Assessment Run 64 2022
PAX8

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for PAX8 used to identify the origin of renal cell and ovarian carcinoma in the diagnostic work-up of cancer of unknown primary (CUP) origin. Relevant clinical tissues, both normal and neoplastic, were selected for a broad spectrum of antigen densities for PAX8 (see below).

Material
The slide to be stained for PAX8 comprised:

1. Fallopian tube, 2. Kidney, 3. Tonsil, 4. Renal clear cell carcinoma (RCC),
5. Ovarian serous adenocarcinoma. 6. Colon adenocarcinoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a PAX8 staining as optimal included:

- A weak to moderate, distinct nuclear staining reaction of the majority of ciliated epithelial cells and a strong nuclear staining of intercalated secretory epithelial cells in the Fallopian tube.
- An at least weak to moderate, distinct nuclear staining reaction in the majority of epithelial cells of the proximal, distal/collecting renal tubules, loops of Henle and the parietal epithelial cells of Bowman's capsule in the kidney.
- A strong, nuclear staining reaction of virtually all neoplastic cells in the ovarian serous adenocarcinoma.
- A moderate to strong, nuclear staining reaction of the majority of neoplastic cells in the RCC.
- No nuclear staining reaction of B-cells. This was expected for antibodies (Ab) raised against the C-terminal part of PAX8 - e.g. mAb clone BC12 and rmAbs clones EP298, SP348, EP331 and ZR-1.
- No staining reaction of neoplastic cells in the colon adenocarcinoma.

  In cells with strong specific nuclear staining reaction, weak to moderate cytoplasmic staining was accepted.

In this assessment cross-reaction with other PAX epitopes e.g. to PAX5 in B-cells and/or PAX6 in neuroendocrine cells was downgraded, due to interpretational challenges especially in the diagnostic work-up of CUP. This applied for polyclonal Abs and mAb clones MRQ-50, PAX8R1 and C12A32. For these Abs the highest score consequently was "Good" providing an otherwise staining pattern as described above was obtained.

Participation

Number of laboratories registered for PAX8, run 62	363
Number of laboratories returning slides	337 (93%)

At the date of assessment, 93% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data was not included in this report.

Results
337 laboratories participated in this assessment. 174 laboratories (52%) achieved a sufficient mark (optimal or good). Abs used and assessment marks are summarized in Table 1 (see page 3)

The most frequent causes of insufficient staining were:
- Use of less successful antibodies primarily mAb clone MRQ-50 and rabbit monoclonal (rm)Ab clone EP331.
- Too short efficient Heat Induced Epitope Retrieval (HIER) time.
- Too low concentration of the primary Ab.

Performance history

This was the seventh NordiQC assessment of PAX8. The pass rate has slowly but consistently been increased since run 56 in 2019. The proportion of participants using the mAb clone MRQ-50 was still relatively high (35% of all participants) and similar to previous runs, this clone was found to be less successful especially when applied on the Ventana BenchMark and Dako Omnis platforms (see Table 5). Furthermore, all results with a cross reaction to other PAX epitopes was downgraded due to interpretational challenges and in total only 27% of the results were scored optimal.

Graph 1. **Proportion of sufficient results for PAX8 in the seventh NordiQC runs performed**

PAX8 performance in NordiQC assessments
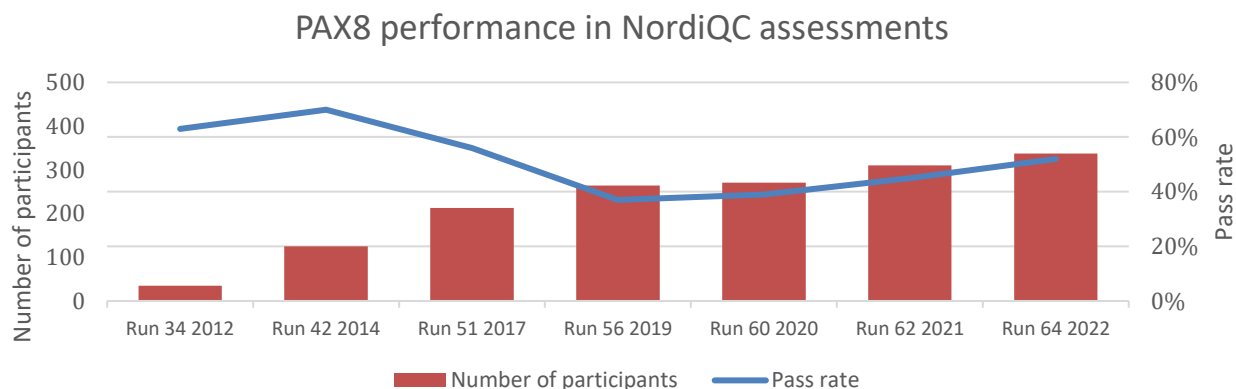
Conclusion

Optimal staining results could be obtained with the rmAb clones **SP348, ZR-1, BP6157, IHC048, QR016** and the mAb clone **BC12**. Irrespective of the clone applied, efficient HIER, use of a sensitive 3-step polymer/multimer based detection system and careful calibration of the primary antibody were the most important prerequisites for an optimal staining result.

