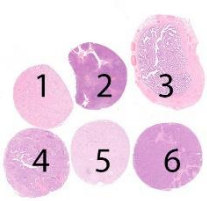
Assessment Run 68 2023

Paired box gene-8 protein (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. Kidney, 2. Tonsil, 3. Fallopian tube, 4. Colon adenocarcinoma,
5. Clear cell renal carcinoma (ccRCC), 6. Ovarian serous 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 ccRCC.
- 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 68	405
Number of laboratories returning slides	368 (91%)

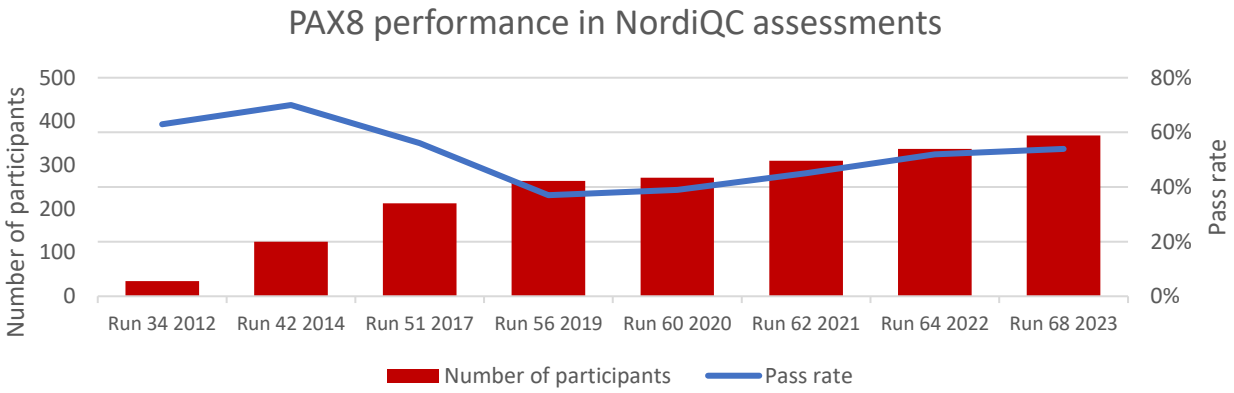
At the date of assessment, 91% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data was not included in this report.

Results
368 laboratories participated in this assessment. 197 laboratories (54%) 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 eight 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 among the RTU users (27% 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 were downgraded due to interpretational challenges and in total only 32% of the results were scored good.

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



Conclusion
Optimal staining results could be obtained with the rmAb clones **SP348, ZR-1, MXR013, GR002, QR016,** and **RM436**. 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. No optimal results were however observed on the Bond Platform. 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 (72%) compared to Ready-To-Use (RTU) Abs (26%), 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 68**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **BC12***	9 4	Biocare Zytomed Systems	-	3	7	3	23%	-
mAb clone **MRQ-50**	16	Cell Marque	-	8	6	2	50%	-
mAb clone **PAX8R1**	1	Abcam	-	-	1	-	-	-
mAb clone **ZM28**	1	Zeta Corporation	-	1	-	-	-	-
rmAb clone **EP298[5]***	1	Epitomics[5]	-	1	-	-	-	-
rmAb clone **EP331***	10 4	Cell Marque Epitomics	-	5	8	1	36%	-
rmAb clone **SP348***	146	Abcam Gennova Spring Bioscience	102	31	9	4	91%	70%
rmAb clone **ZR-1***	2 2	Zeta Corporation BioSite	1	-	2	1	-	-
rmAb clone **BP6157***	2	Biolynx	-	1	1	-	-	-
rmAb clone **QR016***	7	Quartett	3	3	1	-	86%	43%
pAb, **10336-1-AP**	11	Proteintech	-	1	3	7	9%	-
pAb, **363A-15**	1	Cell Marque	-	-	1	-	-	-
pAb, **CP379 AK**	3	Biocare	-	-	1	2	-	-
pAb, **RBK047**	3	Zytomed Systems Diagomics	-	-	3	-	-	-
Conc total	223		106	54	43	20	72%	48%
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone **MRQ-50, 760-4618 (VRPS)[3]**	6	Ventana/Roche	-	-	-	6	0%	0%
mAb clone **MRQ-50, 760-4618 (LMPS)[4]**	49	Ventana/Roche	-	3	34	12	6%	0%
rmAb clone, **EP331* 760-6077(VRPS)[3]**	3	Ventana/Cell Marque	-	1	2	-	-	-
rmAb clone, **EP331* 760-6077(LMPS)[4]**	11	Ventana/Cell Marque	-	4	6	1	36%	0%
mAb clone, **BC12* API438**	6	Biocare Medical	-	2	4	-	33%	0%
mAb clone **IHC008 PII77R06**	3	DCS	-	-	3	-	-	-
rmAb clone **ZR-1* Z2202**	2	Zeta corporation	-	-	1	1	-	-
rmAb clone **SP348* M6481**	3	Spring Bioscience	2	1	-	-	-	-
rmAb clone **2774R ANB31**	1	Biogenex	-	-	1	-	-	-
rmAb clone **GR002* GT210202**	1	GeneTech	1	-	-	-	-	-
rmAb clone **QR016* P-P008**	2	Quartett	1	1	-	-	-	-
rmAb clone **EP331* 363M/AC0338**	12	Cell Marque	-	3	7	2	25%	0%
rmAb clone **SP348* 363R-38**	4	Cell Marque	2	1	1	-	-	-
mAb clone **MRQ-50, 363M-10/17/18**	24	Cell Marque	-	5	13	6	21%	0%
pAb clone **363A-17/18 363A17/18**	4	Cell Marque	-	-	3	1	-	-
mAb clone **MRQ-50, MAD-000550QD**	6	Master Diagnostica	-	4	1	1	67%	0%
rmAb clone **RM436* 8257-C010**	2	Sakura Finetek	1	1	-	-	-	-
rmAb clone **IHC048***	1	GenomeMe	-	-	1	-	-	-
mAb clone **C12A32**	1	Celnovte	-	1	-	-	-	-

