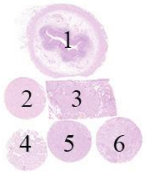
Assessment Run 66 2022
Napsin A

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for Napsin A, identifying cancers of unknown origin and in particular, discriminating lung adenocarcinomas from lung squamous cell carcinomas. Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for Napsin A (see below).

Material
The slide to be stained for Napsin A comprised:



1. Appendix, 2. Kidney, 3. Lung adenocarcinoma, 4. Lung, 5. Lung squamous cell carcinoma 6. Lung adenocarcinoma

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 and convoluted tubules in the kidney.
- A strong, granular cytoplasmic staining reaction of virtually all the neoplastic cells in the lung adenocarcinomas.
- No staining reaction of the neoplastic cells in the lung squamous cell carcinoma.
- No staining reaction of columnar epithelial and stromal cells in the appendix. Dispersed macrophages situated in lamina propria of the appendix could display positive staining reaction. This was accepted, providing that it did not compromise interpretation of the expected reaction pattern.

Participation

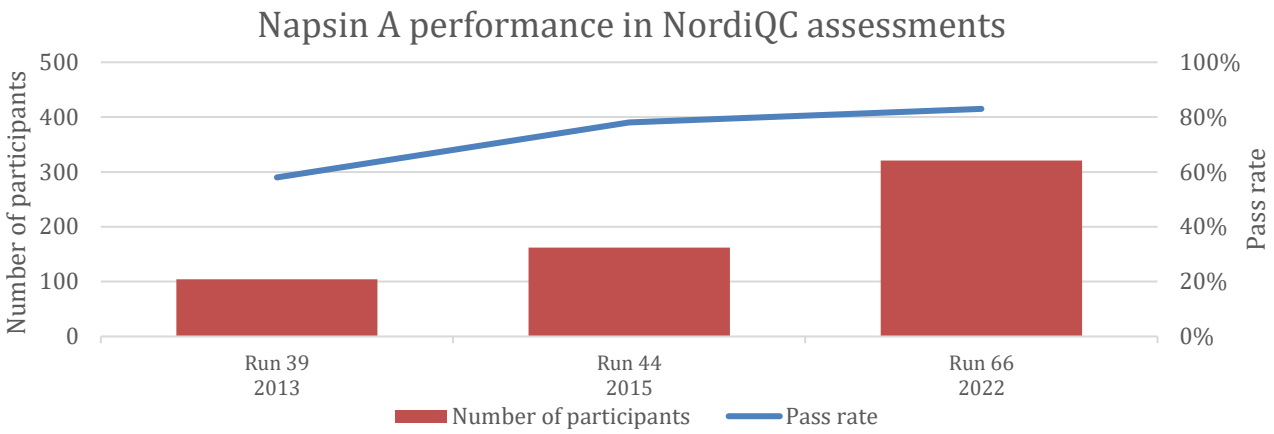
Number of laboratories registered for Napsin A, run 66	343
Number of laboratories returning slides	321 (94%)

Results
321 laboratories participated in this assessment. 266 laboratories (83%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2). 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.

The most frequent causes of insufficient staining were:
- Less successful performance of polyclonal Napsin A antibodies
- Less successful performance of OptiView with amplification as detection system
- Less successful performance of the lot numbers V0002826/27 of the Ready-to-Use (RTU) system 760-4867 based on mAb clone MRQ-60 (Ventana/Roche)
- Too low concentration of the primary Ab

Performance history
This was the third NordiQC assessment of Napsin A. The number of participants has increased significantly and the overall pass rate increased marginally compared to run 44 in 2015 (see Graph 1).

Graph 1. **Proportion of sufficient results for Napsin A in the three NordiQC runs performed**



Conclusion

The mAbs clones **IP64**, **MRQ-60**, **TMU-Ad02**, **BS10, ZM11** and the rmAbs clones **KCG1.1**, **EP205**, **BP6083** could all be used to obtain an optimal result. In general, pAbs should be avoided although one laboratory using the pAb 372A-7X (Cell Marque) were able to produce an optimal result. Both the mAb clone IP64 and MRQ-60 seems very robust for demonstration of Napsin A and were used by the vast majority of laboratories either within a laboratory developed (LD) assay or as RTU formats – in total 82% (264/321) of the protocols. Used as concentrate, the mAb clone IP64 could produce optimal results on all four main IHC systems.

Using vendor recommended protocol settings within a RTU system, the mAb clones IP64 (PA0064, Bond III/MAX, Leica Biosystems) and MRQ-60 (760-4867, Benchmark, Ventana/Roche) were very successful, both providing a pass rate of 100% of which a significant proportion were giving an optimal mark (grouped together 70%).

