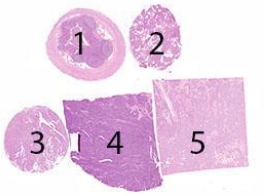
## Assessment Run 71 2024

# Insulinoma-associated protein 1 (INSM1)

**Purpose**
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for INSM1, typically used in the diagnostic work-up of neuroendocrine tumors. Relevant clinical tissues, both normal and neoplastic, were selected to display a broad spectrum of antigen densities for INSM1 (see below).

**Material**
The slide to be stained for INMS1 comprised:




1. Appendix, 2. Pancreas, 3. Lung adenocarcinoma, 4. Small cell lung carcinoma (SCLC) 5. Neuroendocrine carcinoma (mamma).

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing INSM1 staining as optimal included:

- A strong and distinct nuclear staining reaction of neuroendocrine cells in the appendiceal mucosa and endocrine cells in the pancreatic islets of Langerhans.
- An at least moderate to strong, distinct nuclear staining reaction of the vast majority of neoplastic cells in the SCLC.
- An at least weak nuclear staining reaction of the vast majority of neoplastic cells in the neuroendocrine carcinoma.
- No staining reaction of the appendiceal columnar epithelial cells, pancreatic exocrine cells and neoplastic cells in the lung adenocarcinoma.

**KEY POINTS FOR INSM1 IMMUNOASSAYS**
- The rmAb clone **MRQ-70** is recommendable both as a concentrated Ab and an RTU.
- The widely used mAb clone **A-8** seems less reproducible overall and especially gives an inferior performance on Ventana BenchMark platforms.

**Participation**

| | |
|---|---|
| Number of laboratories registered for INSM1, run 71 | 169 |
| Number of laboratories returning slides | 156 (92%) |

All slides returned after the assessment were assessed and received advice if the result being insufficient, but the data were not included in this report.

**Results**
156 laboratories participated in this assessment. 112 (72%) achieved a sufficient mark (optimal or good), see Table 1a (see page 2). Tables 1b and 1c summarizes the antibodies (Abs) used and assessment marks (see page 2 and 3).

The most frequent cause of insufficient staining reactions was:
- Less successful primary Ab format of clone A-8 from Santa Cruz

**Performance history**
This was the first NordiQC assessment of INSM1 and the overall pass rate was 72%.

**Controls**
In appendix, neuroendocrine cells in the appendiceal mucosa should display a moderate to strong nuclear staining reaction. Columnar epithelial cells and smooth muscle cells should be negative. A weak staining reaction can be found in ganglion cells and scattered lymphocytes, but were in this assessment not consistent, and thus not implemented as a criterion for an optimal staining and indicator of low limit of demonstration of INSM1. In pancreas, an least moderate to strong nuclear staining reaction should be seen in the endocrine cells in the islets of Langerhans.

**Conclusion**
The rmAb clone **MRQ-70** was the most successful Ab for the demonstration of INSM1. As concentrated (conc.) format within a laboratory developed assay, optimal results were obtained on all four main stainer platforms. Efficient HIER in an alkaline buffer and carefully calibrated primary Ab together with a sensitive detection system were the most important prerequisites for a sufficient staining. The mAb clone A-8 gave overall an inferior and less reproducible performance.

Table 1a. **Overall results for INSM1, run 71**

| | n | Optimal | Good | Borderline | Poor | Suff.[1] | OR[2] |
|---|---|---|---|---|---|---|---|
| Concentrated antibodies | 104 | 39 | 31 | 20 | 14 | 68% | 38% |
| Ready-To-Use antibodies | 52 | 16 | 26 | 4 | 6 | 81% | 31% |
| Total | 156 | 55 | 57 | 24 | 20 | | |
| Proportion | | 35% | 37% | 15% | 13% | 72% | |

