Assessment Run 53 2018

Octamer transcription factor-3/4 (OCT3/4)

Material
The slide to be stained for **OCT3/4** comprised:

1. Colon, 2. Placenta, 3. Seminoma, 4. Embryonal carcinoma, 5-6. Testicular germ cell neoplasia in situ (GCNIS)

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing an OCT3/4 staining as optimal included:

- A moderate to strong and distinct nuclear staining reaction in virtually all the neoplastic cells of the two GCNIS and the embryonal carcinoma. A diffuse, weak cytoplasmic reaction was accepted.
- An at least weak to moderate, distinct nuclear staining reaction in the majority of neoplastic cells of the seminoma.
- No nuclear staining reaction in the placenta and appendix.
- Cytoplasmic staining of neuroendocrine (NE) cells and fat cells was seen and accepted with the mouse monoclonal antibody (mAb) clone MRQ-10. Weak cytoplasmic staining was also seen and accepted in myofibroblasts.

Participation

Number of laboratories registered for OCT3/4, run 53	194
Number of laboratories returning slides	189 (97%)

Results
189 laboratories participated in this assessment. 94% achieved a sufficient mark. Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:

- Less successful performance of the mAb clone C-10 on the Ventana BenchMark system.

Performance history
This was the third NordiQC assessment of OCT3/4. A significant increase in the pass rate was seen compared to run 35 in 2012. This may be explained by an increased use of high quality ready-to-use (RTU) systems.

Table 2. **Proportion of sufficient results for OCT3/4 in the three NordiQC runs performed**

	Run 24 2008	Run 35 2012	Run 53 2018
Participants, n=	18	43	189
Sufficient results	83%	77%	94%

Conclusion
In general, a very high pass rate was seen, and optimal staining results could be obtained with the mAbs clones **C-10**, **MRQ-10, N1NK, NRG1.1** and **SEMGC**. Irrespective of the clone applied, efficient heat induced epitope retrieval (HIER), preferable in alkaline buffer, use of a sensitive polymer/multimer detection system and careful calibration of the primary Ab were the most important prerequisites for an optimal staining result. The concentrated (Conc) format of the mAbs clones MRQ-10 and N1NK provided a high proportion of optimal staining results on all four main stainer platforms - Omnis (Dako), Autostainer (Dako), Bond (Leica) and BenchMark (Ventana). The mAb clone C-10 also povided optimal staining on all four main platforms, but the proportion of optimals were significantly lower on the Ventana BenchMark and Leica Bond platforms compared to the two Dako platforms.
RTU systems performed slightly better than Conc antibodies. The mAb clone N1NK based Dako Autostainer RTU system provided a perfect score - all protocols (17 of 17) were assessed as optimal. The mAb clone MRQ-10 based Ventana Benchmark RTU system followed closely, with 98% (52 of 53) achieving sufficient staining results with 89% (47 of 53) being optimal.

At present, no easily accessible normal tissue expressing OCT3/4 has been identified, and GCNIS seems to be the preferred recommendable control in which the neoplastic cells must show an as strong as possible nuclear staining reaction (a minimal cytoplasmic staining reaction must be accepted). Placenta or colon/appendix can serve as negative tissue control showing no nuclear staining reaction.

