## Assessment Run 35 2012

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

**Material**
The slide to be stained for OCT3/4 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 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 IGCNs 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 the neoplastic cells of the seminoma
- No nuclear staining reaction in the placenta and in the appendix.

43 laboratories participated in this assessment. 77 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for OCT3/4, run 35**

| Concentrated Abs: | N | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **C-10** | 19<br>1 | Santa Cruz<br>Master Diagnostics | 10 | 4 | 4 | 2 | 70 % | 75 % |
| mAb clone **MRQ-10** | 5<br>2 | Cell Marque<br>Immunologic | 4 | 2 | 1 | 0 | 83 % | 100 % |
| mAb clone **N1NK** | 3 | Leica/Novocastra | 2 | 1 | 0 | 0 | - | - |
| mAb clone **SEMGC** | 1 | Biocare | 0 | 1 | 0 | 0 | - | - |
| rmAb clone **EP143** | 1 | Epitomics | 0 | 0 | 1 | 0 | - | - |
| pAb **ab19857** | 1 | Abcam | 0 | 0 | 1 | 0 | - | - |
| Ready-To-Use Abs: | | | | | | | | |
| mAb clone **MRQ-10 760-4392** | 7 | Ventana/Cell Marque | 4 | 3 | 0 | 0 | 100 % | 100 % |
| mAb clone **C-10** | 1 | Zhongshan | 0 | 0 | 1 | 0 | - | - |
| mAb clone **MRQ-10 309M-17** | 1 | Cell Marque | 0 | 1 | 0 | 0 | | |
| mAb clone **N1NK PA0934** | 1 | Leica/Novocastra | 1 | 0 | 0 | 0 | - | - |
| Total | 43 | | 21 | 12 | 8 | 2 | - | |
| Proportion | | | 49 % | 28 % | 19 % | 4 % | 77 % | |

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

Following central protocol parameters were used to obtain an optimal staining:

**Concentrated Abs**
mAb clone **C-10** (OCT-3/4): 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) (1/2)*, Cell Conditioning 1 (CC1; BenchMark, Ventana) (2/7)*, Bond Epitope Retrieval Solution 2 (BERS 2; Bond, Leica) (2/2)*, Tris-EDTA/EGTA pH 9 (4/6)* or Citrate pH 6 (1/1)* as the retrieval buffer.
The mAb was typically diluted in the range of 1:50-1:250 depending on the total sensitivity of the protocol

employed. Using these protocol settings 12 out of 16 (75%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **MRQ-10** (Oct-4): The protocols giving an optimal result were all based on HIER using either CC1 (BenchMark, Ventana) (2/4), Tris-EDTA/EGTA pH 9 (1/1) or Citrate pH 6.7 (1/1) as the retrieval buffer.
The mAb was typically diluted in the range of 1:20-1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 out of 5 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **N1NK** (Oct-3/4): The protocols giving an optimal result were based on HIER using either Bond Epitope Retrieval Solution 1 (BERS 1; Bond, Leica) (1/1) as the retrieval buffer or TRS pH 9 (Dako) (1/1). The mAb was diluted in the range of 1:50-1:100. Using these settings 2 out of 2 produced an optimal staining.

**Ready-To-Use Abs**
mAb clone **MRQ-10** (prod. no.760-4392, Ventana/Cell Marque): The protocols giving an optimal result were based on HIER using mild or standard CC1, an incubation time of 16-40 min. in the primary Ab and UltraView (760-500) as the detection system. One lab used a combined epitope retrieval with enzymatic pre-treatment in Protease 3 for 8 min. and HIER in short CC1, an incubation time of 16 min. in the primary Ab and OptiView (760-700) as the detection system.
Using these protocol settings 6 out of 6 (100%) laboratories produced a sufficient staining.

mAb clone **N1NK** (product. no. PA0934, Leica/Novocastra): The protocol giving an optimal result was based on HIER using standard CC1, an incubation time of 36 min in the primary Ab and UltraView (760-500) as the detection system.