The main causes (55%) for an insufficient staining result was primarily related to a false positive reaction of cells expected to be negative and was typically related to the use of pAbs, application of OptiView with amplification as detection system or the primary antibody lot numbers V004826/27 of the RTU system 760-4867 (Ventana/Roche) based on clone MRQ-60. 42% of the insufficient results were related to false negative/too weak staining results, and for users of mAb clone IP64 within a LD-assay, the concentration of the primary Ab was important to titrate correctly for optimal performance (dilution range of 1:20-1:150) .

Kidney is recommendable as positive tissue control; virtually all epithelial cells of the convoluted/proximal tubules must show an at least moderate and distinct granular cytoplasmic staining reaction. Appendix can be used as negative tissue control as no staining should be seen in epithelial cells. Dispersed reaction in stromal macrophages might be seen (and should be accepted).

Table 1. **Antibodies and assessment marks for Napsin A, run 66**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **IP64**	105	Leica Biosystems	52	38	13	2	86%	50%
mAb clone **MRQ-60**	27	Cell Marque	6	20	1	0	96%	22%
mAb, clone **TMU-Ad02**	12	Biocare Medical	8	3	1	0	92%	67%
mAb clone **BS10**	4	Nordic Biosite	2	1	1	0	-	-
mAb clone **ZM11**	1	Zeta Corporation	1	0	0	0	-	-
mAb clone **IHC635**	1	GenomeMe	0	1	0	0	-	-
rmAb clone **KCG1.1**	2 2 1 1	Acris Diagnostic Biosystems Zytomed Systems Abcam	1	2	3	0	50%	17%
rmAb clone **EP205**	5	Cell Marque Bio SB	2	3	0	0	100%	40%
rmAb clone **BC15**	1	Zytomed	0	1	0	0	-	-
rmAb clone **BP6083**	1	Biolynx	1	0	0	0	-	-

rmAb clone **EPR6267**	1	Abcam	0	0	1	0	-	-
rmAb clone **QR058**	1	Quartett	0	1	0	0		
pAb **352A-7x**	5	Cell Marque	1	0	3	1	20%	20%
Conc total	170		74	70	23	3	85%	44%
Ready-To-Use antibodies								
mAb clone **MRQ-60 760-4867**[3]	30	Ventana/Roche	22	8	0	0	100%	73%
mAb clone **MRQ-60 760-4867**[4]	85	Ventana/Roche	35	26	21	3	72%	41%
mAb clone **MRQ-60 352M**	2	Cell Marque	0	2	0	0	-	-
mAb clone **BS10 MAD-000752QD**	5	Master Diagnostica	0	4	1	0	-	-
mAb clone **IP64 AM701-5M**	1	BioGenex	1	0	0	0	-	-
mAb clone **IP64 PA0064**[3]	6	Leica Biosystems	4	2	0	0	100%	67%
mAb clone **IP64 PA0064**[4]	8	Leica Biosystems	3	5	0	0	100%	60%
mAb clone **C2C2 CNM-0012**	1	Celnovte	1	0	0	0	-	-
mAb clone **MX015 MAB-0704**	1	Maixin	0	1	0	0	-	-
rmAb clone **BC15 API3043**	1	Biocare	1	0	0	0	-	-
rmAb clone **BC15 RBG059**	1	Zytomed systems	1	0	0	0	-	-
rmAb clone **810B1C8 PA102**	1	Abcarta	1	0	0	0	-	-
rmAb clone **BP6083 I1066E**	1	Biolynx	1	0	0	0	-	-
rmAb clone **EP205 352R**	1	Cell Marque	1	0	0	0	-	-
rmAb clone **EP205 8331-C010**	2	Sakura Finetek	2	0	0	0	-	-
rmAb clone **EP205 PR059**	1	PathnSitu	0	0	1	0	-	-
rmAb clone **EP205**	1	Zybio	0	1	0	0	-	-
pAb **760-4446**	2	Ventana/Roche	0	0	2	0	-	-
pAb **352A**	1	Cell Marque	0	0	1	0	-	-
RTU total	151		73	49	26	3	81%	48%
Total	321		147	119	49	6	-	
Proportion			46%	37%	15%	2%	83%	

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 asessed 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 Napsin A, Run 66
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 (CC1, Ventana/Roche) (22/45)*, Target Retrieval Solution (TRS) (3-in-1) pH 9 (Dako/Agilent) (19/30), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (5/11), Tris-EDTA/EGTA pH 9 (1/1), Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (3/10), Cell

Conditioning 2 (CC2, Ventana/Roche) (1/1), or Target Retrieval Solution pH 6.1 (TRS pH 6.1) (Dako/Agilent) (1/2) as the retrieval buffer. The mAb was typically diluted in the range of 1:20-1:150 depending on the total sensitivity of the protocol employed. Using these protocol settings 56 of 57 (98%) 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 typically based on HIER using TRS (3-in-1) pH 9 (4/13) or CC1 (1/10). The mAb was diluted in the range of 1:300-1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings 11 of 11 (100%) laboratories produced a sufficient staining result. One laboratory obtained an optimal result without performing any pre-treatment at all.