Table 1. **Antibodies and assessment marks for OCT3/4, run 53**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **C-10**	22 1 1 1	Santa Cruz Immunologic Nordic Biosite Zeta Corporation	15	5	4	1	80%	83%
mAb clone **MRQ-10**	28 1	Cell Marque Menarini	26	1	2	0	93%	96%
mAb clone **N1NK**	19 9 3	Leica/Novocastra Dako/Agilent Monosan	26	4	1	0	97%	97%
mAb clone **NRG1.1**	2 1	Diagnostic Biosystems Zytomed	2	1	0	0	-	-
mAb clone **SEMGC**	1	Menarini	1	0	0	0	-	-
pAb **ab19857**	1	Abcam	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone **C-10 MAB-0618**	1	Maixin	1	0	0	0	-	-
mAb clone **C-10 MAD-000239QD**	2	Master Diagnostica	1	0	1	0	-	-
mAb clone **MRQ-10 309M-18**	2	Cell Marque	2	0	0	0	-	-
mAb clone **MRQ-10 760-4392**	53	Ventana/Roche	47	5	1	0	98%	100%
mAb clone **N1NK IR092**	17	Dako/Agilent	17	0	0	0	100%	100%
mAb clone **N1NK IR092** [3]	12	Dako/Agilent	12	0	0	0	100%	100%
mAb clone **N1NK IR092** [4]	1	Dako/Agilent	1	0	0	0	-	-
mAb clone **N1NK PA0193**	2	Leica/Novocastra	2	0	0	0	-	-
mAb clone **N1NK PA0934**	2	Leica/Novocastra	0	2	0	0	-	-
mAb clone **SEMGC PM313AA**	6	BioCare	4	2	0	0	-	-
rmAb **EP143 HAR061**	1	PATH-N-SITU	0	0	0	1	-	-
Total	189		157	21	9	2	-	
Proportion			83%	11%	5%	1%	94%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer) but used by laboratories on the full-automatic Dako Omnis system.
4) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer) but used by laboratories on different platforms (e.g. Ventana Benchmark).

Detailed analysis of OCT3/4, Run 53

The following protocol parameters were central to obtain optimal staining:

Concentrated Antibodies
mAb clone **C-10**: Protocols with optimal results were all based on HIER using Bond™ Epitope Retrieval Solution 2 (BERS2, Leica) (2/5)*, Cell Conditioning 1 (CC1, Ventana/Roche) (2/8), Tris-EDTA/EGTA pH 9 (3/3), TRS High pH (3-in-1) (Dako/Agilent) (4/4) or TRS High pH (Dako/Agilent) (4/4) as retrieval buffer. 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, 20 of 24 (83%) laboratories produced a sufficient staining result (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

mAb clone **MRQ-10**: Protocols with optimal results were all based on HIER using BERS2 (Leica) (3/3), CC1 (Ventana/Roche) (13/15), HIER Buffer M, pH 8 (Thermo Scientific) (1/1), TRS High pH (3-in-1) (Dako/Agilent) (4/4) or TRS High pH (Dako/Agilent) (5/5) as retrieval buffer. The mAb was diluted in the range of 1:20-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 27 of 28 (96%) laboratories produced a sufficient staining result

mAb clone **N1NK**: Protocols with optimal results were all based on HIER using Bond™ Epitope Retrieval Solution 1 (BERS1, Leica) (2/2), BERS2 (Leica) (5/6), CC1 (Ventana/Roche) (10/14), Cell Conditioning 2 (CC2, Ventana/Roche) (3/3), Diva Decloaker (BioCare) (1/1), TRS High pH (3-in-1) (Dako/Agilent) (4/4) or TRS High pH (Dako/Agilent) (1/1) as retrieval buffer. The mAb was diluted in the range of 1:20-1:150 depending on the total sensitivity of the protocol employed. Using these protocol settings, 30 of 31 (97%) laboratories produced a sufficient staining result.

mAb clone **NRG1.1**: Protocols with optimal results were all based on HIER using DBS Montage Citrate Antigen Retrieval Solution (Diagnostic Biosystems) (1/1) or TRS High pH (3-in-1) (Dako/Agilent) (1/1) as retrieval buffer. The mAb was diluted 1:50. Using these protocol settings, 2 of 2 laboratories produced an optimal staining result.

mAb clone **SEMGC**: One lab used the mAb clone SEMGC and achieved an optimal result. The protocol was based on HIER in TRS High pH (3-in-1) (Dako/Agilent). The mAb was diluted 1:100, incubated for 30 min. and visualized with a 3-step polymer conjugate system (EnVision Flex+, Dako/Agilent) on the Dako Autostainer instrument.

