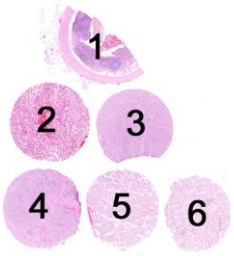
Assessment Run 35 2012

Placental Alkaline Phosphatase (PLAP)

Material
The slide to be stained for PLAP comprised:



1. Appendix, 2. Placenta , 3. Seminoma, 4. Embryonal carcinoma, 5-6. Testicular intratubular
germinal cell neoplasia (IGCN).

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a PLAP staining as optimal included:

- A strong and distinct predominantly membranous but also cytoplasmic staining reaction of virtually all the neoplastic cells of the two IGCNs and the seminoma
- An at least weak to moderate predominantly membranous but also cytoplasmic staining reaction of the majority of the neoplastic cells of the embryonal carcinoma.
- A strong predominantly membranous but also cytoplasmic staining reaction of the placental cytotrophoblasts and syncytiotrophoblasts with no or only a minimal reaction in the stromal cells.

For the mAb clones PL8-F6 (BioGenex) and NB10 (Cell Marque/Ventana) a dot-like intracytoplasmic staining reaction in the epithelial cells of the appediceal mucosa was accepted. This staining pattern is related to the "Mouse-Anti-Golgi" (MAG) staining due to the ascites format of these two antibodies in combination with tissues from patients with blood group A. In this test appendix of blood group A was selected. The MAG staining reaction did not cause downmarking, because it did not interfere with the interpretation.

For the mAb clone 8A9 (many vendors see table 1), an intracytoplasmic staining reaction in smooth muscle cells was accepted. Literature* has shown hat PLAP can be expressed in both normal and neoplastic smooth muscle cells. NordiQC previously downmarked this cross-reaction because we did not find this pattern compatible with an optimal staining, but due to the publications and ongoing studies to investigate if PLAP expression is present in muscle cells, the staining reaction in smooth muscle cell currently has to be accepted.
* Detection and diagnostic utilization of placental alkaline phosphatase in muscular tissue and tumors with myogenic differentiation. Jeffrey D Goldsmith et al. *Am J Surg Pathol 26(12): 1627–1633, 2002.*

132 laboratories participated in this assessment. 91% achieved a sufficient mark. In table 1, the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for PLAP, run 35**

Concentrated Abs	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **8A9**	36 12 1 1	Dako Leica/Novocastra Monosan Thermo/NeoMarkers	20	22	7	1	84 %	89 %
mAb clone **NB10**	1 1	Cell Marque Zytomed	2	0	0	0	-	-
mAb clone **PL8/F6**	28	BioGenex	22	5	1	0	96 %	96 %
mAb clone **SP15**	6 1	Thermo/NeoMarkers Cell Marque	3	4	0	0	100 %	100 %
pAb **A0268**	1	Dako	0	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **8A9 IR/IS779**	18	Dako	12	6	0	0	100 %	100 %

mAb clone **8A9 PA0161**	2	Leica/Novocastra	2	0	0	0	-	-
mAb clone **NB10 760-2664**	20	Ventana/Cell Marque	15	5	0	0	100 %	100 %
mAb clone **NB10 321M-18**	1	Cell Marque	0	0	1	0	-	-
mAb clone **PL8/F6 AM228-5M**	3	BioGenex	1	0	1	1	-	-
Total	132		77	43	10	2	-	
Proportion			58 %	33 %	8 %	1 %	91 %	

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

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

Concentrated Abs
mAb clone **8A9:** The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Target Retrieval Solution pH 9 (3-in-1) (TRS pH 9; Dako) (6/9)*, TRS pH 9 (Dako) (1/3)*, TRS pH 6.1 (3-in-1) (Dako) (1/2)*, Cell Conditioning 1 (CC1; BenchMark, Ventana) (4/17)*, Bond Epitope Retrieval Solution 2 (BERS 2; Bond, Leica) (4/7)*,Tris-EDTA/EGTA pH 9 (3/5)* or Citrate pH 6 (1/2)* as the retrieval buffer.
The mAb was typically diluted in the range of 1:25-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 34 out of 38 (89 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **NB10**: The two protocols giving an optimal result were both based on HIER using either CC1 (BenchMark, Ventana) (1/1) or Tris-EDTA/EGTA pH 9 (1/1) as the retrieval buffer.
The mAb was diluted in the range of 1:50-1:200 depending on the total sensitivity of the protocol employed.