The rmAb clone **SP348** gave encouraging results and a high proportion of sufficient results on the main fully automated platforms and no cross-reaction with e.g. PAX5 was observed. In contrast, the mAb clone **MRQ-50** provided a poor performance especially on the Ventana BenchMark and Dako Omnis platforms and at the same time also labelled PAX5 in B-cells. The **EP331** also provided a low pass rate due to aberrant nuclear staining reaction in non-PAX8 expressing cells and poor signal-to-noise ratio.

Laboratory developed (LD) tests based on concentrated Abs provided a significantly higher proportion of sufficient results (76%) compared to Ready-To-Use (RTU) Abs (17%), mainly related to the most successful Ab rmAb clone SP348 only being available as concentrate and not developed for any RTU system.

Kidney and Fallopian tube are both recommendable as positive tissue controls for PAX8. In kidney, an at least weak to moderate, distinct nuclear staining reaction in the majority of the epithelial cells of the proximal, distal/collecting renal tubules and parietal epithelial cells of Bowman's capsule must be seen. In Fallopian tube, an at least weak to moderate, distinct nuclear staining reaction of the majority of ciliated epithelial cells and a strong nuclear staining of intercalated secretory epithelial cells must be seen. Tonsil can be used as negative tissue control for PAX8, as no staining should be seen in e.g. squamous epithelial cells and lymphocytes (positive nuclear staining in B-cells indicate cross reaction with PAX5).

Table 1. **Antibodies and assessment marks for PAX8, run 64**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **BC12***	6 4	Biocare Zytomed Systems	2	4	4	-	60%	20%
mAb clone **MRQ-50**	25	Cell Marque	-	17	7	1	68%	-
mAb clone **PAX8R1**	3	Abcam	-	1	2	-	-	-
mAb clone **IHC008**	1 1	GenomeMe GeneAb	-	-	1	1	-	-
rmAb clone **EP298⁵***	3 1	Cell Marque Epitomics⁵	-	1	-	3	-	-
rmAb clone **EP331***	9 6	Cell Marque Epitomics	-	8	6	1	53%	-
rmAb clone **SP348***	105 3 3	Abcam Gennova Spring Bioscience	81	24	4	2	95%	73%
rmAb clone **ZR-1***	3 1 1	Zeta Corporation Gene Tech ImmunoForce	2	1	1	1	60%	40%
rmAb clone **BP6157***	1	Biolynx	1	-	-	-	-	-
rmAb clone **IHC048***	1	GenomeMe	1	-	-	-	-	-
rmAb clone **QR016***	2	Quartett	1	1	-	-	-	-
pAb, **10336-1-AP**	13	Proteintech	-	3	6	4	23%	-
pAb, **363A-15**	2	Cell Marque	-	2	-	-	-	-
pAb, **CP379**	2	Biocare	-	-	2	-	-	-
pAb, **RBK047**	2 1	Zytomed Systems Diagomics	-	1	1	1	-	-
Conc total	199		88	63	34	14	76%	44%
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone **MRQ-50, 760-4618 (VRPS)³**	3	Ventana/Roche	-	-	-	3	-	-
mAb clone **MRQ-50, 760-4618 (LMPS)⁴**	55	Ventana/Roche	-	4	33	18	7%	-
rmAb clone, **EP331* 760-6077(VRPS)³**	5	Ventana/Cell Marque	-	-	5	-	-	-
rmAb clone, **EP331* 760-6077(LMPS)⁴**	15	Ventana/Cell Marque	-	-	11	4	-	-
mAb clone, **BC12* API438**	8	Biocare Medical	-	2	1	5	25%	-
rmAb clone **ZR-1* Z2202**	2	Zeta corporation	-	1	1	-	-	-
rmAb clone **SP348 M6481**	1	Spring Bioscience	1	-	-	-	-	-
rmAb **ZR-1* BSB2099**	1	Bio SB	-	-	-	1	-	-
rmAb clone **2774R ANB31**	1	Biogenex	-	-	-	1	-	-
rmAb clone **EP331* 363M/AC0338**	3	Cell Marque	-	-	3	-	-	-
mAb clone **MRQ-50, 363M-10/17/18**	33	Cell Marque	-	8	19	6	24%	-
mAb clone **MRQ-50, MAD-000550QD**	2	Master Diagnostica	-	2	-	-	-	-
mAb clone **MRQ-50, MAD-000550QD**	1	Vitro SA	-	1	-	-	-	-
pAb clone **RBG047**	1	Zytomed	-	-	1	-	-	-
rmAb clone **RM436* 8257-C010**	2	Sakura Finetek	2	-	-	-	-	-
rmAb clone **IHC048***	1	GenomeMe	-	1	-	-	-	-
mAb clone **C12A32**	1	Celnovte	-	1	-	-	-	-
mAb clone **4H7B3***	1	Diagnostic Biosystems	-	-	1	-	-	-
Unknown	2		-	-	1	1	-	-

RTU total	138		3	20	76	39	17%	2%
Total	337		91	83	110	53		
Proportion			27%	25%	33%	15%	52%	

1) Proportion of sufficient stains (optimal or good). (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 assessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 assessed protocols).
5) Ab terminated by vendor.
*Clones that do not show cross reactivity with PAX5.