Table 3. **Proportion of optimal results for OCT3/4 for the most commonly used antibodies as concentrates on the 4 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Dako Omnis**		**Ventana BenchMark GX / XT / Ultra**		**Leica 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 **C-10**	4/4**	-	3/3	-	2/8 (25%)	0/1	2/5 (40%)	-
mAb clone **MRQ-10**	3/3	-	5/5 100%	-	13/15 (93%)	-	3/3	-
mAb clone **N1NK**	3/3	1/1	1/1	-	9/13 (69%)	3/3	5/6 (83%)	2/2

* 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)

Ready-To-Use antibodies and corresponding systems
mAb clone **MRQ-10** product no. **760-4392**, Ventana/Roche, Ventana Benchmark GX/XT/Ultra:
Protocols with optimal results were all based on either HIER in CC1 (Ventana/Roche) (efficient heating time for typically 16-64 min. at 90-100°C) or HIER in combination with proteolysis (efficient heating time 16-24 min. in CC1 proceeded **or** followed by 8 min. in Protease 3 (Ventana/Roche) at 36°C. Incubation time of the primary Ab was 8-40 min. at 36°C and UltraView (760-500, Ventana/Roche) with or without amplification or OptiView (760-700, Ventana/Roche) were used as detection system. Using these protocol settings, 52 of 52 (100 %) laboratories produced a sufficient staining result.

mAb clone **N1NK** product no. **IR092**, Dako/Agilent, Autostainer+/Autostainer Link:
Protocols with optimal results were based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (efficient heating time 10-40 min. at 95-99°C), 10-40 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (Dako/Agilent, K8000/K8002/K8012) as detection system. Using these protocol settings, 17 of 17 (100%) laboratories produced an optimal staining result.

mAb clone **N1NK** product no. **PA0193**, Leica/Novocastra, Leica Bond Max/Bond III:
Protocols with optimal results were based on HIER using BERS2 (Leica) (efficient heating time 20-30 min. at 99°C), 15 min. incubation of the primary Ab and Bond Polymer Refine Detection (Leica, DS9800) as detection system. Using these protocol settings, 2 of 2 laboratories produced an optimal staining result.

mAb clone **SEMGC**, product no. **PM313AA**, Biocare, IntelliPath:
One protocol with an optimal result was based on HIER using Borg Decloaker pH 9.5 (BioCare) in a pressure cooker 15 min. at 110°C and MACH4 (BioCare, M4U534) as detection system.

Table 4 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 accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer instrument are included.

Table 4. **Proportion of sufficient and optimal results for OCT3/4 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/XT mAb clone **MRQ-10** **760-4392**	100% (7/7)	100% (7/7)	98% (45/46)	87% (40/46)
Dako AS mAb clone **N1NK IR092**	100% (8/8)	100% (8/8)	100% (9/9)	100% (9/9)

* 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 >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments
In this assessment, the prevalent feature of an insufficient result was mostly characterized by false positive staining reaction or excessive background staining compromising interpretation. This pattern was seen in 73% of the insufficient results (8 of 11 laboratories). The remaining 27% (3 of 11) of insufficient results were characterized by a too weak staining reaction of cells expected to be demonstrated.

Nearly all of the participating laboratories were able to demonstrate OCT3/4 in high-level antigen expressing structures such as neoplastic cells of the two GCNIS and the embryonal carcinoma, whereas demonstration of OCT3/4 in the seminoma expressing less OCT3/4 was more challenging and required an optimally calibrated protocol (see Figs. 1-2). In pursuit of a strong nuclear staining in the neoplastic cell, a false positive nuclear staining reaction could be introduced in non-neoplastic cells. This pattern was most often seen with the mAbs clones C-10 and MRQ-10 (see Figs. 3-5).