mAb clone **PL8/F6**: The protocols giving an optimal result were all based on HIER using either TRS pH 9 (3-in-1, Dako) (6/6),TRS pH 9 (Dako) (4/4), TRS pH 6.1 (Dako) (1/1), CC1 (BenchMark, Ventana) (6/10), BERS 2 (Bond, Leica) (3/3), Tris-EDTA/EGTA pH 9 (1/1) or Diva Decloaker pH 6.2 (Biocare) (1/1) as the retrieval buffer.
The mAb was typically diluted in the range of 1:40-1:800 depending on the total sensitivity of the protocol employed. Using these protocol settings 25 out of 26 (96 %) laboratories produced a sufficient staining (optimal or good).

rmAb clone **SP15**: The protocols giving an optimal result were all based on HIER using either TRS pH 9 (Dako) (1/1), CC1 (BenchMark, Ventana) (1/3) or Tris-EDTA/EGTA pH 9 (1/2) as the retrieval buffer.
The mAb was diluted in the range of 1:100-1:1.600 depending on the total sensitivity of the protocol employed. Using these protocol settings 4 out of 4 (100 %) laboratories produced a sufficient staining (optimal or good).

Ready-To-Use Abs
mAb clone **8A9** (prod. no. IS/IR779, Dako): The protocols giving an optimal result were all based on HIER in PT-Link (heating time for 10-20 min at 97-98°C) using TRS pH 9 (3-in-1) (Dako) or TRS pH 9 (Dako) as HIER buffer, an incubation time of 20 min in the primary Ab and EnVision Flex (K8000) as the detection system. Using these protocol settings 17 out of17 (100 %) laboratories produced a sufficient staining.

mAb clone **8A9** (prod. no. PA0161, Leica/Novocastra): The protocols giving an optimal result were based on HIER using BERS 2 (Bond, Leica), an incubation time of 15 min in the primary Ab and Bond Polymer Refine Detection (DS9800) as the detection system.

mAb clone **NB10** (prod. no. 760-2664, Ventana/Cell Marque): The protocols giving an optimal result were based on HIER using mild or standard CC1, an incubation time of 16-60 min in the primary Ab and UltraView (760-500) +/- amplification kit or OptiView (760-700) as the detection system. Using these protocol settings 17 out of 17 (100 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient stains were:

- Omission of or insufficient HIER
- Too low concentration of the primary Ab
- Unexplained (appropriate protocol settings giving insufficient results)

In this assessment and in concordance with the previous NordiQC assessment for PLAP, the prevalent feature of an insufficient staining was a too weak or false negative reaction of the cells expected to be demonstrated.
The majority of the laboratories were able to demonstrate PLAP in high antigen expressing structures such as the trophoblasts of the placenta and the neoplastic cells of the seminoma and the two IGCNs, whereas the demonstration of PLAP in the neoplastic cells of the embryonal carcinoma expressing less PLAP was much more challenging and required an optimally calibrated protocol.

Several antibodies could be used to obtain an optimal staining result. In general efficient HIER typically in an alkaline buffer and a carefully calibrated Ab titre were the main parameters to provide an optimal staining. However virtually all the antibodies used gave a different staining pattern in the tissues tested.

The most widely used mAb clone 8A9 (several vendors) gave a weak to moderate intracytoplasmic staining reaction in the smooth muscle cells in e.g. the vessels and lamina muscularis mucosae of the appendix. This staining pattern was only seen for the mAb clone 8A9 and not by any of the other clones giving an optimal staining. As described in the above mentioned scoring criteria for PLAP, this cross reaction was accepted as publications have described and documented this staining pattern in smooth muscle cells. It is still to be investigated by other techniques than IHC and western blotting if muscle cells express PLAP or it is related to a cross reaction of the mAb clone 8A9.