Detailed analysis of PAX8, Run 64
The following protocol parameters were central to optimal staining:

Concentrated Antibodies
rmAb clone **SP348**: Protocols with optimal results were based on HIER using Target Retrieval Solution (TRS) High pH (29/31)* (Dako/Agilent), TRS High pH (3-in-1)(4/8) (Dako/Agilent), or Cell Conditioning 1 (CC1, Ventana/Roche) (45/63) or using HIER in CC1 (Ventana/Roche) in combination with Protease 3 (Ventana/Roche) for 4 min. (3/5). The rmAb was diluted 1:50-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings, 100 of 104 (96%) laboratories produced a sufficient staining result.
*(number of optimal results/number of laboratories using this buffer)

rmAb clone **ZR-1**: Only two protocols produced optimal results. One based on TRS High pH (3-in-1) (Dako/Agilent), and one in Tris-EDTA/EGTA pH 9 buffer. The rmAb was diluted 1:50-100. Using these protocol settings 2 of 2 laboratories produced a sufficient staining result.

mAb clone **BC12:** Only two protocols produced optimal results. Both protocols were based on Bond Epitope Retrieval Solution 2 (BERS2) (Leica Biosystems). The mAb was diluted 1:25-50 in Renoir Red (Biocare). Using these protocol settings 4 of 6 (67%) laboratories produced a sufficient staining result.

Table 3. **Proportion of optimal results for PAX8 for the most commonly used antibodies as concentrate on the four main IHC systems***

Concentrated antibodies	Dako/Agilent Autostainer		Dako/Agilent Omnis		Ventana/Roche BenchMark GX / XT / Ultra			Leica Biosystems Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC1 pH 8.5 + P3	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
rmAb **SP348**	4/8 (50%)	-	29/31 (94%)	0/1	45/63 (71%)	3/5 (60%)	0/2	0/1	-
rmAB **ZR-1**	1/1	-	-	-	0/1		-	0/2	-
mAb **BC12**	-	-	-	-	-	-	-	2/6 (33%)	-

* 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
Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used Ready-To-Use (RTU) systems (≥5 assessed protocols). 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 device are included.

Table 4. **Proportion of sufficient and optimal results for PAX8 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 **MRQ-50** 760-4618	0/3	0/3	4/55 (7%)	0/55
VMS Ultra/XT mAb **EP331** 760-6077	0/5	0/5	0/13	0/13

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and detection kit. Only protocols performed on the specified vendor IHC stainer were included.

Comments

In this assessment and in concordance with the previous NordiQC PAX8 assessments, the prevalent feature of an insufficient result was a too weak or false negative staining reaction of cells expected to be demonstrated. This pattern was seen in 78% of the insufficient results (127 of 163 laboratories). The remaining 22% of insufficient results were characterized by a poor signal-to-noise ratio and/or false positive staining reaction compromising interpretation.

As observed in previous runs, the majority of the participating laboratories were able to demonstrate PAX8 in high-level antigen expressing cells, such as secretory epithelia cells of the Fallopian tube and neoplastic cells of the ovarian serous adenocarcinoma, whereas demonstration of PAX8 in low-level antigen expressing cells as the neoplastic cells of the RCC, epithelial cells of collecting ducts, parietal cells lining the Bowman capsules of the kidney and in particular ciliated epithelial cells of the Fallopian tube and epithelial cells of the proximal tubules in the kidney were more challenging and only seen with appropriate protocol settings (see Fig. 1a - 5b). Cases of insufficient staining due to false positive cytoplasmic and/or aberrant nuclear reaction of cells not expressing PAX8 was also seen. This pattern was typically caused by use of a less successful primary antibody giving cross-reaction with e.g. PAX5.