48% (90 of 189) of the laboratories used Abs as Conc format within laboratory developed (LD) assays for OCT3/4. Irrespective of the clone applied, careful calibration of the titre and efficient HIER (both high and low pH) were the main protocol prerequisites for optimal results. Both 2- and 3-step polymer/multimer based detection systems could be used to provide an optimal result, but the majority of laboratories (73%) used 3-step polymer/multimer based detection systems. The vast majority of laboratories used the mAbs clones C-10, MRQ-10 and N1NK (see Table 1). All three clones could be used to obtain an optimal staining result and the proportion of sufficient staining results was generally very high. The proportion of sufficient staining results for mAbs clones MRQ-10 and N1NK were 93% (27 of 29) and 97% (30 of 31), respectively, whereas the proportion of sufficient staining result for mAb clone C-10 reached 80% (20 of 25). Data focusing on the four main IHC systems (see Table 3) showed that – on the Ventana BenchMark and Leica Bond platforms - the proportion of optimal results using the mAb clone C-10 as a Conc, was significantly lower than using the mAbs clones MRQ-10 and N1NK. In total, only 29% (4 of 14) of the laboratories using mAb clone C-10 as Conc on the Ventana BenchMark or Leica Bond platform obtained an optimal staining result. In contrast, 83% (35 of 42) of the laboratories using the mAb clones MRQ-10 and N1NK as Conc on the same platforms obtained an optimal staining result. These data indicate that mAb clone C-10 is more difficult to optimize on both the Ventana BenchMark and Leica Bond platforms than the clones MRQ-10 and N1NK. In concordance with the previous NordiQC assessment for OCT3/4, run 35 2012, the main reason for insufficient staining results with mAb clone C-10 was either an excessive background staining making interpretation difficult or a false positive nuclear staining reaction especially in cytotrophoblast cells in placenta (see Fig. 7).

52% (99 of 189) of the laboratories used Abs in RTU formats. This was a significant increase compared to the previous OCT3/4 assessment in 2012, where only 23% of the laboratories used the RTU format. The most widely used RTU systems for OCT3/4 were the mAb clone MRQ-10 based **760-4392** from Ventana, intended for use on the Ventana BenchMark system and mAb clone N1NK based **IR092** from Dako/Agilent, intended for use on the Dako Autostainer system. Both RTU systems were highly successful. The proportion of optimal results was 100% (17 of 17) for the IR092 RTU system regardless of the protocol settings used (recommended or LD) (see Table 4). Despite being developed for the Dako Autostainer system, 12 laboratories used IR092 on the fully automated Dako Omnis system, and all (100%) achieved optimal staining results. Similar impressive results were seen with the 760-4392 RTU for the Ventana BenchMark system. Following the recommended protocol settings, 100% (7 of 7) achieved optimal staining results (see Table 4). In comparison 87% (40 of 46) of laboratories using LD protocol settings achieved optimal staining results. Both RTU systems typically rely on efficient HIER at high pH, but 5 laboratories using the mAb clone MRQ-10 based 760-4392 system, modified the retrieval procedure combining HIER in CC1 (high pH buffer) with short (8 min.) proteolysis in Protease 3 ("Weak protease", Ventana/Roche). All 5 laboratories obtained optimal staining results, whereas one Lab, using the mAb clone MRQ-10 in a LD assay with standard (12 min.) proteolysis in Protease 1 ("Strong protease", Ventana/Roche), obtained insufficient staining result due to weak staining reaction and poor morphology (see Figs. 6-7).

Controls

At present, no easily accessible normal tissue expressing OCT3/4 has been identified and GCNIS seems to be the preferred recommendable control in which the neoplastic cells must show an as strong as possible nuclear staining reaction (a minimal cytoplasmic staining reaction must be accepted). Placenta or colon/appendix can serve as negative tissue control showing no nuclear staining reaction.

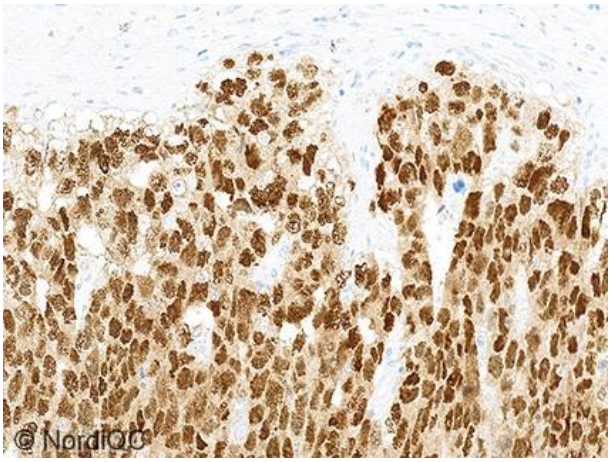

Fig. 1a
Optimal OCT3/4 staining of the embryonal carcinoma using the mAb clone N1NK in a LD assay on the Ventana BenchMark system using a 3-step multimer detection system (OptiView, Ventana). A strong, distinct nuclear staining of virtually all tumour cells is seen. (same protocol used in Fig. 2a). Compare with Fig. 1b.

