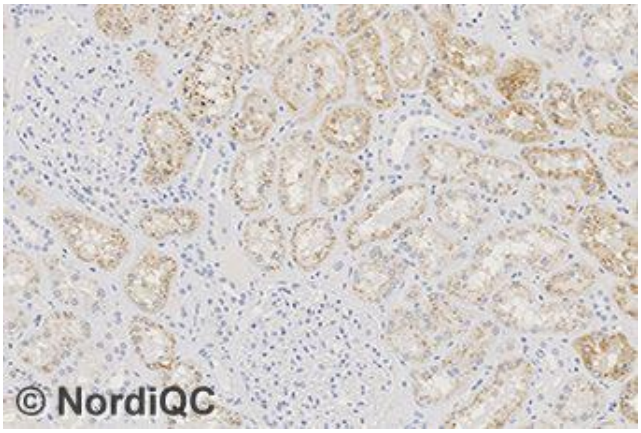


Fig. 1b (x200)
Insufficient Napsin A staining of the kidney using the mAb clone IP64 (Leica) diluted 1:400 and with an incubation time of 15 min. after HIER in a citrate based (pH 6.0) buffer (BERS1, Leica). Staining was performed on the Bond III using a 3-step polymer system (Refine, Leica). The combination of a too low titer of mAb IP64 and the use of HIER in non-alkaline buffer, results in a too weak, granular cytoplasmic staining reaction of the majority of the epithelial cells of the proximal tubules. Compare with Fig. 1a – same field. Also compare with Figs. 2b and 3b – same protocol.

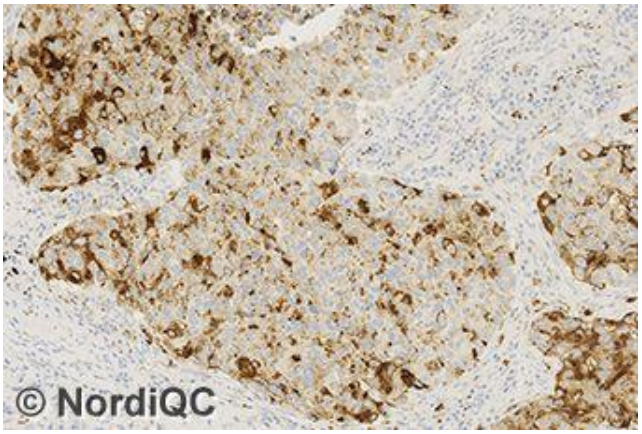


Fig. 2a (x200)
Optimal Napsin A staining of the lung adenocarcinoma (core no. 3) using the same protocol as in Fig. 1a. A strong, granular cytoplasmic staining of virtually all the tumor cells is seen. Compare with Fig. 2b.

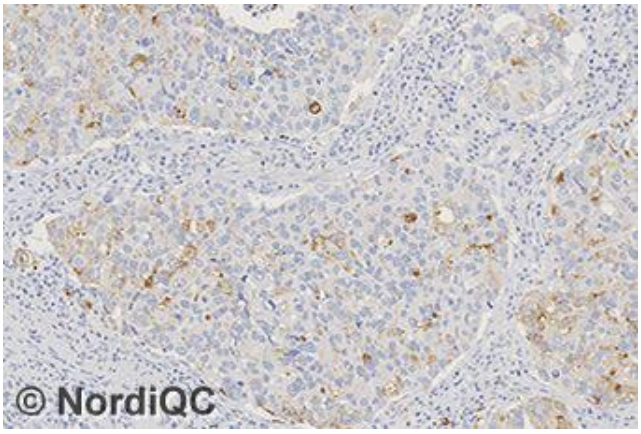


Fig. 2b (x200)
Insufficient Napsin A staining of the lung adenocarcinoma (core no. 3) using the same protocol as in as in Fig. 1b. Only a weak, granular cytoplasmic staining of the tumor cells is seen and only in a minor faction of the cells. Compare with Fig. 2a - same field.

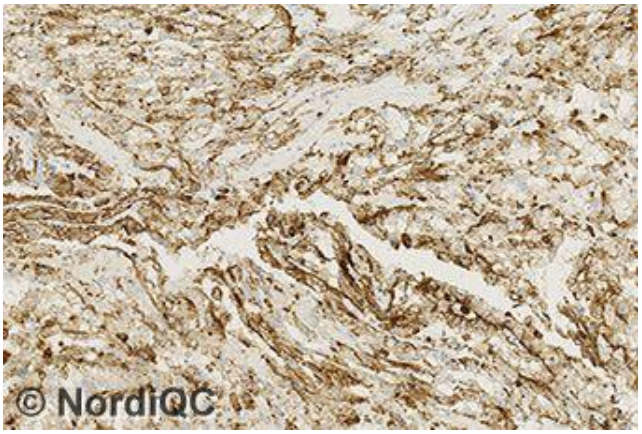


Fig. 3a (x200)
Optimal Napsin A staining of the renal clear cell carcinoma using the same protocol as in Fig. 1a and 2a. A moderate to strong, granular cytoplasmic staining of the majority of the tumor cells is seen. Compare with Fig. 3b.