Cross-reactivity with PAX5 resulting in a distinct nuclear staining reaction of B-cells for antibodies raised against the N-terminal part of PAX8 was seen in 42% (142/337) of the returned slides (see Fig. 5a and 5b). This reaction applied for all polyclonal Abs and mAb clones MRQ-50, C12A32, IHC008, 2774R and PAX8R1. Within the last couple of years well-performing rmAbs without cross reactivity has been introduced to the market (see Table 1). Based on this, cross-reactivity with PAX5 was downgraded due to the risk of misinterpretation in the diagnostic work-up of CUP. The diagnostic challenges and different reaction profiles related to the choice of PAX8 Ab has e.g. been described by Kamaljeet Singh et al.; *AIMM 2020, Aug;28(7):558-561; Comparison of PAX8 Expression in Breast Carcinoma Using MRQ-50 and BC12 Monoclonal Antibodies* and Tacha D et al., *AIMM 2013, Jan;21(1):59-63; PAX8 mouse monoclonal antibody [BC12] recognizes a restricted epitope and is highly sensitive in renal cell and ovarian cancers but does not cross-react with b cells and tumors of pancreatic origin*.

59% (199 of 337) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for PAX8. The mAb clones MRQ-50, BC12 the rmAb clones SP348, EP331, ZR-1 and the pAb 10336-1-AP were the most widely used antibodies (see Table 1). Out of these six Abs only the mAb clone BC12, rmAbs clones SP348 and ZR-1 provided optimal staining results.

Data focusing on the four main IHC systems (see Table 3) showed that the rmAb clone SP348 could be used to obtain optimal results on tree of the four main systems.

The SP348 clone was the most successful concentrate with a pass-rate of 95%, 73% optimal. Within the sufficient protocols the concentrate was used in a range of 1:25-1:2.000 and with HIER in an alkaline buffer as single pre-treatment. 5 laboratories used a proteolytic pre-treatment in combination with HIER for SP348, 3 assessed as optimal and 2 good.

In this assessment optimal results for the SP348 clone were obtained on the Dako/Agilent Autostainer and Omnis and the Ventana/Roche Benchmark platforms. In previous runs limited data was available and it was not possible to conclude if the rmAb clone was able to produce optimal results on either Dako Autostainer or the Leica Biosystems Bond instruments, and for the latter it still has to be clarified if possible.

Virtually all optimal results for clone SP348 were obtained by use of 3-step polymer/multimer based detection systems providing a high level of analytical sensitivity and expected performance in all tissues.

The most widely used concentrate mAb clone MRQ-50 was observed to be inferior to e.g. rmAb clone SP348 and gave a relatively low pass rate of 68%, no optimal. The clone frequently both provided a too low level of analytical sensitivity and cross reaction with e.g. PAX5 in B-cells. Similar to the observations generated in previous runs, the performance of mAb clone MRQ-50 was affected by the choice of platform. In the cumulated data for runs 62 and 64, the MRQ-50 clone only gave a pass rate of 7% on the Ventana BenchMark platform (12/181) and no sufficient mark on Dako Omnis (0/14) (see Table 5).

This inferior performance and reduced analytical sensitivity can potentially be related to the washing conditions and/or influence of elevated temperature settings (32°C on the Dako Omnis and 36°C on the Ventana BenchMark) compared to systems using room temperature for incubation and washings. Whether this is the case with the mAb clone MRQ-50 is so far uncertain. Both the Dako Autostainer and the Leica Biosystems Bond platform could be used to provide sufficient results (see Table 5) using HIER in an alkaline buffer for about 20 min. and the mAb clone MRQ-50 conc. diluted in the range of 1:50-200 with an incubation time of 15-30 min. depending on the total sensitivity of the protocol employed. For both platforms 3-layer detection systems were most successful.

Table 5. Overview of the assessment marks for mAb clone MRQ-50 on the four main IHC instruments in runs 62 and 64 (cumulated data for both RTU and concentrate).

MRQ-50 score	Dako/Agilent Autostainer	Dako/Agilent Omnis	Ventana/Roche BenchMark GX / XT / Ultra	Leica Biosystems Bond III / Max
Optimal	-	-	-	-
Good	22	-	12	31
Borderline	4	11	110	3
Poor	-	3	59	-
Total	26	14	181	34
Sufficient %	85%	0%	7%	91%

The number of participants using the rmAb clone EP331 both as a RTU format and as a concentrate has increased immensely from 4 participants in run 60 to 37 in this present run. None of the participants using this clone have received an optimal mark in NordiQC. In general, it seemed to be challenging to calibrate a protocol based on rmAb clone EP331 to obtain a high level of analytical sensitivity, appropriate specificity and optimal signal-to-noise ratio. If rmAb clone EP331 was used within protocols providing high level technical sensitivity identifying the critical structures as neoplastic cells in the RCC an aberrant nuclear staining was seen in non-PAX8 expressing stromal cells and simultaneously an aberrant cytoplasmic staining in e.g. tonsillar dendritic cells and the neoplastic cells in the colon adenocarcinoma. If the protocol was calibrated to reduce the aberrant background reaction, the critical structures were typically not demonstrated as expected. Sufficient results were only seen on the Dako Omnis, whereas applying the EP331 clone on the Ventana BenchMark, Leica Bond or Dako Autostainer a false positive staining of the tonsil, colon adenocarcinoma and glycocalyx in the kidney was seen for both the concentrate and RTU formats (see Figs. 6a-b).