Clone **MXR013* RMA-1024**	2	Fuzhou Maixin	2	-	-	-	-	-
Clone **H5A8 DTBL0220101**	1	DaTe Bioengineering Technology	1	-	-	-	-	-
Unknown	1		-	-	-	1	-	-
RTU total	145		10	27	77	31	26%	8%
Total	368		116	81	120	51		
Proportion			32%	22%	32%	14%	54%	

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 68

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 (37/49)* (Dako/Agilent), TRS Low pH (3/4) (Dako/Agilent), or Cell Conditioning 1 (CC1, Ventana/Roche) (60/81) or using HIER in CC1 (Ventana/Roche) in combination with Protease 3 (Ventana/Roche) for 4 min. (1/3). The rmAb was diluted 1:50-1:2.000 depending on the total sensitivity of the protocol employed. Using these protocol settings, 122 of 131 (93%) laboratories produced a sufficient staining result.
*(number of optimal results/number of laboratories using this buffer)

rmAb clone **QR016**: three protocols produced optimal results. All 3 were based on HIER in CC1 (Ventana/Roche) (3/5). The rmAb was diluted 1:100. Using these protocol settings 4 of 4 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**	3/7** (43%)	0/1	35/42 (83%)	2/2	60/81 (74%)	1/3	0/2	0/6	-
rmAb **QR016**	-	-	-	-	3/5 (60%)	-	-	0/2	-
rmAb **EP331**	-	-	0/7	-	0/5	-	-	0/2	-
mAb **BC12**	-	0/1	-	-	0/1	-	-	0/8	-
mAb **MRQ-50**	0/3	-	0/2	-	0/1	-	-	0/6	0/2
pAb **10336-1-AP**	0/2	-	0/3	-	0/5	-	-	0/1	-

* 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% (0/6)	0% (0/6)	4% (2/47)	0% (0/47)
VMS Ultra/XT mAb **EP331 760-6077**	33% (1/3)	0% (0/3)	36% (4/11)	0% (0/11)

* 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 72% of the insufficient results (123 of 171 laboratories). The remaining 28% 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 and parietal cells lining the Bowman capsules of the kidney, whereas demonstration of PAX8 in low-level antigen expressing cells as the neoplastic cells of the ccRCC, 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 Figs. 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 34% (125/368) of the returned slides (see Figs. 5a and 5b). This reaction applied for all polyclonal Abs and mAb clones MRQ-50, C12A32, IHC008, H5A8, PAX8R1 and rmAb 2774R. 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*.