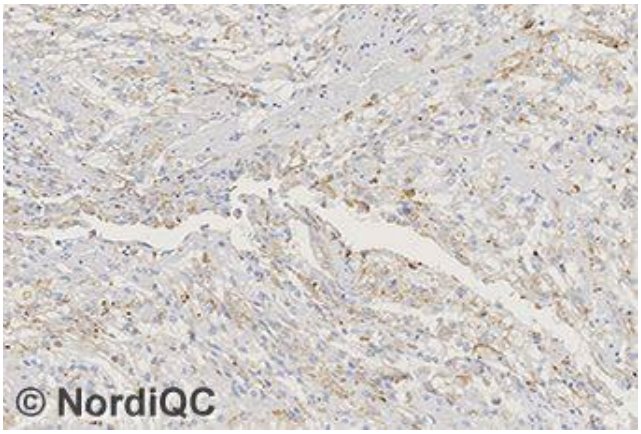


Fig. 3b (x200)
Insufficient Napsin A staining of the renal clear cell carcinoma using the same protocol as in Figs. 1b and 2b. Only a weak, granular cytoplasmic staining of the tumor cells is seen and only in a minor faction of the cells. Compare with Fig. 3a – same field.

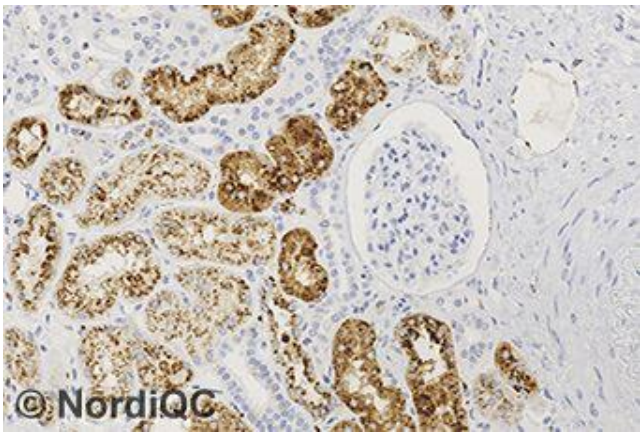


Fig. 4a (x200)
Optimal Napsin A staining of the kidney using the mAb clone IP64 (Leica) diluted 1:100 and with an incubation time of 32 min. after HIER in an alkaline buffer (CC1, Ventana) using a 3-step multimer system (OptiView, Ventana) and performed on the BenchMark Ultra. A moderate to strong, granular cytoplasmic staining reaction of the majority of the epithelial cells of the the proximal tubules is seen. (same protocol used in Figs. 4a - 6a). Compare with Fig. 4b.

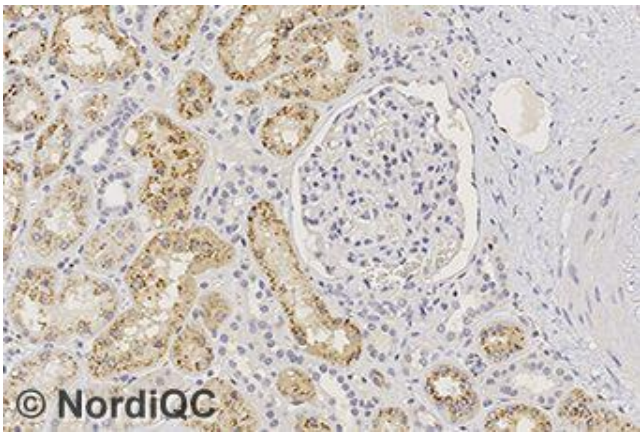


Fig. 4b (x200)
Insufficient Napsin A staining of the kidney using the pAb 760-4446 (RTU, Cell Marque/Ventana) with an incubation time of 28 min. after HIER in an alkaline buffer (CC1, Ventana) using a 2-step multimer system (UltraView, Ventana) and performed on the BenchMark Ultra. Only weak granular cytoplasmic staining reaction of the majority of the epithelial cells of the the proximal tubules is seen. Compare with Fig. 4a – same field. Also compare with Figs. 5b and 6b – same protocol.