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for INSM1, run 71**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | OR[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **A-8** | 1 | Diagnostic Biosystems | 1 | - | - | - | - | - |
| | 5 | Gennova | 1 | 2 | 1 | 1 | 60% | 20% |
| | 1 | Master Diagnostica | - | - | 1 | - | - | - |
| | 1 | Monosan | 1 | - | - | - | - | - |
| | 2 | Nordic Biosite | - | 1 | 1 | - | - | - |
| | 50 | Santa Cruz | 10 | 15 | 13 | 12 | 50% | 20% |
| | 5 | Zeta Corporation | 4 | 1 | - | - | 100% | 80% |
| mAb clone **BSB-123** | 3 | Bio SB | - | 3 | - | - | - | - |
| | 1 | LS Bio | - | 1 | - | - | - | - |
| rmAb clone **MSVA-456R** | 1 | MS Validated Antibodies | - | 1 | - | - | - | - |
| rmAb clone **BP6240** | 1 | Biolynx Biotechnology | 1 | - | - | - | - | - |
| rmAb clone **EPR23199-37-6-1** | 1 | Abcam | - | - | 1 | - | - | - |
| rmAb clone **MRQ-70** | 27 | Cell Marque | 19 | 6 | 2 | - | 93% | 70% |
| rmAb clone **QR116** | 1 | Quartett | - | - | - | 1 | - | - |
| rmAb clone **RBT-INSM1** | 2 | Bio SB | - | 1 | 1 | - | - | - |
| rmAb clone **ZR395** | 2 | Zeta Corportation | 2 | - | - | - | - | - |
| Total | 104 | | 39 | 31 | 20 | 14 | | |
| Proportion | | | 38% | 30% | 19% | 13% | 68% | |

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).

Table 1c. **Ready-To-Use antibodies and assessment marks for INSM1, run 71**

| Ready-To-Use antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | OR[2] |
|---|---|---|---|---|---|---|---|---|
| mAb **A-8**<br>**AMB44-5M** | 2 | BioGenex | 1 | - | - | 1 | - | - |
| mAb clone **A-8**<br>**MAD-000777QD** | 10 | Master Diagnostica/ Vitro SA | 2 | 6 | 2 | - | 80% | 20% |
| mAb clone **A-8**<br>**PDM586** | 3 | Diagnostic Biosystems | 1 | 1 | - | 1 | - | - |
| mAb clone **A-8**<br>**MAB-1017** | 1 | Fuzhou Maixin | - | 1 | - | - | - | - |
| mAb clone **BSB-123**<br>**BSB 3553/4/5** | 11 | Bio SB | - | 7 | 2 | 2 | | |
| mAb clone **DA267**<br>**DMRD0168** | 1 | Dartmon Biotechnology | 1 | - | - | - | - | - |
| mAb clone **BY059**<br>**BFM-0177** | 1 | Bioin Biotechnology | - | 1 | - | - | - | - |
| rmAb clone **IHC741**<br>**IHC741** | 1 | GenomeMe | - | 1 | - | - | - | - |
| rmAb clone **BLR272L**<br>**API3299** | 1 | Biocare Medical | - | 1 | - | - | - | - |
| rmAb clone **315I4E7**<br>**PA598** | 1 | Abcarta | - | 1 | - | - | - | - |
| rmAb clone **GR013**<br>**GT246802** | 1 | Gene Tech | 1 | - | - | - | - | - |
| rmAb clone **INSM1/6286R**<br>**ANC07-5M** | 1 | BioGenex | - | - | - | 1 | - | - |
| rmAb clone **MRQ-70**<br>**475-97/98** | 16 | Cell Marque | 9 | 6 | - | 1 | 94% | 56% |
| rmAb clone **RBT-INSM1**<br>**BSB-3780-3/7/15** | 1 | Bio SB | - | 1 | - | - | - | - |
| rmAb clone **ZR395**<br>**Z2751RP** | 1 | Zeta Corporation | 1 | - | - | - | - | - |
| Total | 52 | | 16 | 26 | 4 | 6 | | |
| Proportion | | | 31% | 50% | 8% | 11% | 81% | |

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).