61% (223 of 368) 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, QR016 and the pAb 10336-1-AP were the most widely used antibodies (see Table 1). Out of these six Abs only the rmAbs clones SP348 and QR016 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 three of the four main systems.

The SP348 clone was the most successful concentrate with a pass-rate of 91%, 70% optimal. Within the sufficient protocols the concentrate was used in a range of 1:50-1:2.000 and with HIER in an alkaline buffer as single pre-treatment. 3 laboratories used a proteolytic pre-treatment in combination with HIER for SP348, 1 result was assessed as optimal and 2 good.

In this assessment optimal results for the SP348 clone were obtained on the Dako/Agilent Autostainer, Dako 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 the Dako Autostainer and the Leica Biosystems Bond instruments. In this run however optimal results were seen on the Autostainer platform in three out of 8 cases, all using the Flex+ as detection system. 6 laboratories used the SP348 clone on the Leica Bond with 4 cases being scored as good, but an overall weak staining reaction. The protocols were based on a dilution in the range of 1:25-100, HIER in BERS 2 pH 9 for 30-40 min. and an Ab incubation time for 15-30 min. These settings are similar to protocols scores as optimal on other platforms. The inferior performance for the rmAb clone SP348 may be related to the Bond Refine detection system which in the cases of rabbit antibodies acts as only a 2-step detection system due to the

post-blocking step or Linker being based on a rabbit anti-mouse antibody amplifying the signal of mouse primary antibodies with no effect on primary rabbit antibodies.

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 second most used concentrate was the mAb clone MRQ-50. This clone was observed to be inferior to e.g. rmAb clone SP348 and gave a relatively low pass rate of 50%, 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 from runs 62, 64 and 68, the MRQ-50 clone only gave a pass rate of 7% on the Ventana BenchMark platform (12/183) and no sufficient mark on Dako Omnis (0/16) (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 have provided 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, 64 and 68 (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	24	-	12	37
Borderline	5	13	111	5
Poor	-	3	60	-
Total	29	16	183	40
Sufficient %	83%	0%	7%	93%

The number of participants using the rmAb clone EP331 both as a RTU format and as a concentrate has declined from 37 to 28 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 of analytical sensitivity identifying the critical structures as neoplastic cells in the ccRCC 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 and Ventana Benchmark platforms, whereas applying the EP331 clone on the 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).

The pAb 10336-1-AP was used by 11 participants, only one provided a sufficient result. The Ab showed both false negative and false positive results including cross-reaction with other PAX subtypes. In the colon adenocarcinoma the neoplastic cells were negative, but both stromal and connective tissue were aberrantly positive (see Figs. 7a and b).

In total 39% (145 of 368) of the laboratories used Abs in RTU formats. This is less than the previous run with 41% RTU users. The most widely used RTU systems for PAX8 were based on the mAb clone MRQ-50, prod. no **760-4618** from Ventana/Roche and prod. no **363M-10/17/18** from Cell Marque. Both RTU products had an alarmingly low pass rate of 6% (3 of 55) and 21% (5 of 24), 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 67 laboratories used the Ventana BenchMark for one of the two RTU formats of mAb clone MRQ-50.
For the Ventana RTU format 760-4618 performed on BenchMark being used by 53 laboratories, only 6 laboratories followed the vendors recommended protocol settings without a sufficient result. The remaining laboratories modified the protocol settings and 4% (2 of 47) achieved a sufficient staining result.

In this assessment an increased number of participants used non-cross-reacting clones for PAX8 as SP348, contributing to the improved pass-rate from 52% to 54%. The overall performance for protocols based on concentrated formats had a pass-rate of 72% compared to protocols based on RTU formats with only a pass-rate of 26% (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 commercially available clones as SP348, QR016 etc. that has the potential to give optimal results on three of the main IHC 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).

