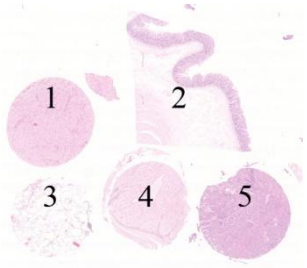
# Napsin A

## Assessment Run 39 2013

The slide to be stained for Napsin A comprised:




1. Kidney, 2. Colon, 3. Lung, 4. Renal clear cell carcinoma, 5. Lung adenocarcinoma
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing the Napsin A staining as optimal were:

- An at least moderate, granular cytoplasmic staining reaction of virtually all type II pneumocytes and alveolar macrophages in the lung.
- An at least weak to 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 lung adenocarcinoma and renal clear cell carcinoma.
- Negative staining reaction of normal columnar epithelial cells and macrophages in lamina propria in the colon.

104 laboratories participated in this assessment. 58 % achieved a sufficient mark (optimal or good). Antibodies (Abs) used and marks are summarized in table 1.

Table 1. **Antibodies and assessment marks for Napsin A, run 39**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **IP64** | 43<br>1 | Leica/Novocastra<br>Zhongshan | 27 | 11 | 5 | 1 | 86 % | 92 % |
| rmAb clone **KCG1.1** | 3<br>1 | Diagnostic Biosystems<br>Acris | 0 | 2 | 2 | 0 | - | - |
| mAb clone **MRQ-60** | 4 | Cell Marque | 2 | 1 | 1 | 0 | - | - |
| mAb clone **TMU-Ad02** | 5<br>3<br>2 | Biocare<br>IBL<br>Novus Biologicals | 0 | 1 | 8 | 1 | 10 % | - |
| rmAb clone **EP205** | 1 | Epitomics | 0 | 1 | 0 | 0 | - | - |
| rmAb clone **EPR6257** | 1 | Epitomics | 0 | 0 | 0 | 1 | | |
| pAb **352A-7x** | 12 | Cell Marque | 1 | 5 | 5 | 1 | 50 % | 100 % |
| pAb **ILP3523 C1** | 1 | Immunologic | 0 | 0 | 1 | 0 | - | - |
| Unknown | 1 | Unknown | 0 | 1 | 0 | 0 | - | - |
| Ready-To-Use antibodies | n | | | | | | | |
| mAb clone **MRQ-60 352M-9x** | 1 | Cell Marque | 0 | 1 | 0 | 0 | - | - |
| mAb clone **MRQ-60 760-4867** | 3 | Ventana/Cell Marque | 0 | 3 | 0 | 0 | - | - |
| mAb clone **TMU-Ad02 PM388** | 1 | Biocare | 0 | 0 | 1 | 0 | - | - |
| rmAb clone **BC15 API 3043** | 1 | Biocare | 0 | 0 | 0 | 1 | - | - |
| pAb **GT210302** | 1 | Gene Tech | 0 | 0 | 1 | 0 | - | - |
| pAb **MAD-005064QD** | 1 | Master Diagnostica | 0 | 0 | 0 | 1 | - | - |
| pAb **760-4446** | 18 | Ventana/Cell Marque | 1 | 3 | 14 | 0 | 22 % | - |
| Total | 104 | | 31 | 29 | 38 | 6 | - | |
| Proportion | | | 30 % | 28 % | 36 % | 6 % | 58 % | |

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 39**

The following protocol parameters were central to obtain an optimal staining:

**Concentrated antibodies**
mAb clone **IP64**: 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) (3/4), Cell Conditioning 1 (CC1; Ventana) (9/14), CC2 (BenchMark, Ventana) (1/2), Bond Epitope Retrieval Solution 1 (BERS1; Leica) (5/8), BERS 2 (Leica) (5/9) or Tris-EDTA/EGTA pH 9 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 35 of 38 (92 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **MRQ-60**: The two protocols with an optimal result were both based on HIER using either TRS pH 9, (3-in-1) (Dako) (1/1) or Citrate pH 6 (1/2) as retrieval buffer. The mAb was diluted in the range of 1:500-1:800 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 of 3 (100 %) laboratories produced a sufficient staining (optimal or good).

pAb **352A-7x**: The protocol with an optimal result was based on HIER using TRS pH 9, (3-in-1) (Dako) (1/2) and a dilution of 1:1.000. Using these protocol settings 2 of 2 laboratories produced a sufficient staining (optimal or good).