**Detailed analysis of INSM1, Run 71**
The following protocol parameters were central to obtain optimal staining:

**Concentrated antibodies**
mAb clone **A-8**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS, Dako/Agilent) High pH (8/16)*, TRS Low pH (1/3) (Dako/Agilent), Cell Conditioning 1 (CC1, Ventana/Roche) (2/30), Cell Conditioning 2 (CC2, Ventana/Roche) (1/2) or Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (5/11) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:1,000. Using these protocol settings, 36 of 62 (58%) laboratories produced a sufficient staining result (optimal or good).
*(number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **MRQ-70**: Protocols with optimal results were based on HIER using TRS High pH (6/11) (Dako/Agilent), CC1 (Ventana/Roche) (12/14) or BERS2 (Leica Biosystems) (1/2) as retrieval buffer. The mAb was diluted in the range of 1:25-1:200. Using these protocol settings, 25 of 27 (93%) laboratories produced a sufficient staining result.

Table 2. **Proportion of optimal results for INSM1 for the most commonly used antibody concentrates on the four main IHC systems***

| Concentrated antibodies | **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 |
| mAb clone **A-8** | 2/5** (40%) | 1/1 | 6/12 (50%) | 0/2 | 2/30 (7%) | 1/2 | 5/10 (50%) | 0/1 |
| rmAb clone **MRQ-70** | 1/2 | - | 5/9 (56%) | - | 12/14 (86%) | - | 1/2 | - |

* 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 Ultra, Ultra plus
3) Bond III

**Ready-To-Use antibodies and corresponding systems**
No Ready-To-Use Abs with a corresponding system (≥5 assessed protocols) were giving optimal results in this assessment.

**Comments**
In this assessment, the prevalent feature of an insufficient result was a false positive staining reaction of cells and structures expected to be negative, e.g. muscle cells, exocrine cells in pancreas and neoplastic cells in the lung adenocarcinoma. This pattern was observed in 57% of the insufficient results (25 of 44). 23% (10 of 44) of the insufficient results was caused by a too weak or completely false negative staining result in structures expected to be positive. Virtually all laboratories were able to demonstrate INSM1 in high-level antigen expressing structures such as neoplastic cells of the SCLC and normal neuroendocrine cells in the appendix and pancreatic Langerhans islets. Demonstration of INSM1 in low-level expressing structures as neoplastic cells of the breast neuroendocrine carcinoma was more challenging and required a carefully calibrated protocol. The remaining insufficient results were caused by either excessive background staining, impaired morphology, excessive counterstaining or poor signal-to-noise ratio (20%).

67% (104 of 156) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for INSM1. The most successful **rmAb clone MRQ-70** was used by 27 participants, giving a pass rate of 93%, 70% optimal (see Table 1b). Optimal results could be obtained on the main platforms from Dako/Agilent, Ventana/Roche and Leica Biosystems (see Table 2).

The **mAb clone A-8** was the most widely used antibody for demonstration of INSM1 and as a concentrate, gave an overall, inferior pass rate of 55%, 26% optimal. The main prerequisites for sufficient staining were use of HIER in an alkaline buffer, careful calibration of the titre of the primary Ab and preferably a 3-step detection system. However, it was observed that despite similar protocol settings were applied by the participants, both sufficient and insufficient results were obtained, and the insufficient results being caused by either false positive or false negative staining reactions. No cause for this irreproducibility has been found in the submitted data. As seen in Table 2, the clone was found challenging on the Ventana BenchMark platform with only 9% optimal in total. It was also observed that the vendor of the clone impacted the performance - data have to be interpreted cautiously due to limited data points. If the mAb clone A-8 was acquired from Zeta Corporation as concentrated format, 100% (5/5) of results based on this product were assessed as sufficient and 4 of these being optimal. In comparison, if the protocols were based on A-8 as concentrate from Santa Cruz, only 50% (25/50) of the results were sufficient. As described above and showed in Figs 1b-5b and 5a, the insufficient results were either false positive or false negative/too weak despite comparable protocols being applied. The less reproducible results for mAb clone A-8 from Santa Cruz do indicate some degree of lot-to-lot variations and in case of inappropriate results with clone A-8 it can be recommended to test a different lot or change to another more successful antibody.