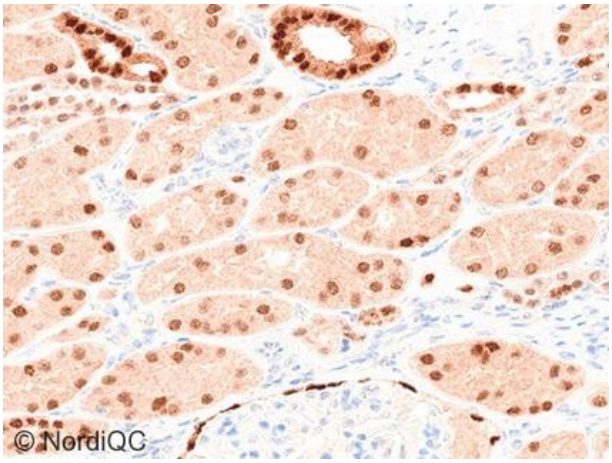


Fig. 1a x200
Optimal PAX8 staining of the kidney using the rmAb clone SP348 (Cell Marque) as a concentrate within a laboratory developed assay optimally calibrated, using HIER in an alkaline buffer and a 3-step polymer based detection system (EnVision Flex++, Dako/Agilent) and performed on the Dako Omnis. A moderate 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 cytoplasmic background staining is seen and accepted in the tubular cells (same protocol used in Figs. 1a - 5a) Compare with Fig. 1b.

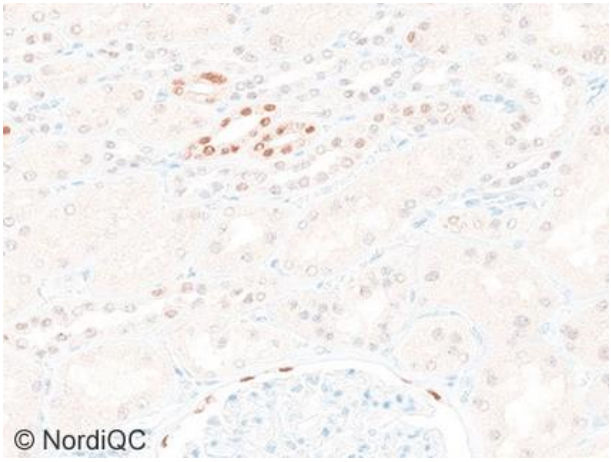


Fig. 1b x200
Insufficient PAX8 staining of the kidney using the mAb clone MRQ-50 (Ventana/Cell Marque) RTU within a laboratory developed assay, using an alkaline HIER pretreatment and a 3-step multimer based detection system (OptiView, Ventana/Roche) and performed on the Ventana BenchMark system. A weak to moderate nuclear staining reaction of the distal/collecting tubular cells and the parietal epithelial cells of Bowman's capsule in the kidney 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.

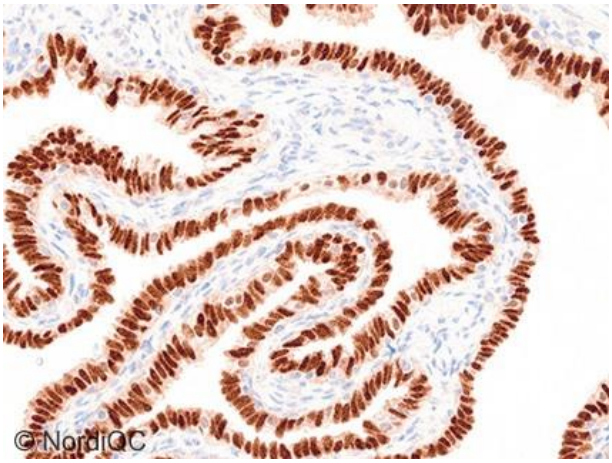


Fig. 2a x200
Optimal PAX8 staining of the Fallopian tube using the same protocol as in Fig. 1a. A weak to moderate, distinct nuclear staining reaction in the majority of the ciliated epithelial cells and a strong nuclear staining reaction of the intercalated secretory epithelial cells is seen. A weak cytoplasmic staining reaction is seen and accepted in the epithelial cells. Compare with Fig. 2b.

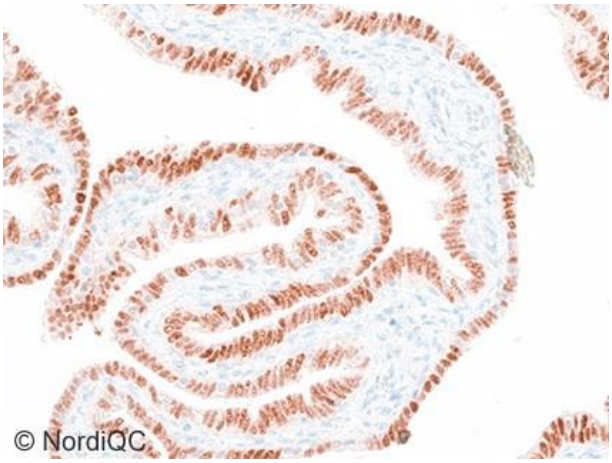


Fig. 2b x200
Insufficient PAX8 staining of the Fallopian tube using the same protocol as in Fig. 1b. A moderate nuclear staining of the intercalated secretory epithelial cells is seen whereas the number and intensity of ciliated epithelial cells is decreased. Compare with Fig. 2a.