**Table 2. Optimal results for Napsin A using concentrated antibodies 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 **IP64** | 83 % 5/6** | 0 % 0/1 | 64 % 9/14 | 50 % 1/2 | 83 % 5/6 | 71 % 5/7 |

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

**Ready-To-Use Antibodies**
pAb prod. no. **760-4446** (Ventana/Cell Marque): The protocol with an optimal result was based on HIER using mild Cell Conditioning 1, 16 min. incubation of the primary Ab and UltraView (760-500) plus amplification kit as detection system.

The most frequent causes of insufficient staining were:

- Less successful primary Ab
- Too low concentration of the primary Ab

In this assessment, the prevalent features of an insufficient staining were either a false positive staining reaction and/or a generally too weak staining reaction.
False positive staining reaction was seen in 55 % of the insufficient results (24 of 44), 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 high proportion of insufficient results as 5 of 12 and 12 of 18 protocols, respectively, gave a false positive staining reaction of columnar epithelial cells of the colon. The mAb clone TMU-AdD02 typically 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. False positive staining reaction was also seen with the mAb clones MRQ-60 and KCG1.1, but less frequently.
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 and the normal epithelial cells of the renal proximal tubules. A too weak staining was most frequently caused by a too low titre of the primary Ab.

The mAb clone IP64 gave the highest proportion of sufficient and optimal results, as seen in table 1.
Optimal result could be obtained on the 3 most widely used IHC platforms, as shown in table 2. Both HIER in alkaline and non-alkaline buffers in combination with either 2-step or 3-step polymer/multimer based detection systems could be used to obtain optimal results. Biotin based detection systems cannot be recommended as granular cytoplasmic staining reaction for Napsin A is similar to unspecific staining pattern of endogenous biotin.

**Controls**
Both kidney and lung can be used as positive tissue control for Napsin A. However, in this test, kidney was found slightly 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. In this assessment it was observed that an acceptable staining reaction could be seen in the normal lung, while a too weak staining reaction was seen in the neoplasias included in the test material circulated, underlining that normal lung is not reliable as positive tissue control for an appropriate calibration of a Napsin A protocol.
Colon is useful as negative control; no staining should be seen in the columnar epithelial cells and macrophages.

**Conclusion**
In this assessment the mAb clone **IP64** was the most robust and specific Ab for the demonstration of Napsin A. 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 TMA-Ad02, pAb 352A-X (Cell Marque) and pAb760-4446 (Ventana) gave a high proportion of false positive staining reactions. Kidney is recommendable as positive tissue control; virtually all epithelial cells of the proximal tubules must show an at least weak-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.

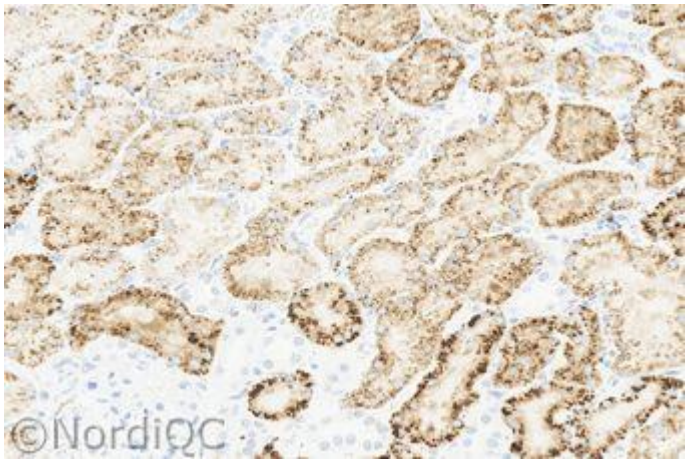


Fig. 1a
Optimal Napsin A staining of the kidney using the mAb clone IP64 optimally calibrated, HIER in an alkaline buffer and a polymer based detection kit. A weak to moderate, granular cytoplasmic staining reaction is seen in the vast majority of the epithelial cells of the proximal tubules. Also compare with Figs. 2a - 4a, same protocol.