The RTU format of the rmAb clone **MRQ-70** (**475-97/98**) from Cell Marque gave a high proportion of sufficient and optimal results as shown in Table 1c. Optimal and sufficient results were seen on the fully automated platforms from Dako/Agilent, Ventana/Roche and Leica Biosystems, with similar protocol settings as for the concentrated format.

Overall, for both concentrated and RTU Abs, the rmAb clone MRQ-70 gave a superior performance with a pass rate of 93% (40 of 43), 65% optimal (n=28) compared to the mAb clone A-8 with a 59% pass rate (48 of 81), 26% optimal (n=21).

In this first assessment of INSM1, various Ab clones were used (see Table 1b and 1c). Despite limited data, more clones seem promising: e.g. the **mAb clone BSB-123** gave an overall pass rate of 73% (11 of 15), no optimal results though, and the **rmAb clone ZR395** was used by 3 participants, all optimal.

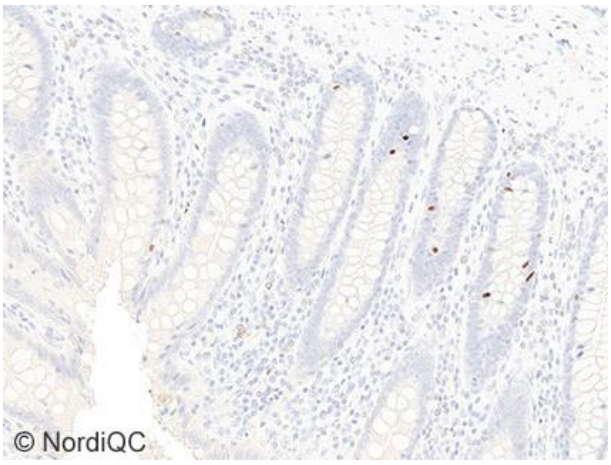



Fig. 1a
Optimal INSM1 staining reaction of the appendix mucosa using the rmAb clone MRQ-70 in a concentrated format (1:25), using HIER at high pH for 48 min., 32 min. incubation of the primary Ab, OptiView as detection system and performed on BenchMark Ultra.
The neuroendocrine cells show an intense, distinct nuclear staining reaction, whereas epithelial cells are negative.
Also compare with Figs. 2a - 5a – same protocol.

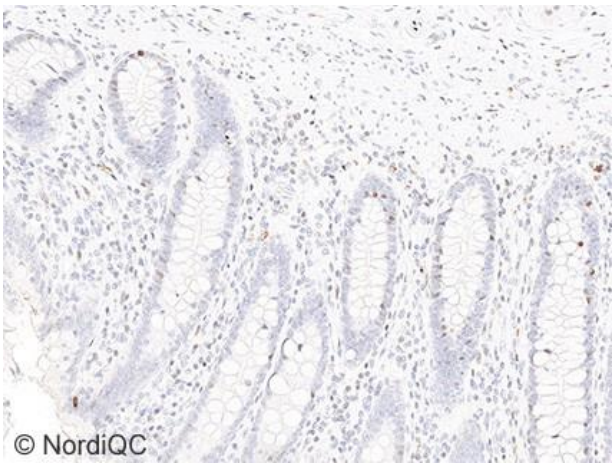



Fig. 1b
Insufficient INSM1 staining reaction of the appendix mucosa using a protocol not calibrated appropriately. In addition to the positive staining reaction in neuroendocrine cells, lymphocytes and smooth muscle cells are also aberrantly weakly positive.
The protocol was based on the mAb clone A-8 as a concentrated format (1:100) using HIER at high pH for 32 min., 32 min. incubation of the primary Ab, OptiView as detection system and performed on BenchMark Ultra.
Also compare with Figs. 2b - 5b – same protocol.