The mAb clone PL8/F6, BioGenex in general gave an intracytoplasmic dot-like staining reaction (MAG) in the epithelial cells of the appendix, which was obtained from a patient with blood group A. This was also accepted, as this did not compromise the interpretation of the PLAP staining. However it was observed that some lots of the mAb clone PL8/F6 also gave a weak to moderate aberrant intracytoplasmic staining reaction in the satellite cells of the peripheral nerves in the appendix, whereas these cells were negative by other lots of the mAb and otherwise applying fully comparable protocol settings for the different lots.

The rmAb clone SP15, Thermo & Cell Marque gave a pass rate of 100 % (concentrated format) and gave a very distinct staining result for PLAP with a high signal-to-noise ratio with no MAG or smooth muscle cell staining. However for unexplained reasons, an excessive background staining and staining reaction in the normal mature spermatocytes were seen with some protocols. The aberrant staining pattern was seen despite the protocol settings were identical to the settings giving an optimal staining result. Due to the limited number of participants it was not possible to see if the different staining patterns were related to a lot-to-lot variation for the rmAb clone SP15.

In this assessment the Ready-To-Use (RTU) systems in general showed a superior pass rate for PLAP compared to the pass rates for the concentrated formats with an in-house validated assay. E.g. the Dako RTU system based on the mAb clone 8A9 gave a pass rate of 100% and if the same mAb clone was used by an in-house validated assay, a pass rate of 84 % was seen.

We have not identified any normal tissues with a low and consistent expression of PLAP that could be used as critical staining quality indicator (CSQI) for PLAP. Placenta is for the moment still the best control. The cytotrophoblasts and syncytiotrophoblasts must show an as strong as possible predominantly membranous but also cytoplasmic staining reaction, while no staining should be seen in the stromal cells. As this assessment has revealed a great variation in staining patterns for many of the used Abs and even with the same Ab, tissues expected to be PLAP negative, such as appendix, must also be used to evaluate the specificity of the used Abs.

This was the 3rd assessment of PLAP in NordiQC (Table 2). A significantly higher pass rate was achieved compared to those seen in the previous 2 runs.

Table 2. **Proportion of sufficient results for PLAP in the three NordiQC runs performed**

	Run 14 2005	Run 24 2008	Run 35 2012
Participants, n=	77	115	132
Sufficient results	73 %	53 %	91 %

The significant improvement of the pass rate for PLAP was highly influenced by the modified scoring criteria allowing both the MAG staining reaction and the cross reaction of the mAb clone 8A9 in the smooth muscle cells. It was also related to the high quality of the Ready-To-Use (RTU) systems for PLAP from the main providers as Dako, Leica & Ventana. The RTU systems in this assessment showed a pass-rate of 100% thus being superior to the pass-rates for the in-house validated protocols for PLAP.

Conclusion

The mAb clones **8A9**, **NB10** and **PL8-F6** and the rmAb clone **SP15** can all be used to give an optimal staining result for PLAP. HIER, preferable in an alkaline buffer, is mandatory to obtain an optimal result. Placenta is the best choice regarding positive control, in which the cytotrophoblasts and syncytiotrophoblasts must show an as strong as possible predominantly membranous but also cytoplasmic staining reaction, while no staining should be seen in the stromal cells.
Appendix may be used as negative control, in which no structures/cells should show any staining reaction.

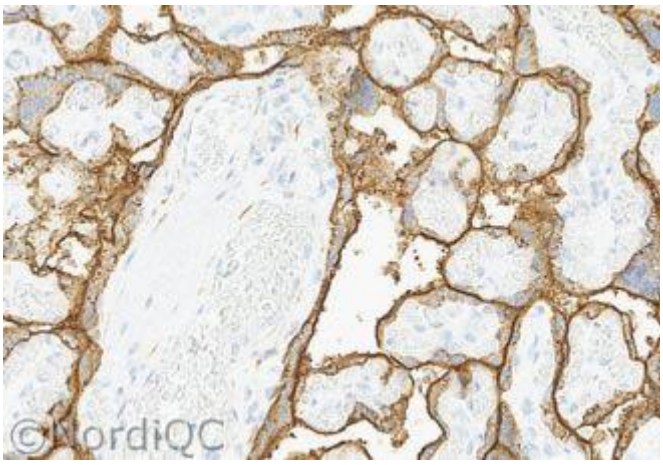


Fig. 1a
Optimal staining for PLAP of the placenta using the mAb clone PL8-F6 optimally calibrated, HIER in an alkaline buffer and a 2-step polymer conjugate.
A strong predominantly membranous but also cytoplasmic staining reaction is seen in virtually all the placental cytotrophoblasts and syncytiotrophoblasts..
Also compare with Figs. 2a – 3a, same protocol.