The most frequent causes of insufficient stainings were:

- Less successful primary Ab
- Less successful performance of the mAb clone C-10 1 on the Ventana BenchMark platform

In this assessment and in concordance with the previous NordiQC assessment for OCT3/4, run 24 2008, the prevalent feature of an insufficient staining was a too weak or false negative staining reaction of the cells expected to be demonstrated. This was seen in 70 % of the insufficient results (7 out of 10). In the remaining 30 % a false positive staining reaction was seen.
The majority of the laboratories were able to demonstrate OCT3/4 in high antigen expressing structures such as the neoplastic cells of the two IGCNs and the embryonal carcinoma, whereas the demonstration of OCT3/4 in the seminoma expressing less OCT3/4 was much more challenging and required an optimally calibrated protocol. The tissue core of the seminoma showed a staining gradient characterized by a strong nuclear staining reaction in the neoplastic cells located at the upper edge and a reduced staining throughout the core towards the opposite edge. This most likely was related to a delayed fixation of the central part of the tumour specimen, which in internal tests has shown to deteriorate many nuclear antigens inclusive OCT3/4. However in the optimal staining results, a distinct positive nuclear staining reaction was seen in the area with a reduced antigen expression.
The most widely used mAb clone C-10, Santa Cruz applied as a concentrate gave an overall pass rate of 70 %. To obtain an optimal result efficient HIER in an alkaline buffer combined with a sensitive detection system was required. For yet unknown reasons the mAb clone C-10 in some laboratories gave an aberrant false positive nuclear staining reaction in virtually all cells including lymphocytes, stromal cells and columnar epithelial cells of the appendix. The insufficient results, both false negative and false positive, for the mAb clone C-10 were mainly seen by the use of the Ventana IHC platforms, BenchMark XT and Ultra.
In total only 1 laboratory out of 6 laboratories using the mAb clone C-10 on a Ventana IHC platform produced a sufficient staining result (optimal). The optimal result was based on the newly launched OptiView detection kit with amplification (tyramide based).
Although the numbers are small, the newly launched mAb clones MRQ-10, Cell Marque & Ventana and N1NK, Leica look promising. In this test a pass rate of 100% was seen for both the concentrated formats and the Ready-To-use formats of these two clones. An optimal result could be obtained on all the IHC platforms used including Autostainer (Dako & LabVision), BenchMark XT and Ultra (Ventana) and Bond (Leica).
It was observed, that the mAb clone MRQ-10 gave a moderate to strong cytoplasmic staining reaction in the neuroendocrine cells in the appendiceal mucosa, scattered follicular dendritic cells in the germinal centres of the appendix and myofibroblasts. This was accepted as this was a cytoplasmic staining reaction not complicating the interpretation of the specific nuclear staining for OCT3/4.

At present no easily accessible normal tissue expressing OCT3/4 has been identified and IGCN seem 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), while other cells shall show no nuclear staining reaction.

This was the 2nd assessment of OCT3/4 in NordiQC (Table 2). A slightly reduced pass rate was seen, which both can be related to a significant increase in many new participants and/or new challenging material circulated for this assessment.

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

| | Run 24 2008 | Run 35 2012 |
|---|---|---|
| Participants, n= | 18 | 43 |
| Sufficient results | 83 % | 77 % |

**Conclusion**

The mAb clones **C-10**, **MRQ-10** and **N1NK** are all recommendable Abs for OCT3/4.
For all the three Abs a combination of efficient HIER and the application of a sensitive IHC system seem to be mandatory for an optimal performance. In this assessment the mAb clones **MRQ-10** and **N1NK** gave a pass rate of 100 %. The two clones could be used both as a concentrate and as Ready-To-use format.
IGCN is recommended as a positive control in which the staining must be as strong as possible in the nuclei of the neoplastic cells, whereas no nuclear staining should be seen in other cells.