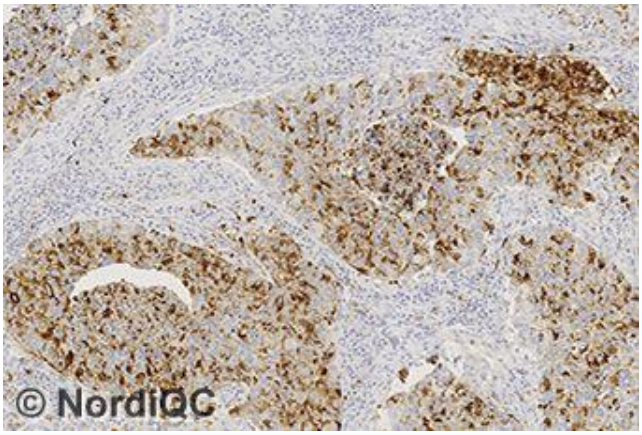


Fig. 5a (x100)
Optimal Napsin A staining of the lung adenocarcinoma (core no. 3) using the same protocol as in Fig. 4a. A strong, granular cytoplasmic staining of virtually all the tumor cells is seen. Compare with Fig. 5b.

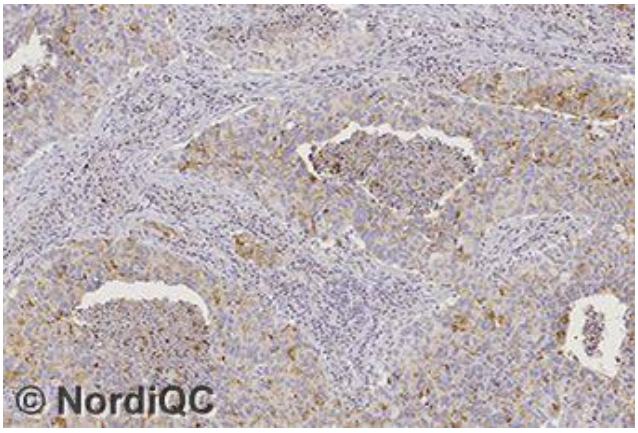


Fig. 5b (x100)
Insufficient Napsin A staining of the lung adenocarcinoma (core no. 3) using the same protocol as in as in Fig. 4b. Only a weak, granular cytoplasmic staining of the tumor cells is seen. Compare with Fig. 5a - same field.

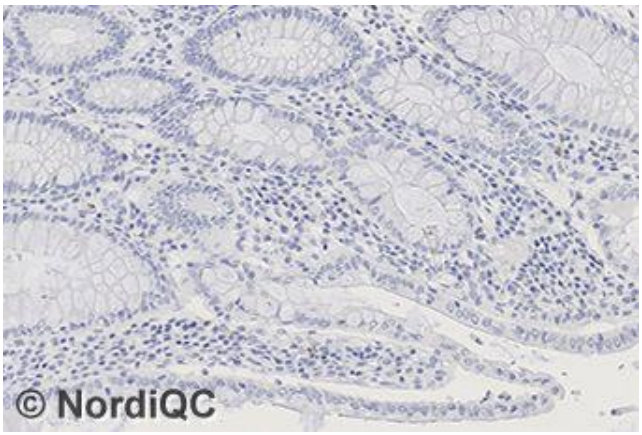


Fig. 6a (x200)
Optimal Napsin A staining in the colon using the same protocol as in Figs. 4a and 5a. No false positive reaction is seen. Compare with Fig 6b.

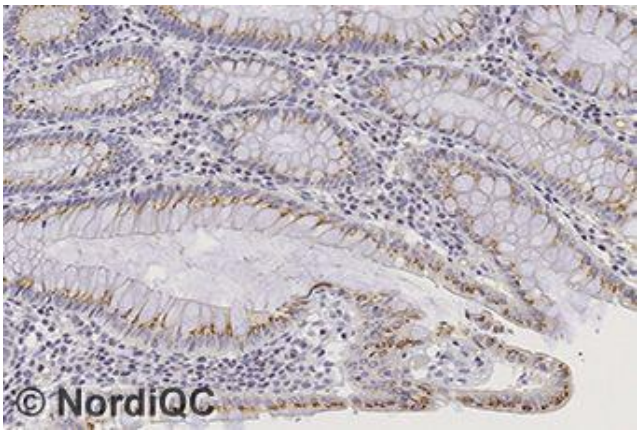


Fig. 6b (x200)
Insufficient Napsin A staining in the colon using the same protocol as in Figs. 4b and 5b. False positive, granular cytoplasmic staining of virtually all the columnar epithelial cells is seen. Compare with Fig. 6a – same field.

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