mAb clone **TMU-Ad02**: Protocols with optimal results were all based on HIER using TRS (3-in-1) pH 9 (8/8). The mAb was diluted in the range of 1:50-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 11 of 11 (100%) laboratories produced a sufficient staining result.

mAb clone **BS10:** Protocols with optimal results were based on HIER using BERS2 (1/1) or TRS (3-in-1) pH 9 (1/1). The mAb was diluted in the range of 1:400-1:700 depending on the total sensitivity of the protocol employed. Using these protocol settings, 2 of 2 laboratories produced an optimal staining result.

mAb clone **ZM11:** One protocol with an optimal result was based on HIER using TRS (3-in-1) pH 9 as retrieval buffer. The mAb was diluted 1:100 and Envision FLEX (Dako/Agilent) was used as the detection system.

rmAb clone **KCG1.1:** One protocol with an optimal result was based on HIER using TRS (3-in-1) pH 9 (1/1) as retrieval buffer. The rmAb was diluted 1:100 and Envision FLEX+ (Dako/Agilent) was used as the detection system.

rmAb clone **EP205:** Protocols with optimal results were based on HIER in CC1 (2/2). The rmAb was diluted in the range of 1:100-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 2 of 2 laboratories produced an optimal staining result.

rmAb clone **BP6083:** One protocol with an optimal result was based on HIER using TRS (3-in-1) pH 9 as retrieval buffer. The rmAb was diluted 1:200 and Envision FLEX was used as the detection system.

Table 2 summarizes the overall proportion of optimal staining results for the most frequently used concentrated antibodies on the four most commonly used IHC platforms.

Table 2. **Proportion of optimal results for Napsin A for the two most commonly used antibody concentrates on the 4 main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark Ultra/XT/GX**		**Leica Biosystems 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	ER2 pH 9.0	ER1 pH 6.0
mAb clone **IP64**	1/2**	-	14/15 (93%)	1/1	20/30 (67%)	(1/1)	3/3	2/3
mAb clone **MRQ-60**	4/5 (80%)	-	0/4	-	1/2	-	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 (RTU) antibodies and corresponding systems
mAb clone **MRQ-60** product no. 760-4867, Ventana/Roche, Ventana Benchmark GX/XT/Ultra:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min. at 95-100°C), 8-32 min. incubation of the primary Ab. and UltraView (760-500)/UltraView with amplification (760-500 + 760-080) or OptiView (760-700) as detection systems. Using these protocol settings, 60 of 67 (90%) laboratories produced a sufficient staining result.

mAb clone **IP64**, product no. **PA0064**, Leica Biosystems, Bond-III/MAX:
Protocols with optimal results were based on HIER using BERS1 (efficient heating time 40 min. at 100°C), 15 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. Using these protocol settings, 6 of 6 (100%) laboratories produced a sufficient staining result.

mAb clone **C2C2**, product no. **CNM-0012**, Celnovte, CNT330-Stainer:
One protocol with an optimal result was based on HIER using TRIS/EDTA pH 9 (efficient heating time 20 min. at 100°C), 30 min. incubation of the primary Ab. and CNTVision Super Poly (SD5300) as detection systems.

rmAb clone **810B1C8**, product no. **PA102**, Abcarta, FAIP-48T-Stainer:
One protocol with an optimal result was based on HIER using Abcarta-EDTA/ER2 pH 9 (efficient heating time 20 min. at 100°C), 15 min. incubation of the primary Ab. and Abcarta-HRP Polymer (PS300) as detection systems.

rmAb clone **BP6083**, product no. **I10662E**, Biolynx, LYNX480-Stainer:
One protocol with an optimal result was based on HIER using Antigen Retrieval 2 (EDTA) (efficient heating time 30 min. at 100°C), 30 min. incubation of the primary Ab. and BXV Visualization System (I20032C) as detection systems.

rmAb clone **EP205**, product no. **8331-C010**, Sakura Finetek, Tissue-Tek Genie Advanced stainer:
Protocols with optimal results were based on HIER using Tissue-Tek Genie High pH Antigen Retrieval (efficient heating time 45 min. at 98°C), 30 min. incubation of the primary Ab and Tissue-Tek Genie Pro Detection Kit, DAB (8826-K250) as detection system. Using these protocol settings, two of two protocols were assessed as optimal.

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 Napsin A for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Leica BOND III/MAX mAb IP64 **PA0064**	100% (6/6)	67% (4/6)	100% (7/7)	29% (2/7)
VMS Ultra/XT/GX mAb MRQ-60 **760-4867**	100% (30/30)	73% (22/30)	73% (58/80)	43% (34/80)

* 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.

