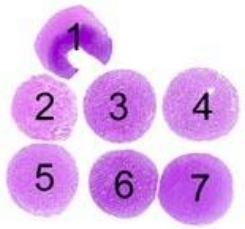
Assessment Run 14 2005

Placental alkaline phosphatase (PLAP)

The slide to be stained for Placental alkaline phosphatase PLAP comprised:
1: Appendix, 2: Striated muscle (tongue), 3-4: Placenta, 5: Seminoma/intratubular germ cell neoplasia (ICGN), 6: Embryonal carcinoma, 7: Breast carcinoma.



Criteria for assessing a PLAP staining as optimal included:

- A strong and distinct predominantly membranous but also cytoplasmic reaction of the embryonal carcinoma, ICGN, and breast carcinoma (heterogeneous pattern)
- A strong predominantly membranous but also cytoplasmic staining of the trophoblastic and syncytiotrophoblastic cells in the two placental specimens with no or minimal reaction in the stromal cells
- All cells in the appendix and striated muscle should be negative

77 laboratories participated in the assessment. 16 achieved optimal staining (21 %), 40 good (52 %), 11 borderline (14 %) and 10 (13 %) poor staining.

The following Abs were used:
mAb clone **8A9** (DakoCytomation, n=31; Novocastra, n=8; NeoMarkers, n=1; Ventana, n=1)
mAb clone **8B6** (DakoCytomation, n=2)
mAb clone **NB10** (Ventana, n=5)
mAb clone **PL8-F6** (BioGenex, n=16)
mAb clone **SP15** (NeoMarkers, n=1)
pAb **0099** (Zymed, n=2)
pAb **258-01** (Signet, n=1)
pAb **A0268** (DakoCytomation, n=9)

Optimal staining in this assessment was obtained only with the clones **PL8-F6** (15 out of 16 were optimal), and **NB10** (1 out 5 was optimal).

With clone **PL8-F6** all optimal protocols were based on HIER using Tris-EDTA/EGTA pH 9. PL8-F6 was used either as a concentrate typically diluted in the range of 1:200 – 1:1000 or as a Ready-To-Use product, which was diluted by the users 1:5 – 1:10.
With clone **NB10** the protocol giving an optimal staining was based on HIER , CC1 in the Ventana Benchmark. **NB10** was applied as a Ready-To-Use product.

The most frequent causes of insufficient staining were (often in combination):
- Too low concentration of the primary antibody
- Too high concentration of the primary antibody
- Omission of epitope retrieval
- Apparently inappropriate choice of primary Ab

The prevalent feature of an insufficient staining was a too weak or completely negative reaction in the two placentas and the neoplasias. Typically there was no significant difference in the intensity in the normal placental and neoplastic cells indicating, that placental tissue can serve as a reliable control for PLAP. The trophoblastic cells should be as strongly stained without reaction of stromal cells.

Clone **8A9** was the only Ab reacting distinctively with both smooth and striated muscle cells, very similar to a desmin staining. In this assessment it did not affect the interpretation of the PLAP staining, but in a diagnostic setting, the cross-reactivity may have an impact on the final diagnosis. It was decided by the core group that staining with this cross-reactivity could not be assessed as optimal, but should be assessed as good, if the other tissues were optimally stained.

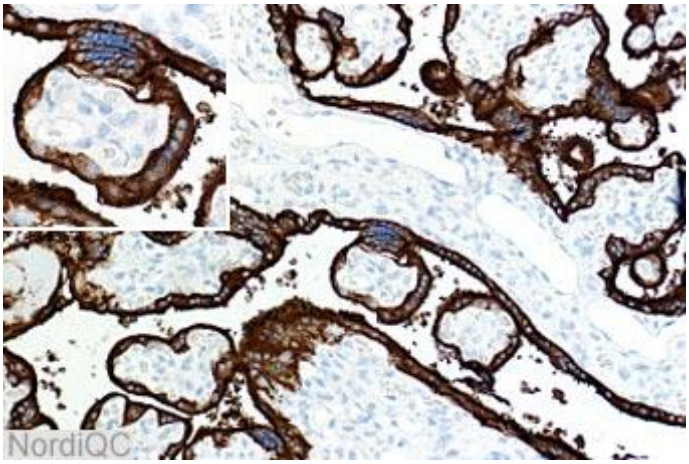


Fig. 1a
Optimal staining for PLAP (mAb clone PL8-F6) in the placenta. All trophoblastic cells are strongly stained.

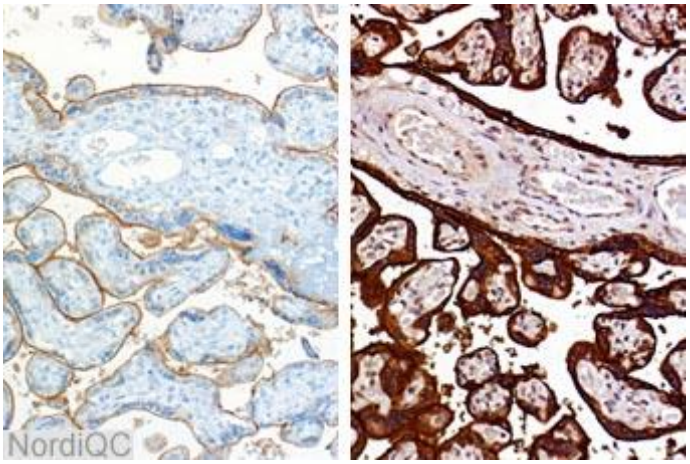


Fig. 1b
Insufficient staining for PLAP in the placenta.
Left: The trophoblastic cells are only weakly demonstrated. Also compare Fig. 2b left.
Right: The trophoblastic cells are strongly stained but also a moderate stromal reaction is seen. Also compare Fig. 2b right.