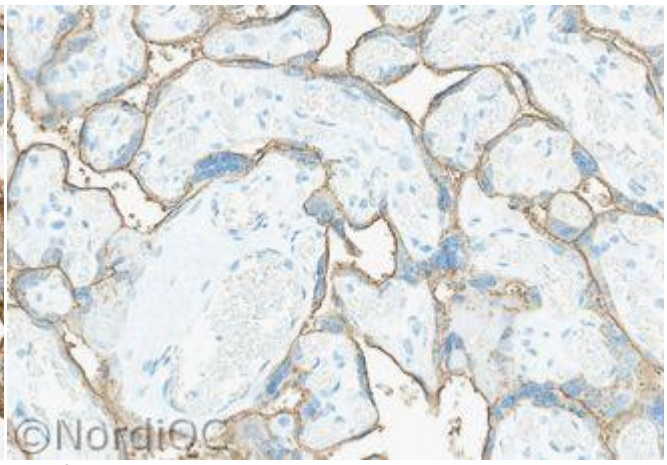


Fig. 1b
Insufficient staining for PLAP of the placenta using the mAb clone PL8-F6 with a protocol giving a too low sensitivity, primarily due to a too low. conc. of the primary Ab.
The intensity of the staining reaction in the placental cytotrophoblasts and syncytiotrophoblasts is reduced compared to the result obtained in Fig. 1a.
Only the membranous staining reaction can be identified.
Also compare with Figs. 2b - 3b, same protocol.

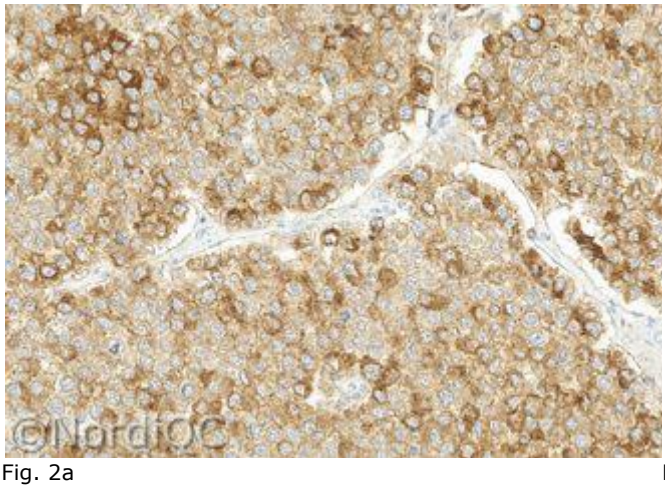


Fig. 2a
Optimal staining for PLAP of the seminoma using same protocol as in Fig. 1a.
Virtually all the neoplastic cells show a moderate to strong predominantly cytoplasmic staining reaction.
No background staining is seen.

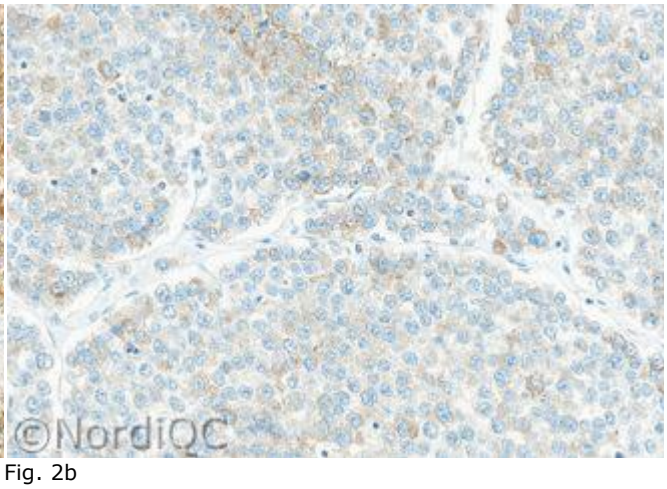


Fig. 2b
Insufficient staining for PLAP of the seminoma using same protocol as in Fig. 1b - same field as in Fig. 2a.
The proportion of positive cells and the intensity of the staining reaction are significantly reduced compared to the result obtained in Fig. 2a. Only dispersed neoplastic cells show an equivocal staining reaction.