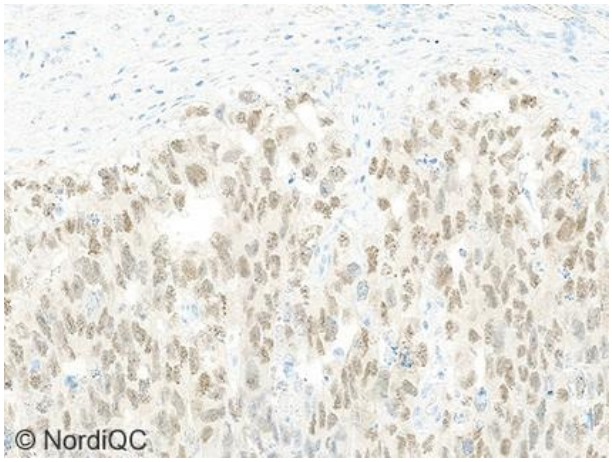

Fig. 1b
Insufficient OCT3/4 staining of the embryonal carcinoma using the mAb clone N1NK in a LD assay on the Ventana BenchMark system using similar protocol setting as in Fig. 1a, but with the use of a less sensitive 2-step multimer detection system (UltraView, Ventana). Only faint nuclear staining of tumour cells is seen. Compare with Fig. 1a. – same field. Also compare with Figs. 2b – same protocol.

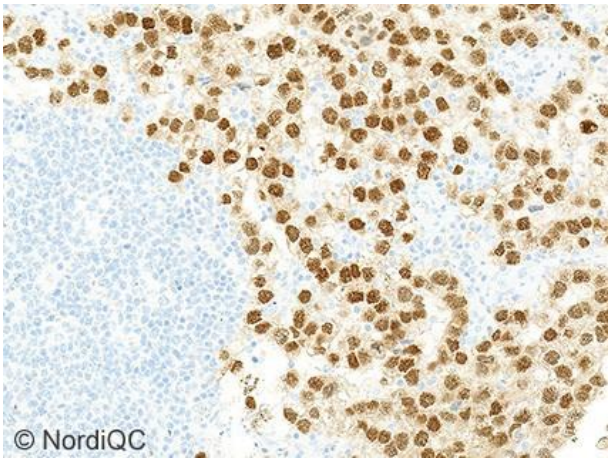

Fig. 2a
Optimal OCT3/4 staining of the seminoma using the same protocol as in Fig. 1a. A moderate to strong, distinct nuclear staining of virtually all tumour cells is seen. Compare with Fig. 2b.

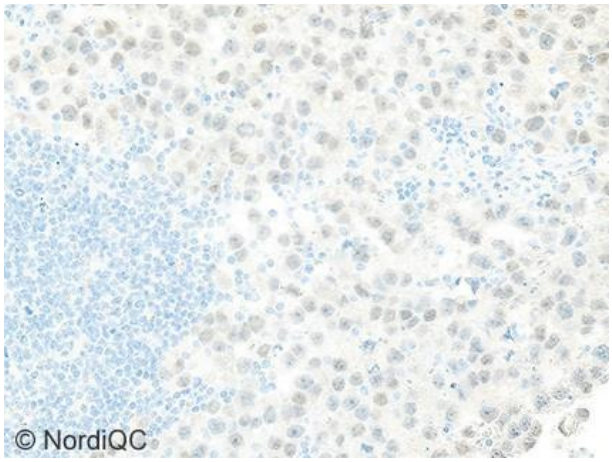

Fig. 2b
Insufficient OCT3/4 staining of the seminoma using the same protocol as in Fig. 1b. The tumour cells are virtually negative. Compare with Fig. 2a - same field.