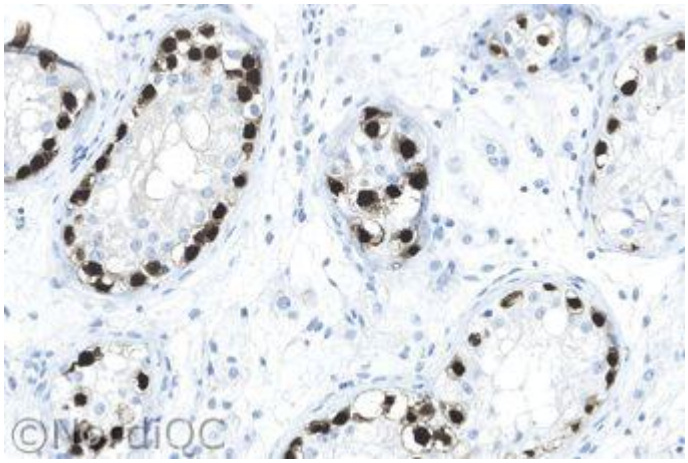


Fig. 1a
Optimal OCT3/4 staining of the intratubular germ cell neoplasia using the mAb clone MRQ-10 optimally calibrated and with HIER in an alkaline buffer. The neoplastic cells show a strong distinct nuclear staining reaction and no background staining is seen.
Also compare with Fig. 2a, same protocol.

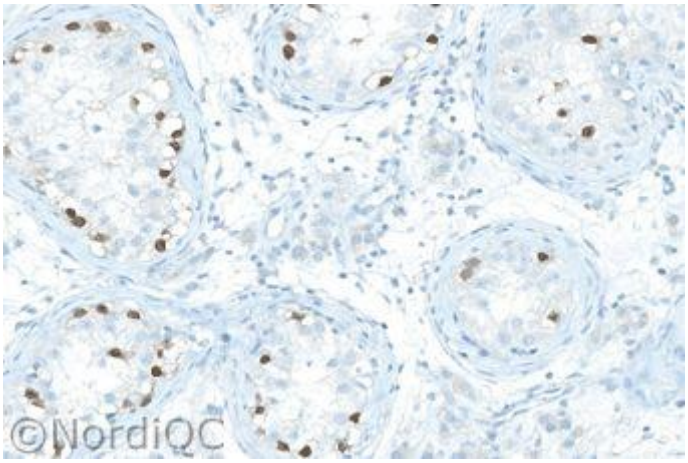


Fig. 1b
Staining for OCT3/4 of the intratubular germ cell neoplasia using the mAb clone C-10 with a protocol/detection kit giving a too low sensitivity.
The intensity of the nuclear staining reaction in the neoplastic cells is reduced compared to the result obtained in Fig. 1a. However also compare with Fig. 2b, same protocol.

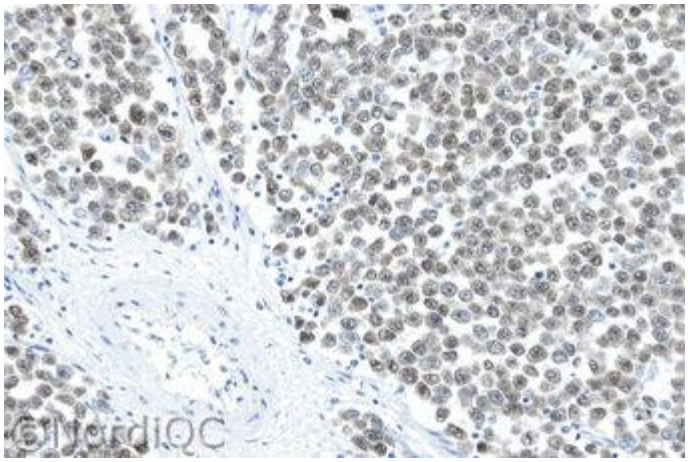


Fig. 2a
Optimal staining for OCT3/4 of the seminoma using same protocol as in Fig. 1a.
Virtually all the neoplastic cells show a weak to moderate and distinct nuclear staining reaction.
No background staining is seen.

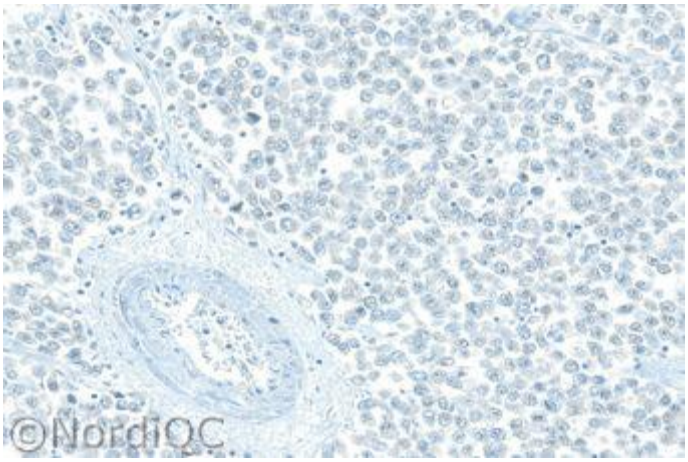


Fig. 2b
Insufficient staining for PLAP of the seminoma using same protocol as in Fig. 1b - same field as in Fig. 2a.
The neoplastic cells are all virtually false negative.