In total 41% (138 of 337) of the laboratories used Abs in RTU formats. This is lesser that the previous run with 46% RTU users. The most widely used RTU systems for PAX8 were based on the mAb clone MRQ-50, prod. no **760-4616** from Ventana/Roche and prod. no **363M-18** from Cell Marque. Both RTU products had an alarmingly low pass rate of 7% (4 of 58) and 24% (8 of 33), respectively. However, these data are in line with the observation for the MRQ-50 based LD assays and supports the observation that the mAb clone MRQ-50 is very difficult to optimize on the Ventana BenchMark platform. In total 78 laboratories used the Ventana BenchMark for one of the two RTUs formats of mAb clone MRQ-50.
For the Ventana RTU format 760-4618 performed on BenchMark being used by 58 laboratories, only 3 laboratories followed the vendors recommended protocol settings without a sufficient result. The remaining 54 laboratories modified the protocol settings and 7% (4 of 54) achieved a sufficient staining result. One laboratory using the Ventana platform produced a sufficient result with a laboratory modified protocol using HIER (CC1, Ventana) for 56 min. and a primary Ab incubation for 32 min. and OptiView as detection system. The protocol still had difficulties demonstrating PAX8 in the proximal tubular cells in the kidney and the neoplastic cells in the ovarian serous adenocarcinoma showed a significantly reduced intensity. These settings tended to produce a poor signal-to-noise ratio and/or in some cases a false positive staining result in other cases.

In this assessment an increased number of participants used non-cross-reacting clones for PAX8 as SP348, contributing to the improved pass-rate from 45% to 52%. The overall performance for protocols based on concentrated formats had a pass-rate of 76% compared to protocols based on RTU formats with only a pass-rate of 17% (see Table 1). The pass-rate is still at a very low level and the combination of limited access to non-cross reacting RTU systems for PAX8 and the platform depending performance of MRQ-50 complicates the optimization and validation process for the laboratories. However, there are still available clones as SP348, ZR1, BC12 etc. that has the potential to give optimal results on all platforms and at present seem to be the preferred choice.

Controls
Kidney and Fallopian tube are both recommended as positive tissue controls for PAX8. In kidney, optimally calibrated protocols must provide an at least weak to moderate, distinct nuclear staining reaction in the majority of epithelial cells of the proximal and distal renal tubules, loops of Henle, collecting ducts, and the parietal epithelial cells of Bowman's capsule. A weak to moderate cytoplasmic staining reaction in the same cells can be expected. In Fallopian tube, the protocol must be calibrated to provide an at least weak to moderate, distinct nuclear staining in the majority of the ciliated epithelial cells and a strong nuclear staining of the intercalated secretory epithelial cells. A weak cytoplasmic staining in the intercalated secretory epithelial cells can be expected and must be accepted. Internal observations show that inadequate fixation (too short time / delayed) in formalin can reduce epitope availability in low-level PAX8 expressing structures.

Tonsil can be used as negative tissue control for PAX8, as no staining should be seen in e.g. squamous epithelial cells and lymphocytes (positive nuclear staining in B-cells indicate cross reaction with PAX5).
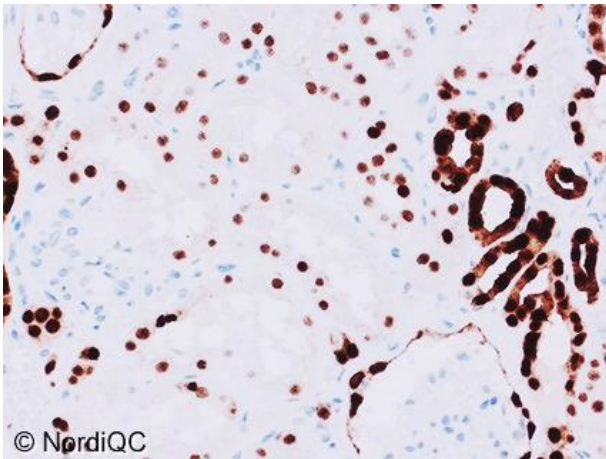