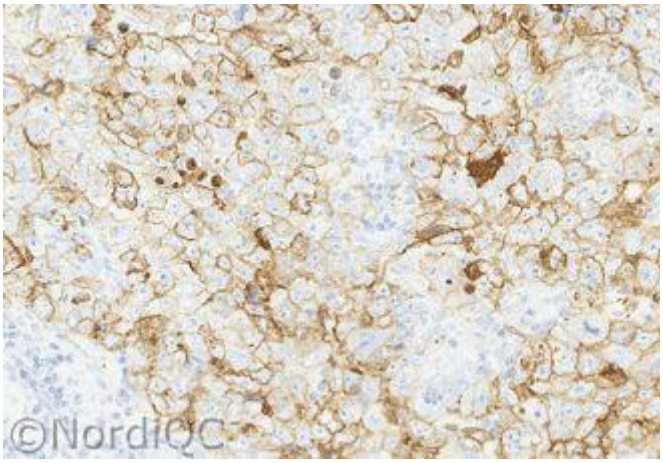


Fig. 3a
Optimal staining for PLAP of the embryonal carcinoma using same protocol as in Figs. 1a - 2a. The majority of the neoplastic cells show a moderate to strong and distinct staining reaction. No background staining is seen.

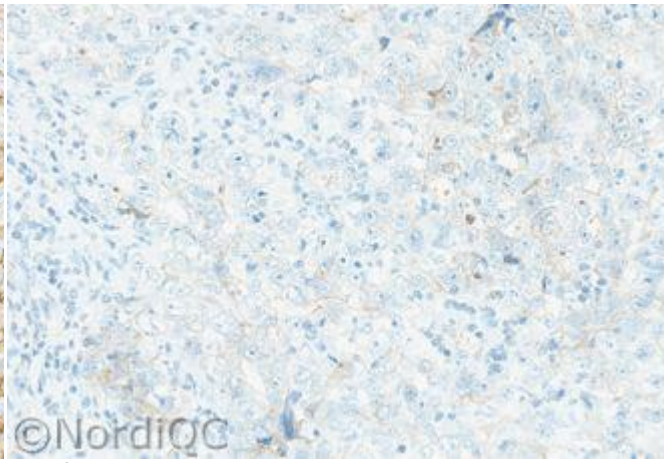


Fig. 3b
Insufficient staining for PLAP of the embryonal carcinoma using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a.
Only dispersed neoplastic cells show a weak and equivocal staining reaction.

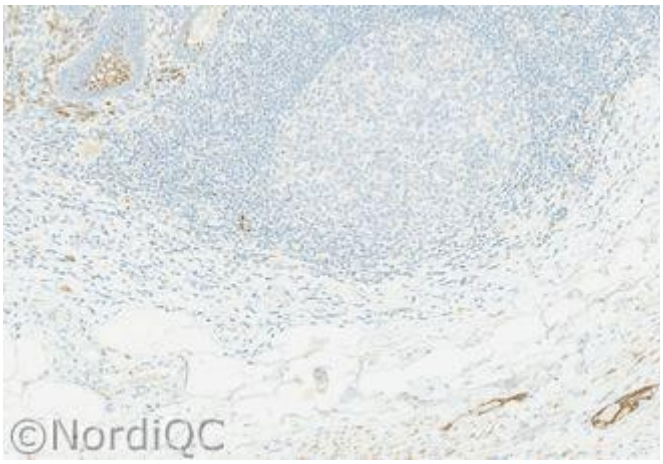


Fig. 4a
Staining for PLAP of the appendix, blood type A using the mAb clone PL8-F6 lot no. MU22808.
The epithelial cells, top left corner, show a distinct intracytoplasmic dot-like staining reaction due to the Mouse-Ascites-Golgi (MAG) staining of the ascites fluid based antibody, while the peripheral nerves, bottom right corner show a moderate to strong aberrant cytoplasmic staining reaction.
Also compare with Fig. 4b, same clone but different lot.