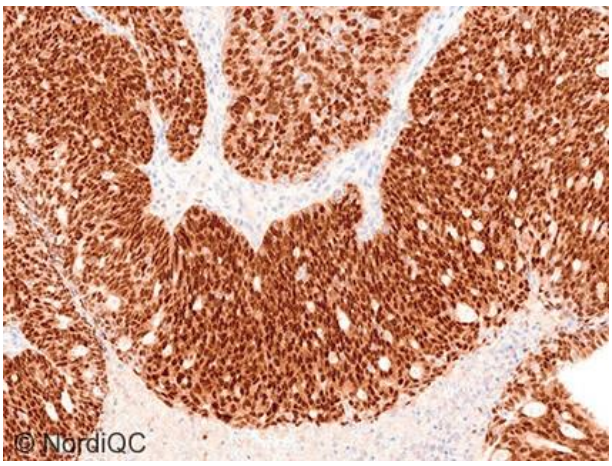


Fig. 3a x100
Optimal PAX8 staining of the ovarian serous adenocarcinoma using the same protocol as in Figs. 1a and 2a. A very strong, nuclear staining reaction is seen in virtually all the neoplastic cells. Compare with Fig. 3b.

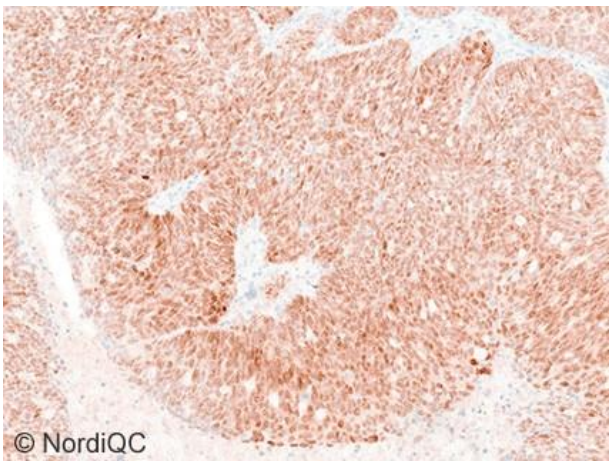


Fig. 3b x100
PAX8 staining of the ovarian serous adenocarcinoma using the same insufficient protocol as in Figs. 1b and 2b. The majority of the neoplastic cells are demonstrated but display only a moderate nuclear staining reaction. Compare with Fig. 3a.

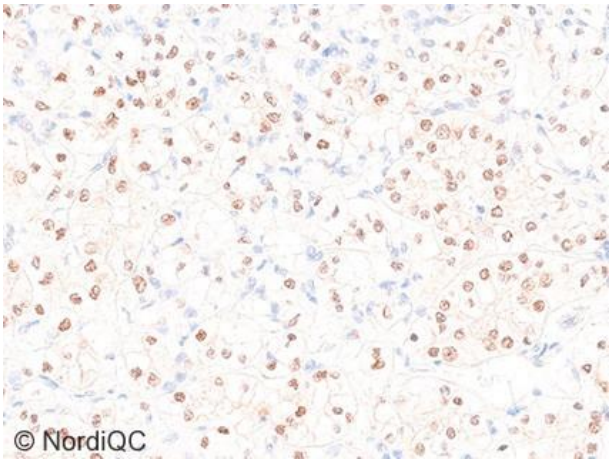


Fig. 4a x200
Optimal PAX8 staining of the ccRCC using the same protocol as in Figs. 1a-3a. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. Compare with Fig. 4b.