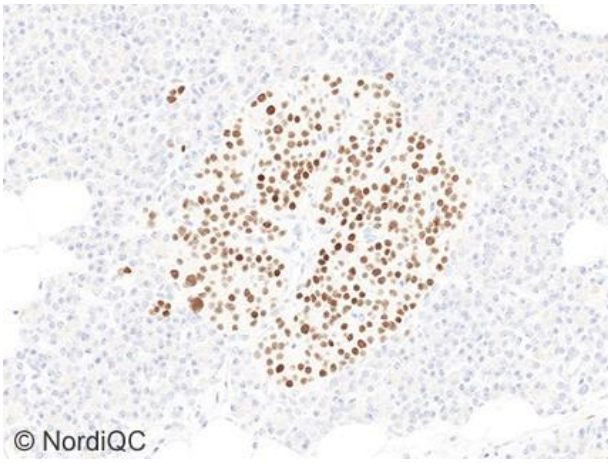



Fig. 2a
Optimal INSM1 staining reaction of the pancreas using same protocol as in Fig. 1a. The vast majority of endocrine islet cells show a strong and distinct nuclear staining reaction.
Also compare with Figs. 3a - 5a – same protocol.

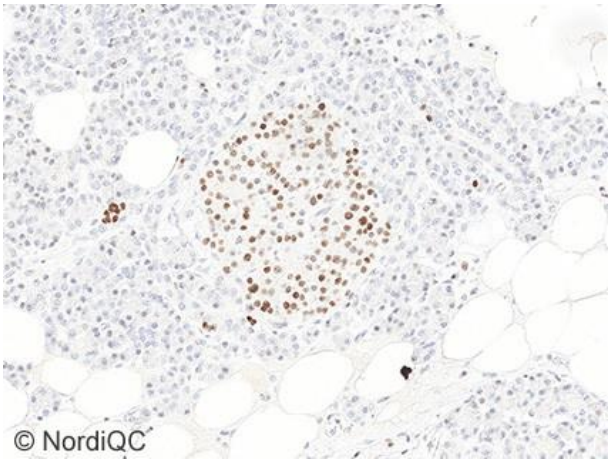



Fig. 2b
Insufficient INSM1 staining reaction of the pancreas using same protocol as in Fig. 1b – same field as in Fig. 2a.
The endocrine islet cells are positive as expected. However, scattered exocrine cells are false positive.
Also compare with Figs. 3b - 5b – same protocol.

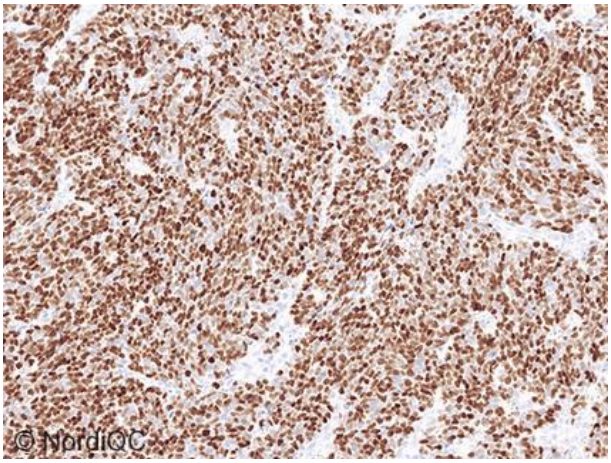



Fig. 3a
Optimal INSM1 staining reaction of the SCLC using same protocol as in Figs. 1a and 2a.
Virtually all the neoplastic cells show a strong and distinct staining reaction.