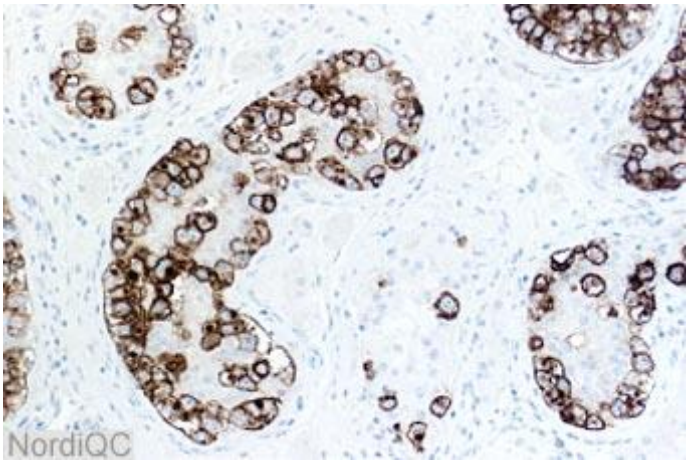


Fig. 2a
Optimal staining for PLAP (mAb clone PL8-F6) in the ICGN. The majority of the neoplastic cells show a moderate to strong and distinct membranous staining.

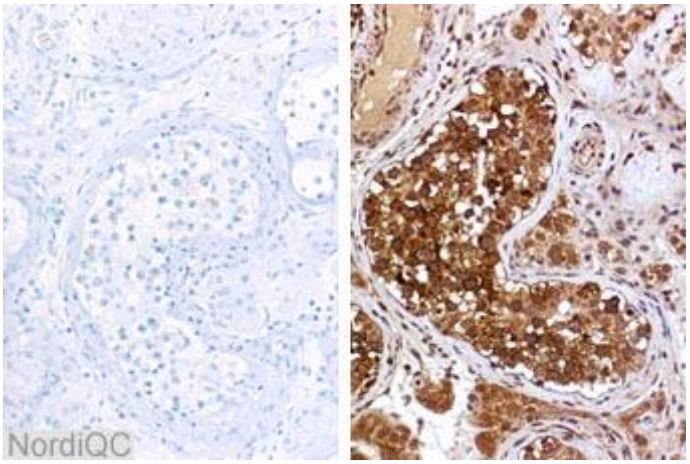


Fig. 2b
Insufficient staining for PLAP in the ICGN.
Left: The neoplastic cells are virtually negative (same protocol as in Fig. 1b left).
Right: The neoplastic cells are strongly stained, but also the unspecific background reaction is seen (same protocol as in Fig. 1b right).

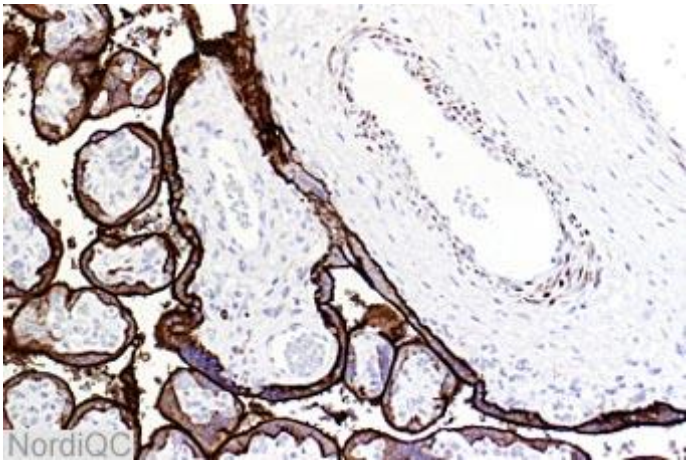


Fig. 3a
Staining for PLAP using the mAb clone 8A9 in the placenta. All trophoblastic cells are strongly stained. However also a sub-population of smooth muscle cells are stained.

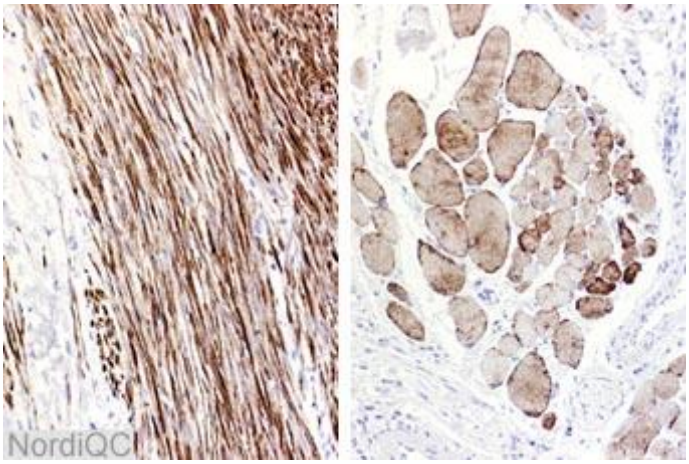


Fig. 3b
Left: Staining for PLAP using the mAb clone 8A9 in the tongue. The striated muscle cells show a distinct positive reaction.
Right: Staining for PLAP using the mAb clone 8A9 in the appendix. The smooth muscle cells show a distinct positive reaction.

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