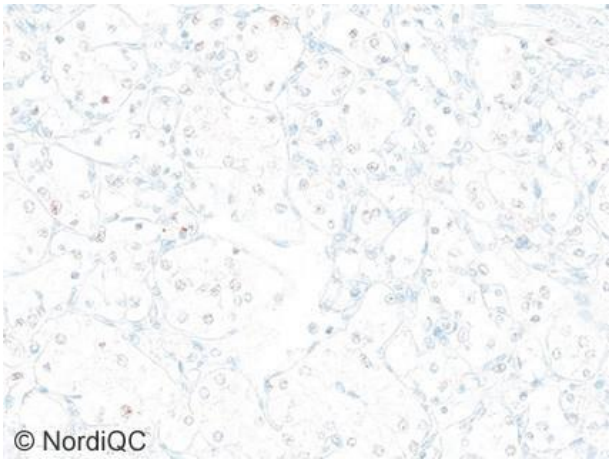


Fig. 4b x200
Insufficient PAX8 staining of the ccRCC using the same protocol as in Figs. 1b-3b.
Only a faint nuclear staining is seen in some of the neoplastic cells. Compare to Fig. 4a.

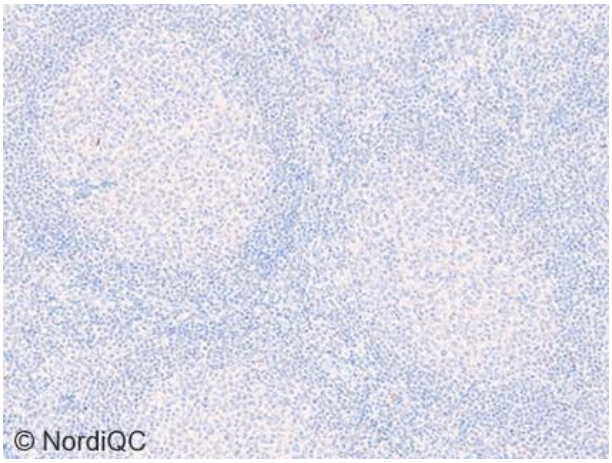


Fig. 5a x100
PAX8 staining without PAX5 cross reactivity. PAX8 staining in tonsil using the same protocol as in Figs. 1a-4a. The rmAb clone SP348 do not cross-react with PAX5, leaving the B-cells unstained. Compare with Fig. 5b.

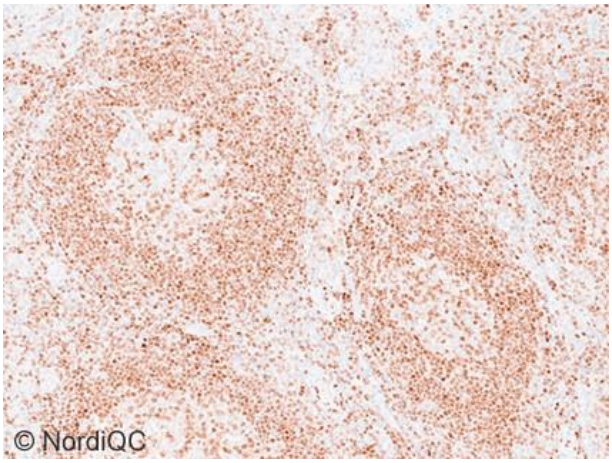


Fig. 5b x100
PAX8 staining with PAX5 cross reactivity. PAX8 staining in tonsil using the same protocol as in Figs. 1b-4b. The mAb clone MRQ-50 cross-reacts with PAX5 resulting in nuclear staining reaction in virtually all B-cells. Compare with Fig. 5a.

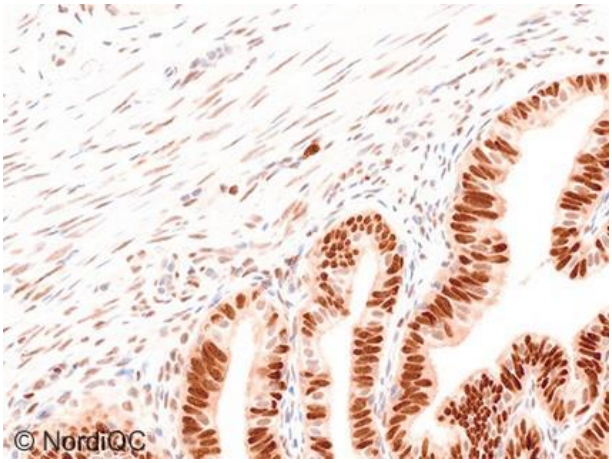


Fig. 6a x200
Insufficient staining result for PAX8 of the Fallopian using a protocol based on the pAb clone 363A-17/18 as RTU (Cell Marque) within a laboratory developed assay, using HIER in an alkaline buffer as pretreatment, a 3-step polymer based detection system (Bond Refine, Leica/Novocastra) and performed on the Leica Bond 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.