Comments
In concordance with the previous NordiQC assessments for Napsin A, the prevalent feature of the insufficient results was either a false positive staining reaction and/or a generally too weak staining reaction. False positive staining reaction, often seen in combination with weak and/or excessive background staining, was observed in 55% (30/55) of the insufficient results. The false positive staining pattern was typically seen as a granular deposit of cellular structures expected to be negative as the columnar epithelial cells of the appendix and the neoplastic cells of the lung squamous cell carcinoma. The main causes for this aberrant staining pattern were primarily related to the use of polyclonal antibodies (20%, 6/30), use of OptiView with amplification (37%, 11/30) and use of the following lot numbers, V0002826 and V0002827 (20%, 6/30), both correlated to the RTU format 760-4867 based on the mAb MRQ-60 (Roche/Venana). Two protocols were assessed as insufficient due to excessive background staining and in the remaining insufficient protocols (42%, 23/55), a too weak or false negative staining results was seen. A too weak staining result was typically characterized by a reduced staining intensity and proportion of tissue structures expected to be demonstrated. This was in particular observed in the normal epithelial cells of the renal convoluted/proximal tubules and the neoplastic cells of the two lung adenocarcinomas.

The mAb clones IP64 and MRQ-60 were the most widely used antibodies for demonstration of Napsin A and were used by 82% (264/321) of the laboratories (see Table 1). Used as concentrated format within laboratory developed (LD) assays, the mAb clone IP64 gave a pass rate of 86% (90/105) and 50% (52/105) of the protocols resulted in an optimal mark. As shown in Table 2, the mAb clone IP64 provided optimal results on all main automatic platforms. Both HIER in alkaline and non-alkaline buffers could be used to obtain optimal results. The choice of detection systems, both 2-step and 3-step polymer/multimer system, were of less importance for the outcome if the titer of the primary Ab was calibrated correctly.

In fact, all (10/10) protocols based on a 2 polymer/multimer detection system and using the concentrate of the mAb clone IP64 in the "optimal" dilution range 1:20-1:150 (average dilution factor 1:49), were assessed as optimal. However, the single factor that influenced the performance of the LD assays mostly, was use of too diluted primary antibody. Using all protocol settings, and for protocols assessed as insufficient (in total 15 protocols), the average dilution factor was 1:363 (dilution range 1:50-1:800). In comparison and using same conditions as described above except for focusing on protocols assessed as optimal (in total 52 protocols), the average dilution factor was 1:101 (dilution range 1:20-1:400) - showing that the working concentration of the primary Ab had a significant influence on the performance of the assays.

Among LD assays, the mAb clone MRQ-60 gave the highest pass rate of 96% (26/27) but only 22% (6/27) being optimal. The optimal results were primarily obtained on the semi-automatic platform Autostainer (4/6) (Dako/Agilent) and based on HIER in TRS (3-in-1) pH 9 or TRS pH 9, dilution range of 1:300-1:500 and EnVision FLEX as the detection system (3/4). One protocol used the same settings except for applying Envision FLEX+ as the detection systems. Due to the low quantity of optimal results, the antibody seems difficult to calibrate correctly and require that all settings are adjusted cautiously to achieve an optimal result. Overall, no difference in performance was observed for 2-step versus 3-step polymer/multimer based detection systems and providing the optimal dilution range of 1:300-500 a pass rate of 100% (11/11), and 36% (4/11) being optimal was seen.

The mAb clone TMU-Ad02 within LD-assays also provided a high pass rate of 92% (11/12). In addition, the proportion of optimal results was 67% (8/12) which was significant higher compared to other main LD-assays (see Tabel 1) and an improvement in performance to the previous run 44, 2015 (pass rate was 43%). Virtually all laboratories (11/12) used this antibody on the Autostainer and Omnis platforms (both Dako/Agilent), and optimal results were typically obtained with protocol settings applying HIER in TRS (3-in-1) pH 9, a dilution range between 1:50-1:200 of the primary antibody and use of EnVision FLEX+ as the detection system. The one protocol assessed as insufficient was stained on the Benchmark Ultra (Ventana/Roche), giving a false positive staining result.