Fig. 1a x200
Optimal PAX8 staining of the kidney using the rmAb clone SP348 (Abcam) within a laboratory developed assay optimally calibrated, using HIER in an alkaline buffer and a 3-step multimer based detection system (OptiView, Ventana/Roche) and performed on the Ventana BenchMark. A weak to strong, distinct nuclear staining reaction is seen in the majority of epithelial cells of the proximal, distal/collecting renal tubules, loops of Henle and the parietal epithelial cells of Bowman's capsule in the kidney. A weak cytoplasmic background staining is seen and accepted in the tubular cells (same protocol used in Figs. 1a - 5a) Compare with Fig. 1b.
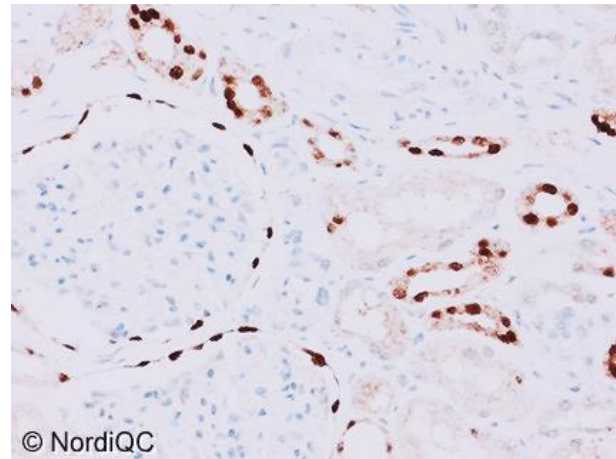
Fig. 1b x200
Insufficient PAX8 staining of the kidney using the mAb clone MRQ-50 (Ventana/Cell Marque) RTU within a laboratory developed assay, using an alkaline HIER pretreatment and a 3-step multimer based detection system (OptiView, Ventana/Roche) and performed on the Ventana BenchMark system. A weak to moderate nuclear staining reaction of the distal/collecting tubular cells is seen. The proximal tubular cells are virtually negative. Compare with Fig. 1a. This was the typical pattern for the MRQ-50 clone when applied on the Ventana Benchmark and Dako Omnis platform. Also compare with Figs. 2b-5b – same protocol.
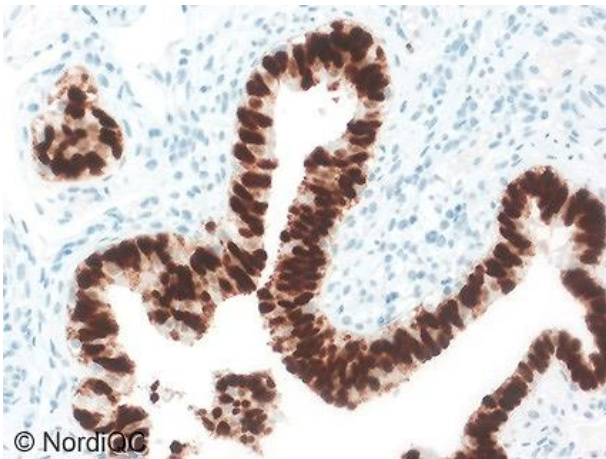
Fig. 2a x200
Optimal PAX8 staining of the Fallopian tube using the same protocol as in Fig. 1a. A weak to moderate, distinct nuclear staining reaction of the majority of the ciliated epithelial cells and a strong nuclear staining reaction of the intercalated secretory epithelial cells is seen. A weak cytoplasmic staining reaction is seen and accepted in the epithelial cells. Compare with Fig. 2b.
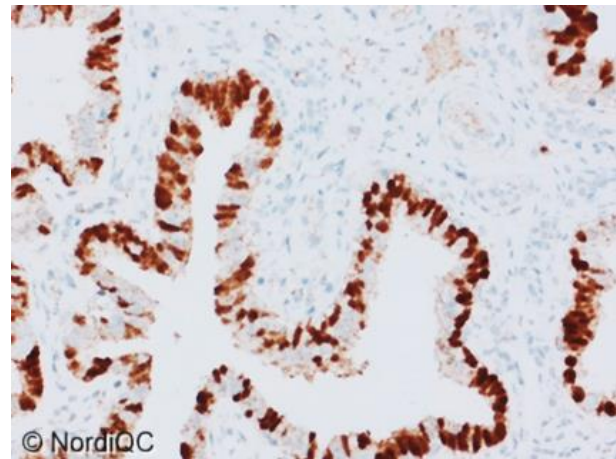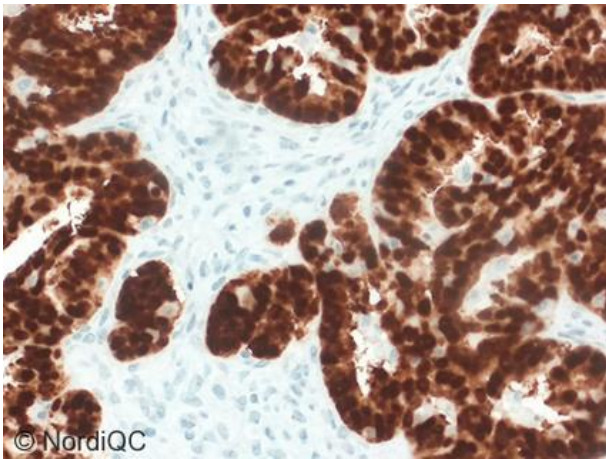