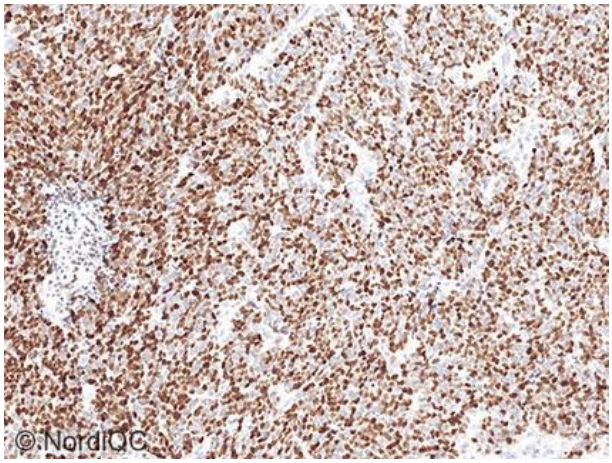



Fig. 3b
INSM1 staining reaction of the SCLC using the same insufficient protocol as in Figs. 1b and 2b – same field as in Fig. 3a.
Also compare with Fig. 4b and 5b – same protocol.

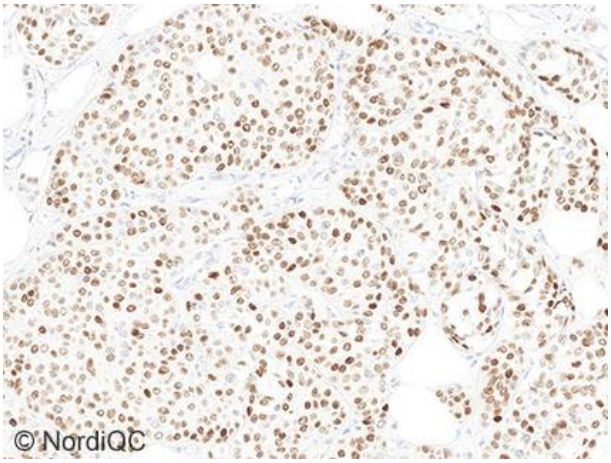



Fig. 4a
Optimal INSM1 staining reaction of the neuroendocrine carcinoma using same protocol as in Figs. 1a – 3a.
The neoplastic cells show a weak to moderate nuclear staining reaction.

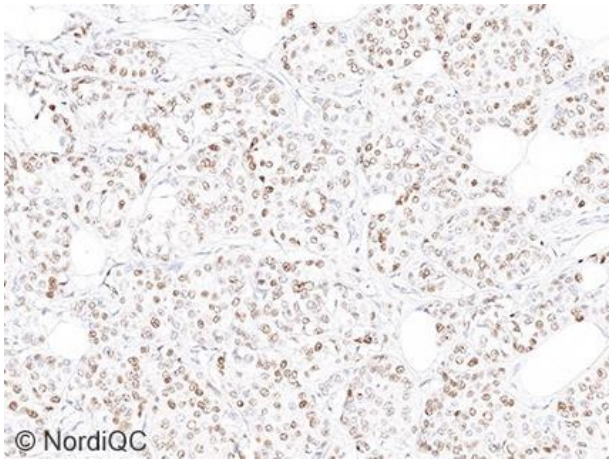



Fig. 4b
INSM1 staining reaction of the neuroendocrine carcinoma, using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a.
In general, a weaker staining reaction is seen.

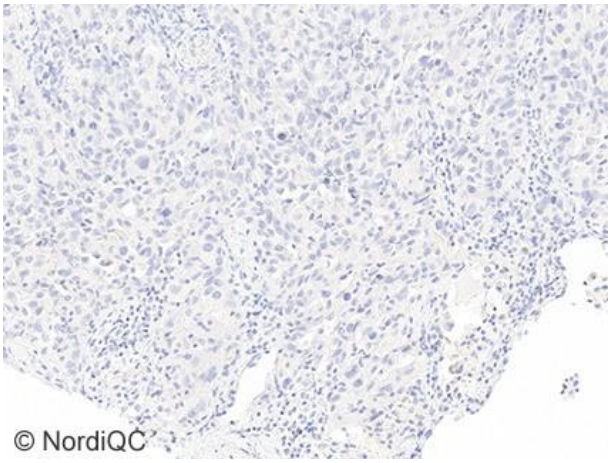



Fig. 5a
Optimal INSM1 staining reaction of the lung adenocarcinoma using same protocol as in Figs. 1a – 4a.
No staining reaction is observed.