In this assessment, 47% (151/321) of the laboratories used a RTU format for demonstration of Napsin A. The RTU format, 760-4867 (Ventana/Roche) based on the mAb clone MRQ-60 was most widely applied and used by 76% (115/151) of the participants (see Table 1) using RTU formats. Five laboratories used this RTU product off-label on other platforms giving a pass rate of 60% (3/5) – only 20% (1/5) being optimal. Used on the intended platform, and as shown in Table 3, both vendor and laboratory modified protocol settings could produce optimal results. However, vendor recommended protocol settings based on OptiView as detection system displayed superior performance as 100% (8/8) of the results were assessed as optimal and thus, are highly recommended for demonstration of Napsin A. Substituting OptiView with UltraView, and applying vendor recommended protocol settings for this particular detection system, the proportion of optimal results declined to 63% (14/22), but still, all (22/22) protocols were assessed as sufficient. In total (see Table 3), 79% (87/110) obtained an optimal mark which is a significant improvement compared to the previous run 44 (2015) in which only 6% (1/18) of the protocols were assessed as optimal. This encouraging development in performance for this RTU system is difficult to elucidate upon, but the acceptance of focal staining of subtypes of macrophages outside lung tissue and laboratories following general advices given by NordiQC to protocol optimization (increase of analytical sensitivity) in former runs as also now officially recommended by vendor to use OptiView as detection system, could explain for higher proportion of optimal results obtained in this assessment. The main problems seen with this RTU system was related to the use of OptiView with amplification and to the use of the primary antibody lot numbers V0004826/V0004827, accounting for 45% (10/22) and 23% (5/22) of the insufficient results, respectively. As described above, the typically reaction pattern seen in these insufficient results was a granular false positive staining reaction in cellular structures expected to be negative e.g., neoplastic cells of the squamous cell carcinoma and epithelial cells of the appendix.

Within the RTU formats for Napsin A, 9% (14/151) of the laboratories used the RTU product PA0064 based on the mAb clone IP64 (Leica Biosystems). As shown in Table 3 and used within a system applying vendor recommended protocol settings, both the proportion of sufficient and optimal results were high, 100% (6/6) and 67% (4/6), respectively. In total, all (13/13) protocols produced a sufficient result, but proportion of optimal results was significant lower using laboratory modified protocol settings (27%, 2/7) and thus, laboratories are encouraged to follow recommendation given by the vendor. No single parameters could be identified explaining for the difference in optimal performance between vendor and laboratory modified protocol settings.

This was the third NordiQC assessment of Napsin A and the pass rate increased marginally from 78% in run 44 (2015) to 83% in this run 66 (2022) – see Graph 1. The most prevalent features influencing the

pass rate in negative direction was use of; 1) a polyclonal antibody (see Table 1) - 86% (6/7) of the protocols being insufficient, 2) OptiView with amplification - 52% (11/21) of the protocols being insufficient, 3) primary antibody (MRQ-60) lot numbers V0002826/27 developed for the RTU system 760-4867 (Ventana/Roche) - 100% (6/6) of the protocols being insufficient and 4) too diluted primary Ab based on the mAb clone IP64 as concentrate (dilution range 1:175-1:800) in which 30% (14/47) of the protocols gave an insufficient staining result. Importantly, the primary Abs must be carefully calibrated according to the expected antigen level of the recommended control material (see below).

Controls

Kidney can be used as positive tissue control for Napsin A. Virtually all epithelial cells of the convoluted/proximal tubules must show an at least moderate, distinct granular cytoplasmic staining reaction. Appendix is useful as negative tissue control; no staining reaction should be seen in the columnar epithelial cells. Dispersed macrophages might display a cytoplasmic reaction for Napsin A.

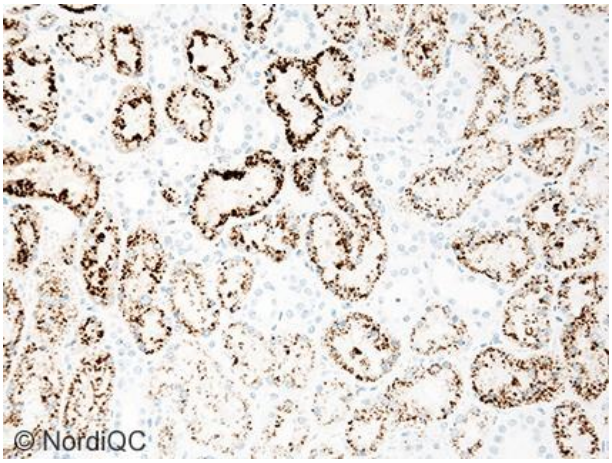


Fig. 1a (x200)
Optimal staining reaction for Napsin A of the kidney using the mAb clone IP64 as concentrate (optimally calibrated 1:50) on the BenchmarK Ultra (Ventana/Roche), efficient HIER in CC1 and OptiView as detection system.
The majority of epithelial cells of the convoluted/proximal tubules display an at least moderate and distinct cytoplasmic granular staining reaction. Same protocol used in Figs. 2a - 4a.