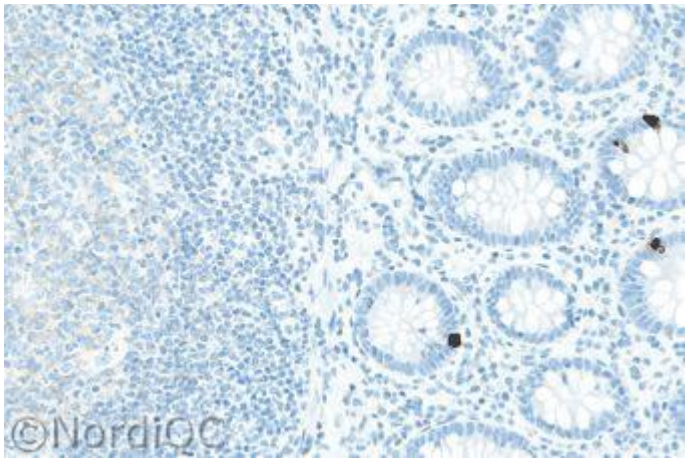


Fig. 3a
Staining pattern for the mAb clone MRQ-10 in the appendix.
The endocrine cells show a strong intracytoplasmic staining reaction, while a weak intracytoplasmic staining reaction is seen in the follicular dendritic cells in the germinal centre.
The overall staining reaction was assessed as optimal.

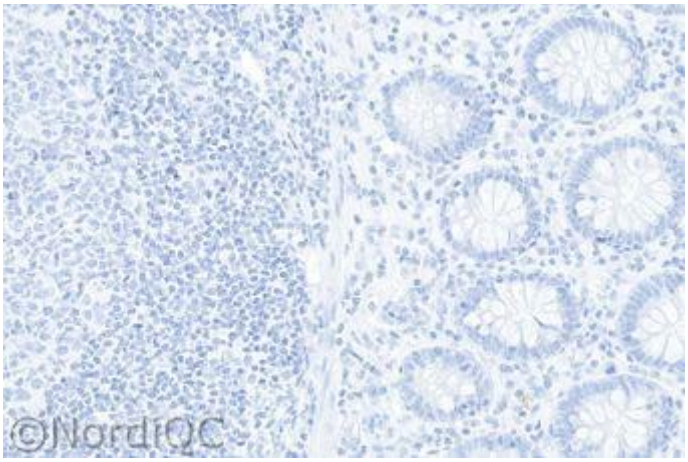


Fig. 3b
Staining pattern for the mAb clone C-10 in the appendix. No intracytoplasmic or nuclear staining reaction is seen – same field as in Fig. 3a.
The overall staining reaction was assessed as optimal.

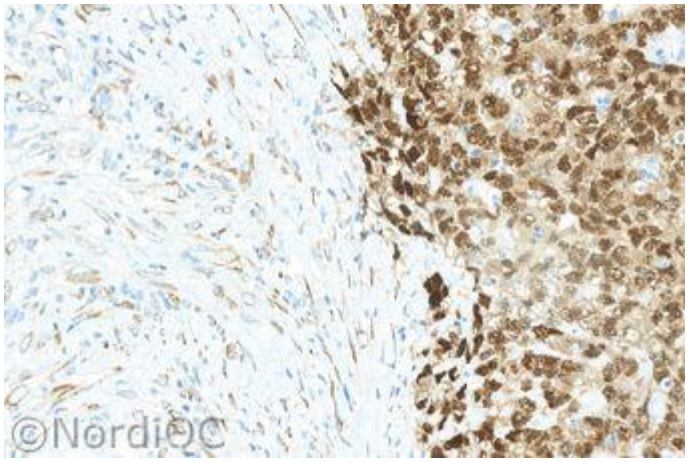


Fig. 4a
Staining pattern for the mAb clone MRQ-10 in the embryonal carcinoma, same protocol as in Fig. 3a. The neoplastic cells show a strong nuclear staining reaction, while a weak to moderate intracytoplasmic staining reaction is seen in the myofibroblasts.