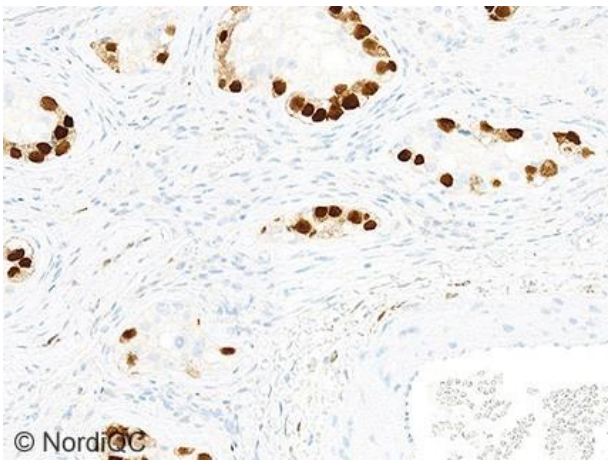

Fig. 3a
Optimal OCT3/4 staining of the GCNIS using the mAb clone MRQ-10 in dilution 1:45 in a LD assay on the Ventana BenchMark system using a 3-step multimer detection system (OptiView, Ventana). A strong, distinct nuclear staining of virtually all tumour cells. Faint cytoplasmic staining in stromal cells is seen and accepted (same protocol used in Fig. 4a). Compare with Fig. 3b.

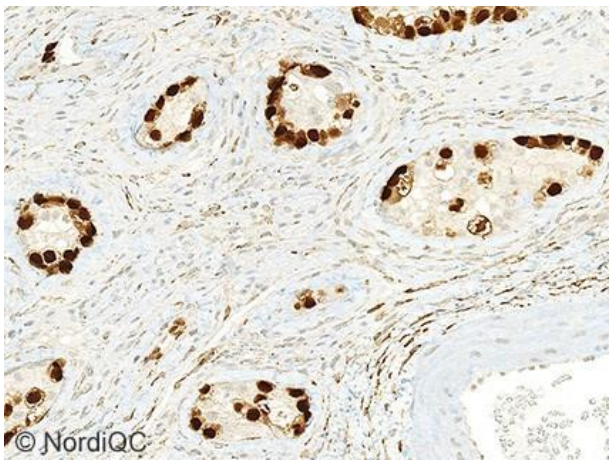

Fig. 3b
Insufficient OCT3/4 staining of the GCNIS using the mAb clone MRQ-10 using similar protocol settings as in Fig. 3a. Only difference being the use of a higher concentration of the primary Ab (1:20). This results in a moderate to strong cytoplasmic and weak nuclear staining in the majority of stromal cells making interpretation difficult. Compare with Fig. 3a – same field.

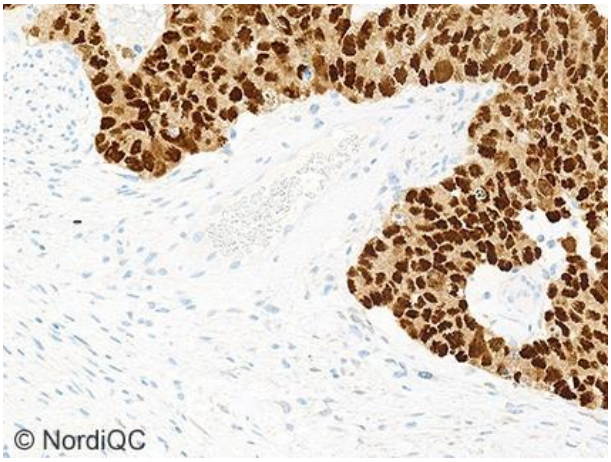

Fig. 4a
Optimal OCT3/4 staining of the embryonal carcinoma using the same protocol as in Fig. 3a. A strong, nuclear staining of virtually all the tumour cells is seen. At the same time, no background staining is seen in the stromal cells. Compare with Fig. 4b.