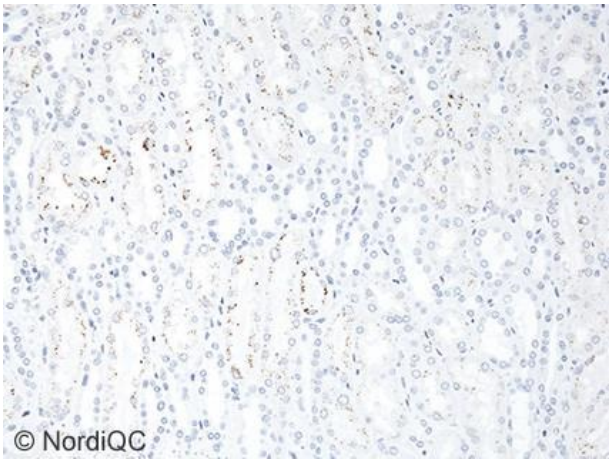


Fig. 1b (x200)
Insufficient staining reaction for Napsin A of the kidney using the mAb clone IP64 as concentrate, but with protocol settings, providing too low analytical sensitivity on the Benchmark Ultra e.g., too diluted primary Ab (1:400) in combination with UltraView as the detection system. The staining intensity and proportion of epithelial cells is significantly reduced - compare with Fig. 1a.

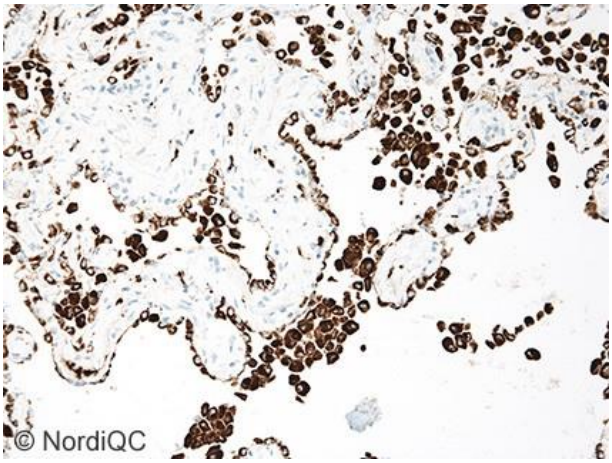


Fig. 2a (x200)
Optimal staining reaction for Napsin A of the lung tissue using same protocol as in Fig. 1a. Virtually all alveolar

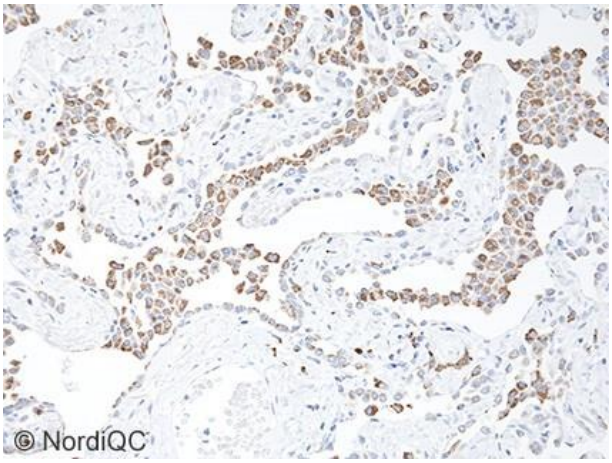


Fig. 2b (x200)
Insufficient staining reaction for Napsin A of the lung tissue using same protocol as in Fig. 1b. The staining intensity of alveolar macrophages is significantly reduced

macrophages and type II pneumocytes show a strong and distinct granular cytoplasmic staining reaction.

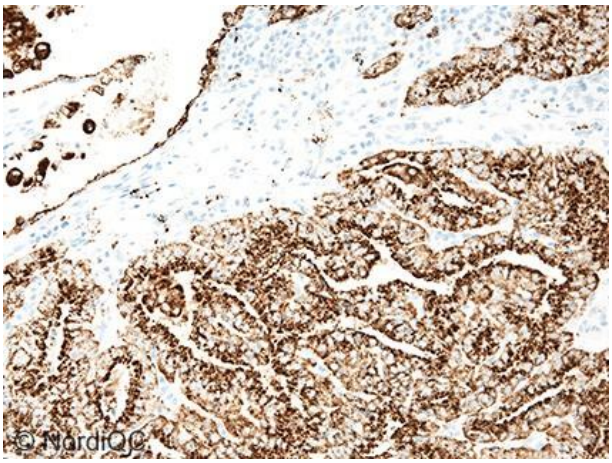


Fig. 3a (x200)
Optimal staining reaction for Napsin A staining of the lung adenocarcinoma (tissue core 3) using same protocol as in Figs. 1a and 2a. All the neoplastic cells display a strong and distinct granular cytoplasmic staining reaction.

and the majority of type II pneumocytes are false negative or only display a faint focal staining reaction– compare with Fig. 2a.