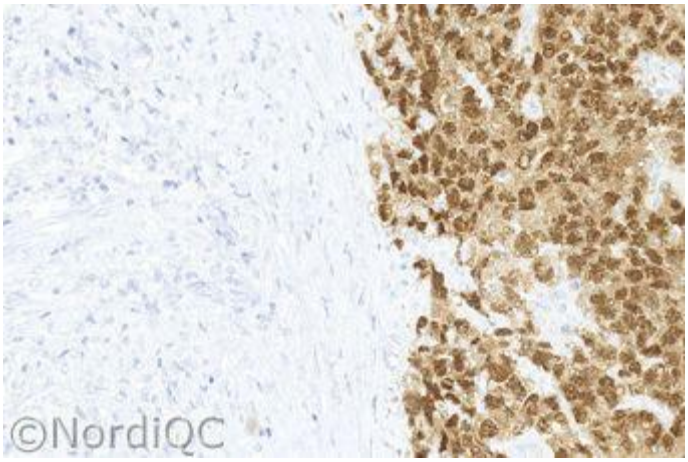


Fig. 4b
Staining pattern for the mAb clone C-10 in the embryonal carcinoma, same protocol as in Fig. 3b. The neoplastic cells show a strong nuclear staining reaction, and no intracytoplasmic staining reaction is seen in the myofibroblasts - same field as in Fig. 4a.

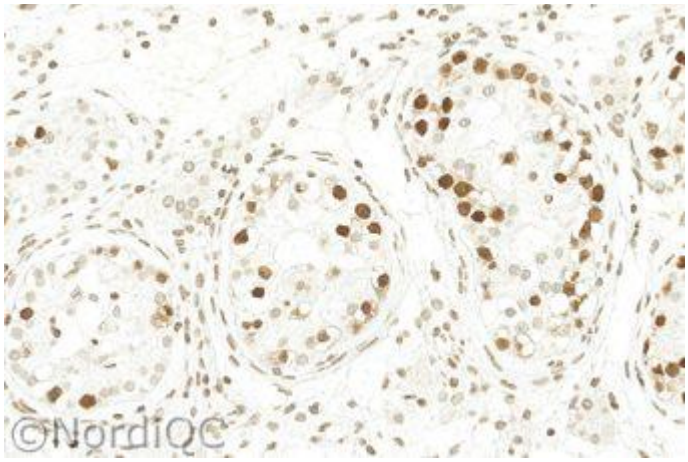


Fig. 5a
Insufficient staining reaction for OCT3/4 of the intratubular germ cell neoplasia using the mAb clone C-10. Virtually all cells show a positive nuclear staining reaction. The neoplastic cells show a strong staining intensity, but e.g. also the stromal cells are demonstrated.
The aberrant nuclear staining reaction most likely was caused by a too high concentration of the primary Ab.
Also compare with Fig. 5b - same protocol.

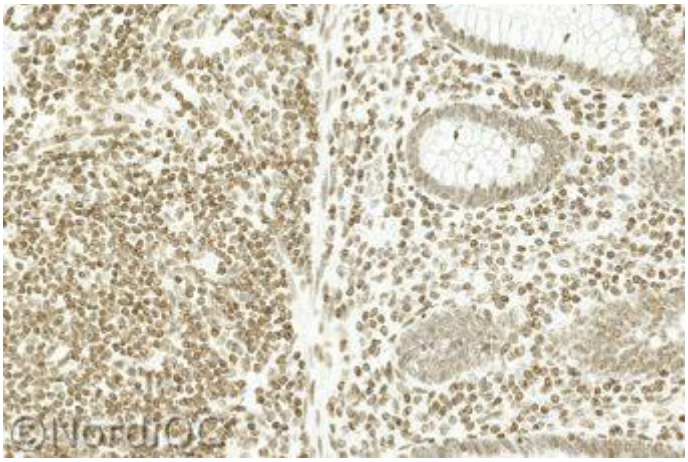


Fig. 5b
Insufficient staining of the appendix using the mAb clone C-10, same protocol as in Fig. 5a.
Virtually all cells show a false positive nuclear staining reaction.

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