Fig. 2b x200
Insufficient PAX8 staining of the Fallopian tube using the same protocol as in Fig. 1b. A moderate nuclear staining of the intercalated secretory epithelial cells is seen whereas the number and intensity of ciliated epithelial cells is significantly decreased. Compare with Fig. 2a.
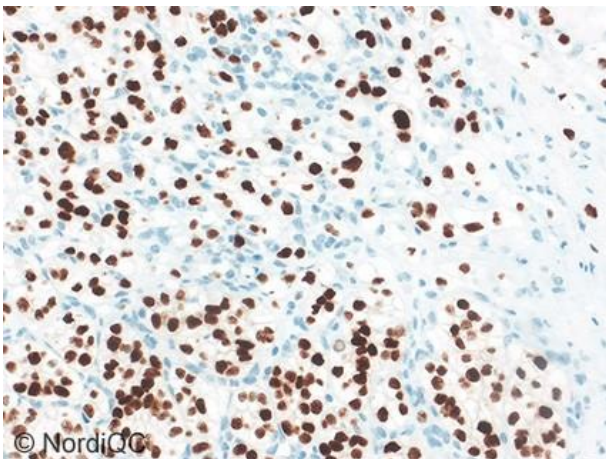
Fig. 3a x200
Optimal PAX8 staining of the ovarian serous adenocarcinoma using the same protocol as in Figs. 1a and 2a. A very strong, nuclear staining reaction is seen in virtually all the neoplastic cells. Compare with Fig. 3b.
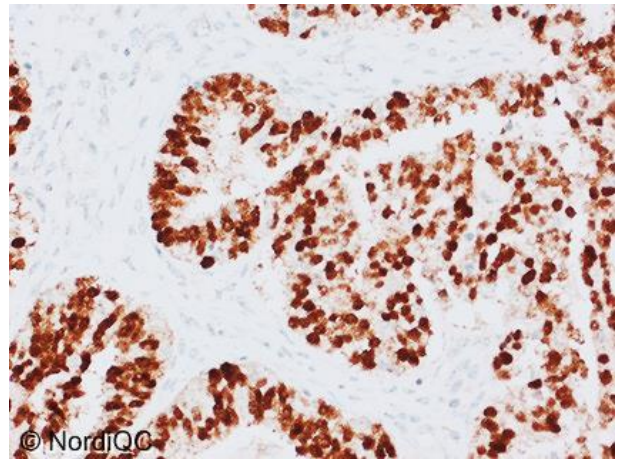

Fig. 3b x200
PAX8 staining of the ovarian serous adenocarcinoma using the same insufficient protocol as in Figs. 1b and 2b. The majority of the neoplastic cells are demonstrated but display only a weak to moderate nuclear staining reaction. Compare with Fig. 3a.

Fig. 4a x200
Optimal PAX8 staining of the RCC using the same protocol as in Figs. 1a-3a. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. No background staining is seen. Compare with Fig. 4b.
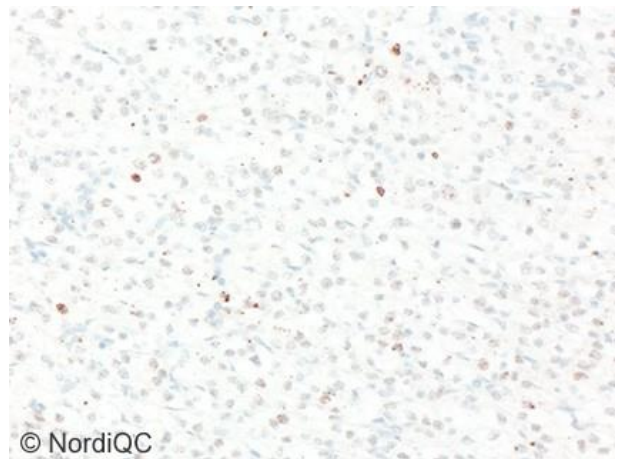