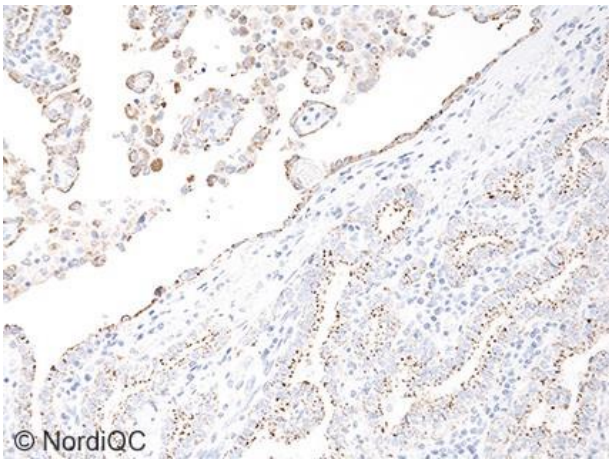


Fig. 3b (x200)
Insufficient staining reaction for Napsin A of the lung adenocarcinoma (tissue core 3) using same protocol as in Figs. 1b and 2b. The staining intensity of the neoplastic cells is significantly reduced – compare with Fig. 3a. The overall low analytical sensitivity of the protocol influence outcome in this case and could potentially be a problem in more challenging neoplastic tissue as e.g., renal cell carcinomas or lung adenocarcinomas with low expression of Napsin A.

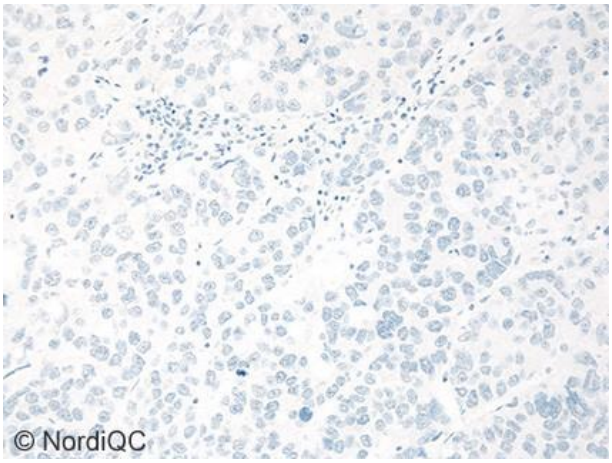


Fig. 4a (x200)
Optimal staining reaction for Napsin A of the lung squamous cell carcinoma using same protocol as in Figs. 1a – 3a. All neoplastic cells are negative as expected.

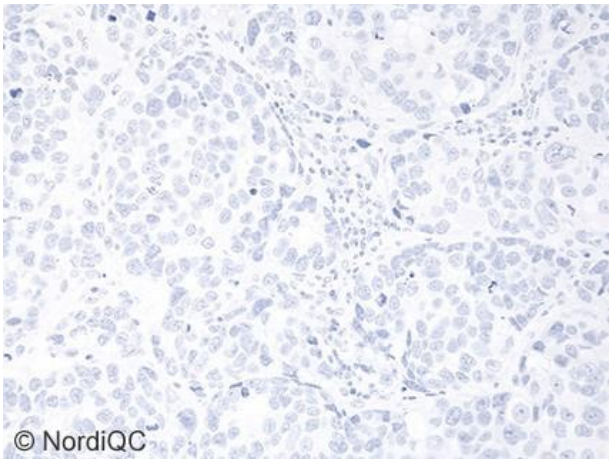


Fig. 4b (x200)
Staining for Napsin A of the lung squamous cell carcinoma using same insufficient protocol as in Figs. 1b and 3b. Although the protocol gave the expected reaction pattern, the protocol does not provide appropriate analytical sensitivity for demonstration of Napsin A – compare with Fig 1a-3b.

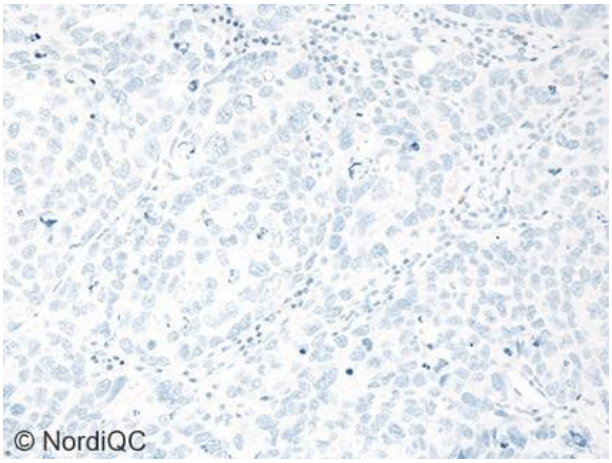


Fig. 5a (x200)
Optimal staining reaction for Napsin A of the lung squamous cell carcinoma using the RTU system 760-4867 (Benchmark Ultra, Ventana/Roche) based on the mAb clone MRQ-60. Vendor recommended protocol settings were applied and OptiView was used as the detection system. All (8/8) protocols based on the same conditions, gave an optimal result. The protocol also provided the same reaction patterns as illustrated in Fig 1a-3a. and as expected, a negative result in the neoplastic cells of the lung squamous cell carcinoma.