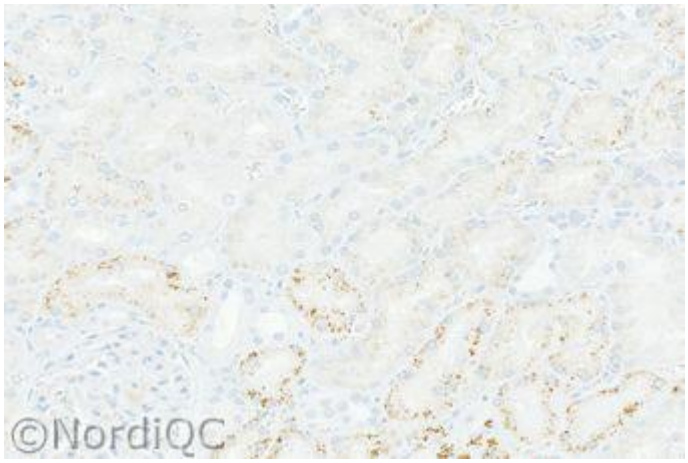


Fig. 1b
Insufficient Napsin A staining of the kidney using the mAb clone IP64 by protocol settings giving a too low sensitivity - same field as in Fig. 1a. Also compare with Figs. 2b - 3, same protocol.

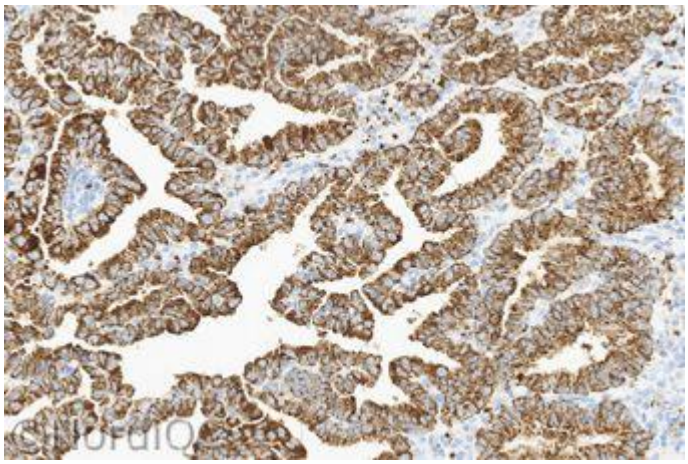


Fig. 2a
Optimal Napsin A staining of the lung adenocarcinoma using same protocol as in Fig. 1a. The majority of the neoplastic cells show a moderate to strong granular cytoplasmic staining reaction. No background staining is seen.

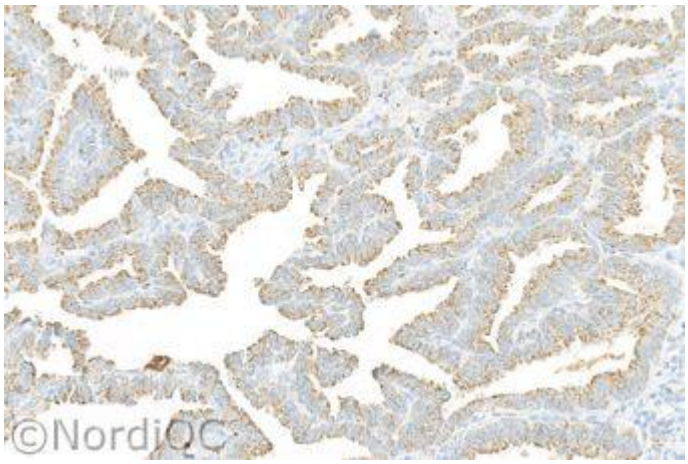


Fig. 2b
Insufficient Napsin A staining of the lung adenocarcinoma using same protocol as in Fig. 1b - same field as in Fig. 2a. The intensity and proportion of cells demonstrated is significantly reduced compared to the level expected and obtained in Fig. 2a.

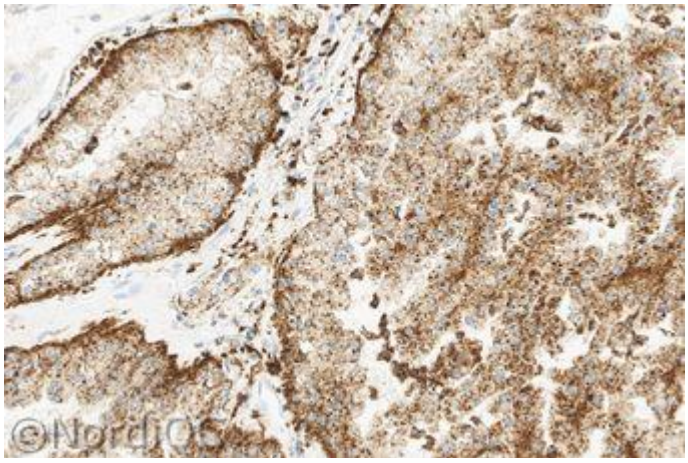



Fig 3a
Optimal Napsin A staining of the renal clear cell carcinoma using same protocol as in Figs. 1a & 2a. The majority of the neoplastic cells show a moderate granular cytoplasmic staining reaction.