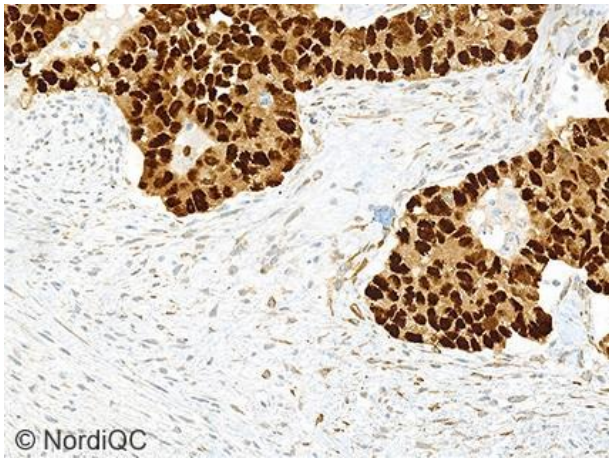

Fig. 4b
Insufficient OCT3/4 staining of the embryonal carcinoma using the same protocol as in Fig. 3b. The strong, nuclear staining reaction of the tumour cells is disturbed by a weak to moderate cytoplasmic and nuclear staining in the majority of stromal cells. Compare with Fig. 4a - same field.

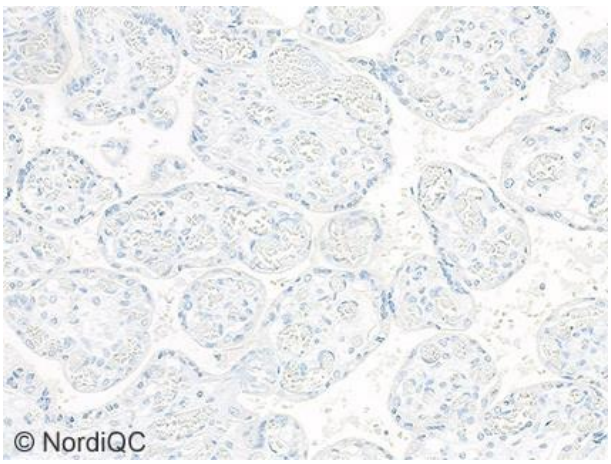

Fig. 5a
Optimal OCT3/4 staining of placenta using the same protocol as in Fig. 3a. In the optimally calibrated MRQ-10 protocol, no background staining is seen in the placenta. Compare with Fig. 5b.

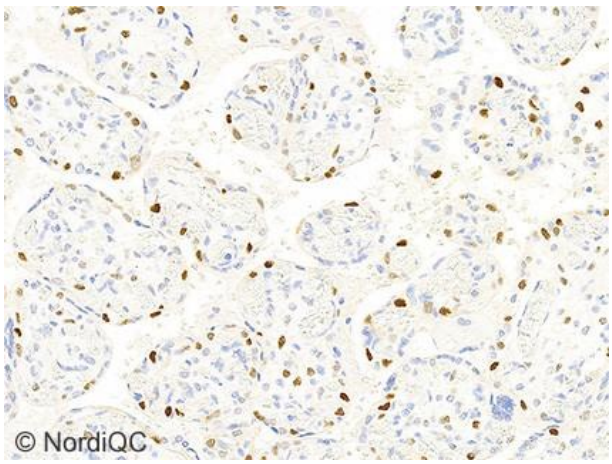

Fig. 5b
Insufficient OCT3/4 staining of placenta using the mAb clone C-10 at a too high concentration on the Ventana BenchMark platform. A false positive nuclear staining is seen in the majority of cytotrophoblast cells. This relates to the use of too high concentration of the primary Ab. Compare with Fig. 5a – same field.

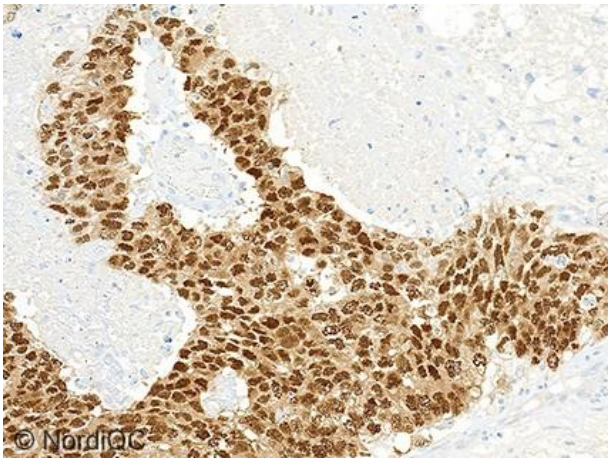

Fig. 6a
Optimal OCT3/4 staining of the embryonal carcinoma using an optimally calibrated MRQ-10 protocol based on HIER pretreatment. A strong, nuclear staining of virtually all the tumour cells is seen. Compare with Fig. 6b. Also compare with Fig. 7a – same protocol.