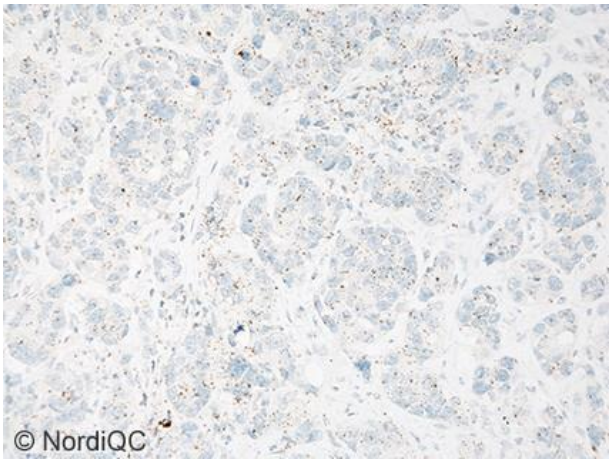


Fig. 5b (x200)
Insufficient staining reaction for Napsin A of the lung squamous cell carcinoma using the same RTU product as in Fig. 5a, but with extended HIER time in CC1 (64 min. at 98°C) extended incubation time in primary Ab (32 min.) and OptiView with amplification as detection system. The neoplastic cells display a false positive and granular staining reaction. This problem was often seen with tyramide signal amplification. Laboratories should be cautious using this amplification step due to the granular deposit of the reaction product, that erroneously can be interpreted as a specific signal for Napsin A - compare with Fig 5a.

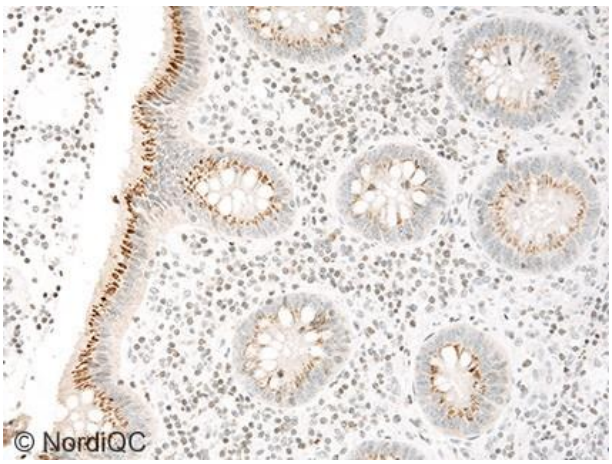


Fig. 6a (x200)
Insufficient staining reaction for Napsin A of the appendix using the RTU system 760-4446 (Benchmark Ultra, Ventana/Roche) based on a polyclonal antibody. The protocol gave an aberrant granular dot-like cytoplasmic staining reaction (false positive) of the epithelial cells in the appendix. In general, polyclonal antibodies should be substituted with more robust and specific antibodies as e.g., mAb clone MRQ-60 or IP64.

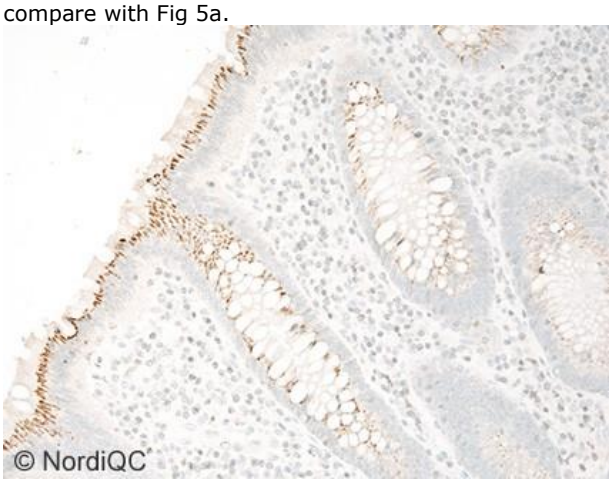


Fig. 6b (x200)
Insufficient staining reaction for Napsin A of the appendix using the same RTU system as in Fig 5a. The aberrant staining result in the epithelial cells is similar to the pattern seen for polyclonal antibodies as illustrated in Fig. 6a and was seen for the lot numbers V0004826 and V0004827 of the RTU system 760-4867 (Ventana/Roche). This deviating reaction pattern might be related to contamination with a polyclonal antibody and was not seen for other lots. All 5/5 protocols based on one of these two lots gave same insufficient result.

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