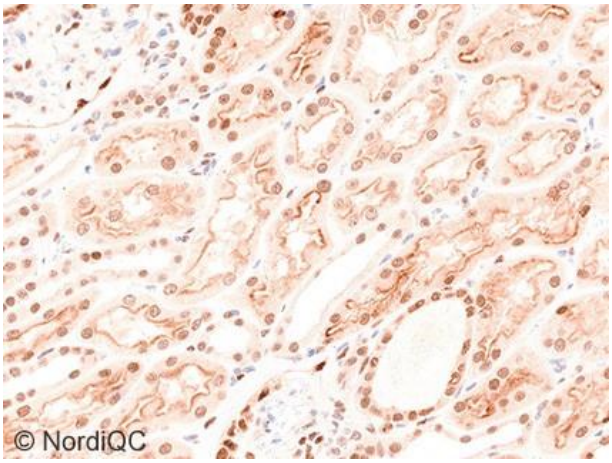


Fig. 6b x200
Staining result of the kidney using same protocol as in Fig. 6a.
Compaired to the optimal result of kidney shown in Fig. 1a, this protocol provides an granulated cytoplasmic staining reaction and a moderate staining of the glycocalyx in the proximal tubules and at the same time a reduced nuclear staining reaction in the cells expected to be demonstrated. Same result was typically also seen on the Dako Omnis platforms and with the rmAb EP331 clone using similar protocol settings.

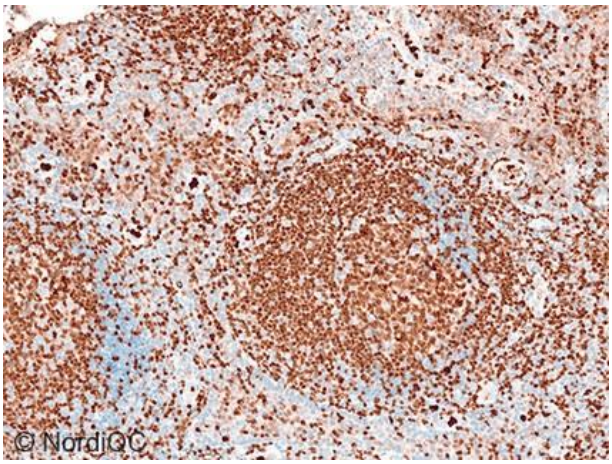


Fig. 7a x100
Insufficient PAX8 staining of the tonsil using the pAb clone 10336-1-AP (Protein Group) concentrate within a laboratory developed assay, using HIER in an alkaline buffer and a 3-step multimer based detection system (OptiView, Ventana/Roche) and performed on the Ventana BenchMark system. A cross reaction to PAX2 and PAX5 is seen along with a general background reaction and strongly stained macrophages. Also compare with Fig 7b – same protocol.

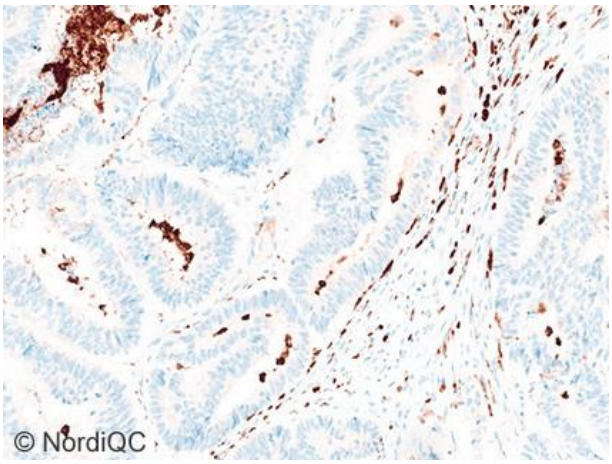


Fig 7b x100
Insufficient staining of the colon adenocarcinoma using same protocol as in Fig. 7a.
The neoplastic cells are negative but the B-cells and macrophages in lamina propria show a moderate to strong positive staining reaction.

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