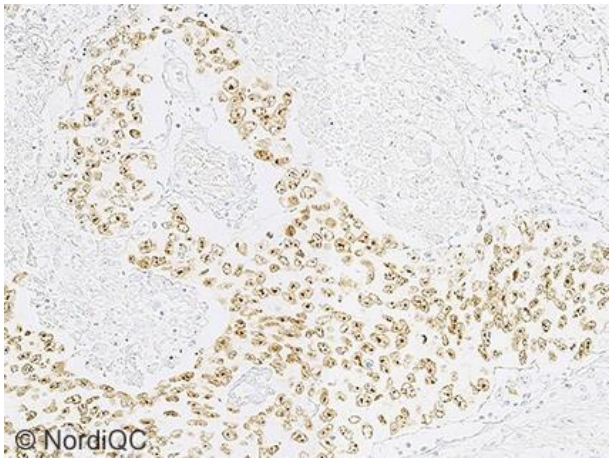

Fig. 6b
Insufficient OCT3/4 staining of the embryonal carcinoma using a protocol with similar settings as in Fig. 6a. Only difference was the use of proteolytic pretreatment instead of HIER. The use of 12 min. Protease 1 (Ventana, Roche) results in both poor morphology and a too weak staining reaction in the tumour cells. Compare with Fig. 6a - same field. Also compare with Fig. 7b – same protocol.

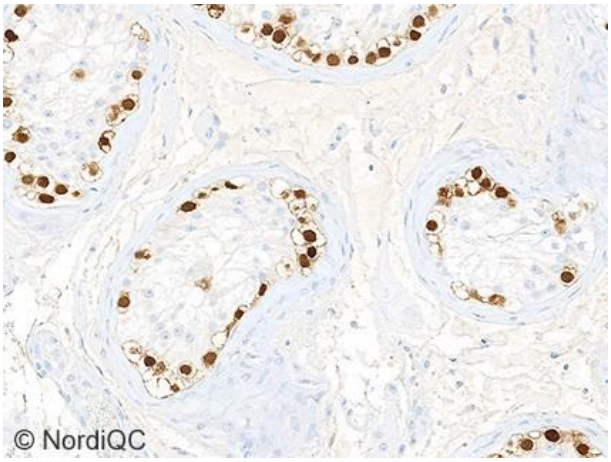

Fig. 7a
Optimal OCT3/4 staining of the GCNIS using the same protocol as in Fig. 6a. A strong, distinct nuclear staining of virtually all tumour cells is seen. Compare with Fig. 7b.

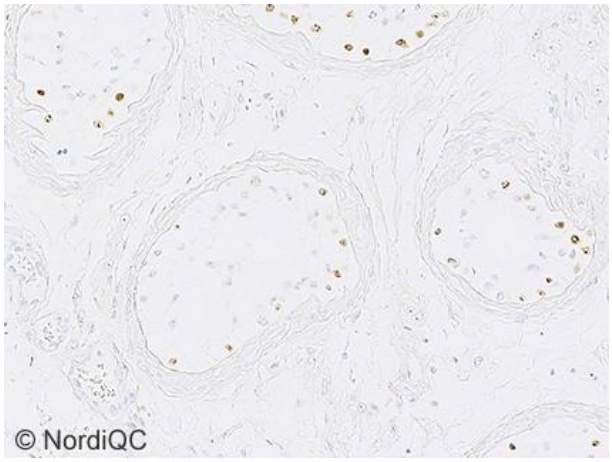

Fig. 7b
Insufficient OCT3/4 staining of the GCNIS using the same protocol as in Fig. 6b. Both poor morphology and a too weak staining reaction in the tumour cells is the result of the proteolytic pretreatment. Compare with Fig. 7a.

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