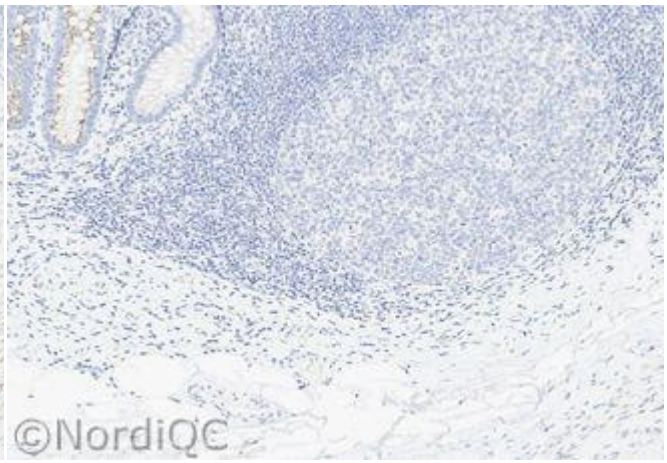


Fig. 4b
Staining for PLAP of the appendix, blood type A using the mAb clone PL8-F6 lot no. MU22612 - same field as in Fig. 4a.
The epithelial cells, top left corner, show a distinct intracytoplasmic dot-like staining reaction due to the MAG staining, while the peripheral nerves, bottom right corner are negative.
Virtually same protocol settings were applied for the two protocols, except two different lots of the primary Ab.

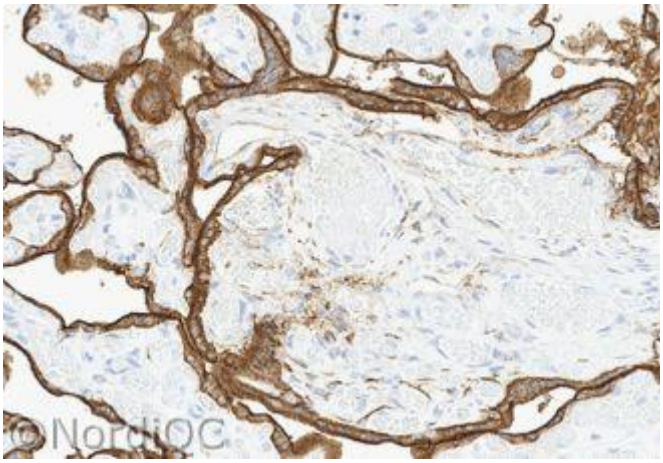


Fig. 5a
Staining for PLAP of the placenta using the mAb clone 8A9.
The placental cytotrophoblasts and syncytiotrophoblasts show a strong cytoplasmic and membranous staining reaction. A moderate cytoplasmic staining reaction is seen in the smooth muscle cells in the stroma. This staining reaction was only seen by the mAb clone 8A9.
Also compare with Fig. 5b - same protocol.

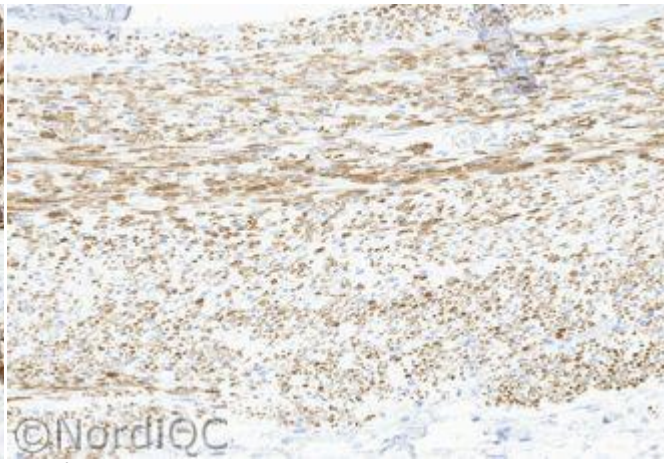


Fig. 5b
Staining for PLAP of the appendix using the mAb clone 8A9, same protocol as in Fig. 5a.
Virtually all the smooth muscle cells of muscularis propria show a moderate cytoplasmic staining reaction. This staining reaction was only seen by the mAb clone 8A9.

SN/MV/LE 28-5-2012