Fig. 4b x200
Insufficient PAX8 staining of the RCC using the same protocol as in Figs. 1b-3b.
Only a faint nuclear staining is seen in the vast majority of neoplastic cells. Compare to Fig. 4a.
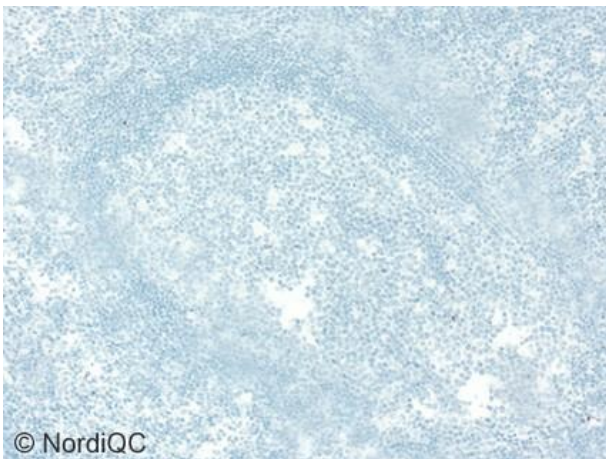
Fig. 5a x100
PAX8 staining without PAX5 cross reactivity. PAX8 staining in tonsil using the same protocol as in Figs. 1a-4a. The rmAb clone SP348 do not cross-react with PAX5, leaving the B-cells unstained. Compare with Fig. 5b.
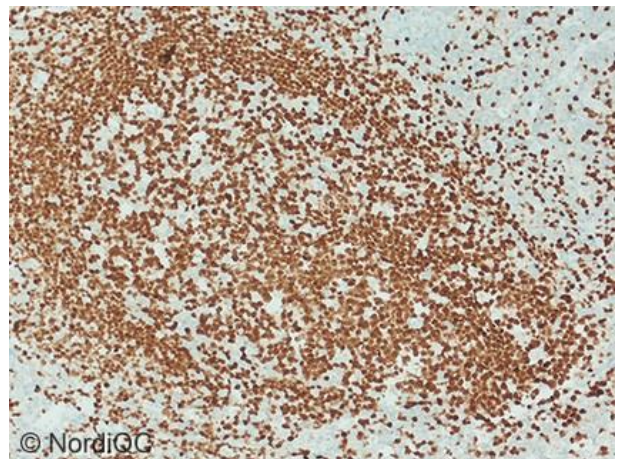
Fig. 5b x100
PAX8 staining with PAX5 cross reactivity. PAX8 staining in tonsil using the same protocol as in Figs. 1b-4b. The mAb clone MRQ-50 cross-reacts with PAX5 resulting in nuclear staining reaction in virtually all B-cells. Compare with Fig. 5a.
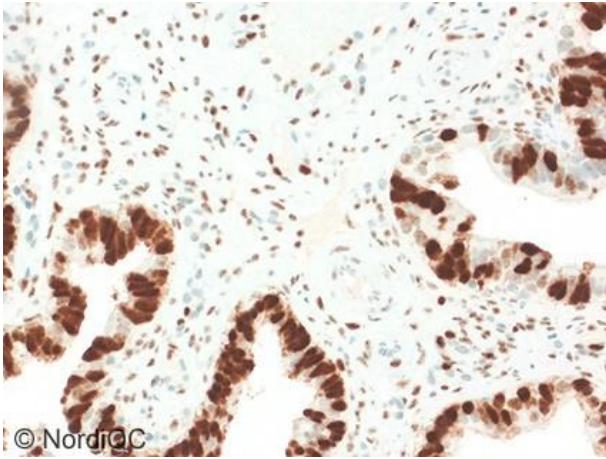
Fig. 6a x200
Staining result for PAX8 of the Fallopian using an insufficient protocol based on the rmAb clone EP331 as RTU (760-6077, Ventana/Cell Marque) within a laboratory developed assay, using HIER in an alkaline buffer as pretreatment, a 3-step multimer based detection system (OptiView, Ventana/Roche) and performed on the Ventana BenchMark system. A weak to moderate, distinct nuclear staining reaction of the majority of the ciliated epithelial cells and a strong nuclear staining of the secretory epithelial cells is seen. The staining pattern in the epithelial cells is as expected and similar to Fig 2a., but in addition a moderate to strong distinct aberrant staining of the nuclei of the stromal cells is seen. Also compare with Fig 6b – same protocol.
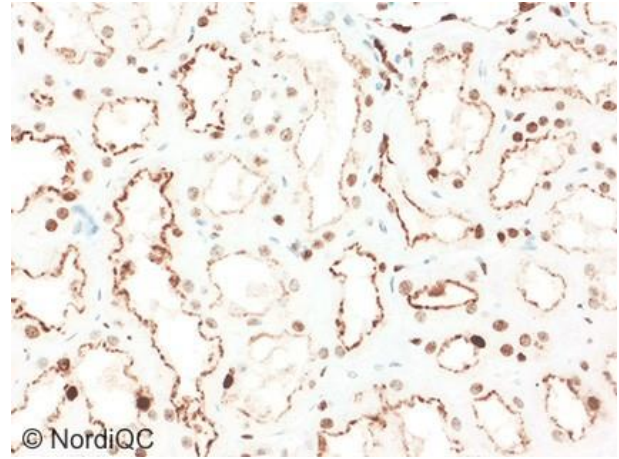

Fig. 6b x200
Insufficient staining of the kidney using same protocol as in Fig. 6a.
Compaired to the optimal result of kidney shown in Fig. 1a, this protocol provides an excessive granulated cytoplasmic staining reaction and a strong staining of the glycocalyx in the proximal tubules and at the same time a reduced nuclear staining reaction in the cells expected to be demonstrated. Same result was typically also seen on the Leica BOND and Dako Omnis platforms using similar protocol settings.

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