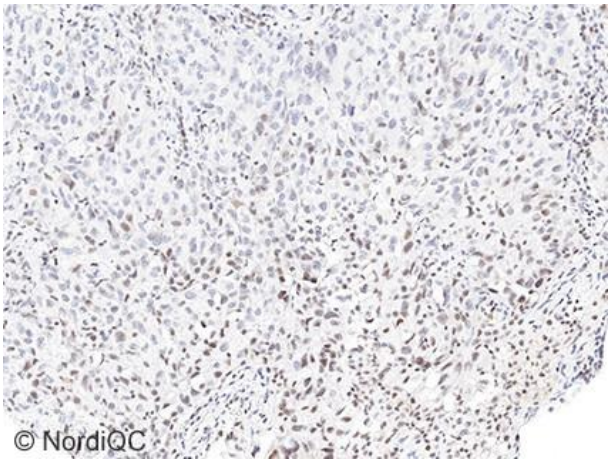



Fig. 5b
Insufficient INSM1 staining reaction of the lung adenocarcinoma using same protocol as in Figs. 1b - 4b.
An aberrant weak to moderate nuclear staining reaction is seen in neoplastic cells expected to be negative. Compare with optimal result in Fig. 5a.

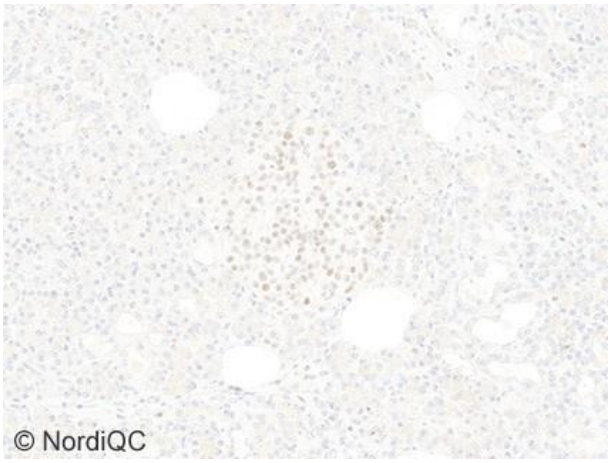



Fig. 6a
Insufficient INSM1 staining reaction of pancreas using exactly same protocols as in Figs. 1b – 5b, But in a different laboratory.
A too weak staining reaction is seen in the endocrine islet cells. Compare with optimal result in Fig. 2a.
The mAb clone A-8 was found to give less reproducible results among the participants. Same protocol settings could give the expected results, results characterized as false positive or false negative.

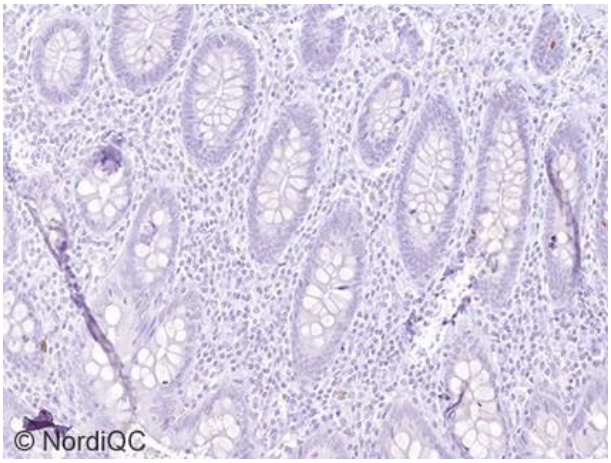



Fig. 6b
Insufficient INSM1 staining of the appendix mucosa.
The protocol was based on the mAb clone A-8 as a concentrated format (1:150) using HIER at high pH for 64 min., 48 min. incubation of the primary Ab, OptiView as detection system and performed on BenchMark Ultra.
The excessive counterstaining masks the weakly positive neuroendocrine cells.

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