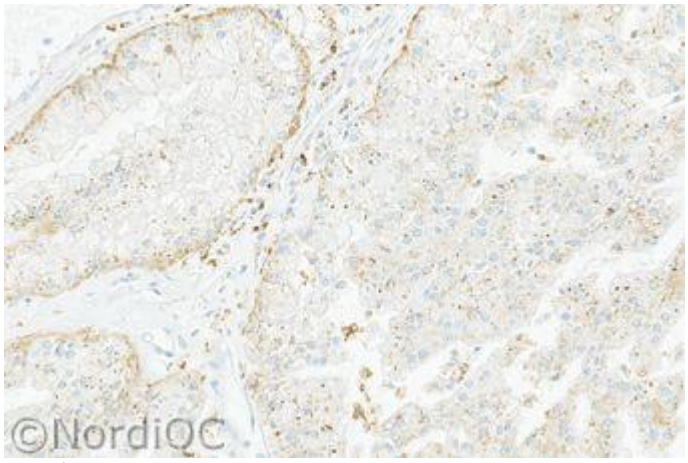



Fig. 3b
Insufficient Napsin A staining of the renal clear cell carcinoma using same protocol as in Figs. 1b & 2b - same field as in Fig. 3a. Only a weak and equivocal staining reaction in scattered cells is seen.

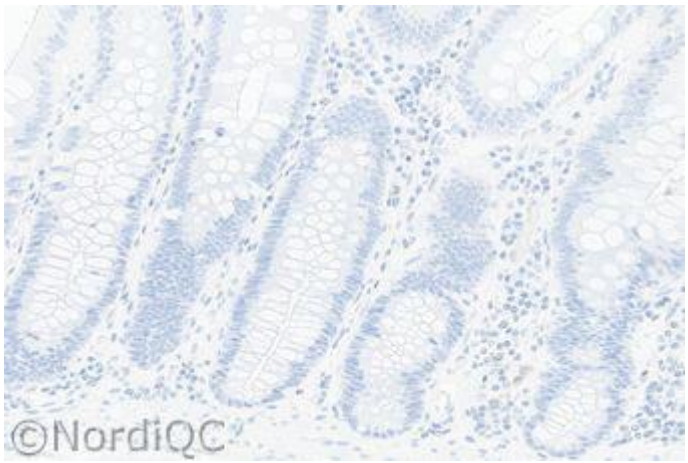



Fig. 4a
Optimal Napsin A staining for Napsin A of the colon using same protocol as in Figs. 1a - 3a. No staining in epithelial cells or macrophages is seen.

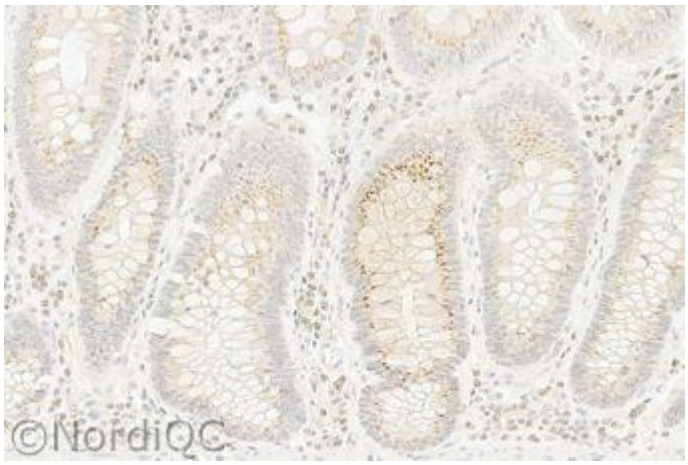



Fig. 4b
Insufficient Napsin staining of the colon - same field as in Fig. 4a. The protocol was based on a polyclonal Ab (Ventana / Cell Marque) giving an aberrant granular cytoplasmic staining reaction in the epithelial cells not supposed to be demonstrated.

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