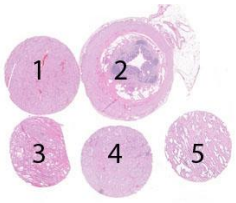
Assessment Run 49 2017

Prostate Specific Antigen (PSA)

Material
The slide to be stained for PSA comprised:

1. Kidney, 2. Appendix, 3-4. Prostate adenocarcinoma, 5. Prostate hyperplasia



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a PSA staining as optimal included:

- A strong, predominantly cytoplasmic staining reaction of all epithelial cells of the hyperplastic prostate glands.
- An at least weak to moderate, predominantly cytoplasmic staining reaction of virtually all neoplastic cells of the prostate adenocarcinoma, core 4.
- A strong, predominantly cytoplasmic staining reaction of all neoplastic cells in the prostate adenocarcinoma, core 3.
- No staining reaction of epithelial cells in the kidney and appendix.

A staining reaction of a pigment-like substance in the kidney was accepted, provided that the epithelial cells of the tubuli were completely negative.

Participation

Number of laboratories registered for PSA, run 49	304
Number of laboratories returning slides	284 (93%)

Results
284 laboratories participated in this assessment. 254 (89%) achieved a sufficient mark (optimal or good). Table 1 summarizes the used antibodies (Abs) and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too high or too low concentration of the primary antibody
- Too short incubation time of primary Ab
- Insufficient HIER (too short heating time)
- Unexplained technical issues

Performance history
This was the fourth NordiQC assessment of PSA. The overall pass rate was high and has increased significantly compared to the result obtained in run 40, 2014 (see table 2).

Table 2. **Proportion of sufficient results for PSA in the four NordiQC runs performed**

	Run 12 2004	Run 27 2009	Run 40 2014	Run 49 2017
Participants, n=	79	126	237	284
Sufficient results	90%	76%	74%	89%

Conclusion
The mAb clones **35H9**, **ER-PR8,** rmAb clone **EP109** and **pAb 0452** could all be used to obtain an optimal staining for PSA. As concentrated formats within a laboratory developed (LD) test, mAb clone **35H9** and rmAb **EP109** were most successful, the former providing optimal results on the three main full-automated IHC systems (Omnis, Benchmark and BOND).
The Ready-To-Use (RTU) systems from Dako/Agilent (**GA514** and **IS/IR514**), Roche/Ventana (**760-2506** and **760-4271**) and Leica/Novocastra (**PA0431**) all provided a high proportion of sufficient and optimal results.
Prostate hyperplasia is recommended as positive tissue control provided that the epithelial cells show an as strong as possible cytoplasmic staining reaction (weak to moderate staining of the stroma must be accepted). Kidney/appendix is recommended as negative tissue control, as no staining reaction should be seen in the epithelial cells.

Table 1. **Antibodies and assessment marks for PSA, run 49**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **35H9**	21 1 1 1 1	Leica/Novocastra Monosan Abnova Diagnostic Biosystem Gene Tech	21	3	1	0	96%	95 %
mAb clone **ER-PR8**	31 3 1 1	Dako/Agilent Cell Marque Zeta Zytomed Systems	19	11	6	0	82%	82%
mAb clone **ER-PR8+A67-B/E3***	1	Biocare Medical	1	0	0	0	-	-
mAb clone **28A4***	1	Leica/Novocastra	0	0	1	0	-	-
rmAb clone **EP109**	5 1	Biocare Medical Cell Marque	6	0	0	0	100%	100%
pAb **0562**	62	Dako/Agilent	33	16	12	1	79%	85%
Ready-To-Use antibodies								
mAb clone **35H9 PA0431**	11	Leica Biosystems	6	5	0	0	100%	100%
mAb clone **35H9 PDM087**	1	Diagnostic biosystems	1	0	0	0	-	-
mAb clone **ER-PR8 760-4271**	18	Ventane/Roche	8	8	2	0	89%	87%
mAb clone **ER-PR8 760-4930**	3	Cell Marque	1	2	0	0	-	-
mAb clone **ER-PR8 324M-17/18**	2	Cell Marque	1	1	0	0	-	-
mAb clone **ER-PR8 AM014-10M**	2	Biogenex	2	0	0	0	-	-
mAb clone **ER-PR8 MAD-000532QD**	2	Master Diagnostica	1	1	0	0	-	-
mAb clone **ER-PR8 MAB-0146**	1	Maixin	1	0	0	0	-	-
rmAb clone **EP109 PME390**	1	Biocare medical	1	0	0	0	-	-
pAb **760-2506**	51	Ventana/Roche	34	11	5	1	88%	93%
pAb **IS/IR514**	33	Dako/Agilent	31	2	0	0	100%	100%
pAb **IS/IR514**[3]	5	Dako/Agilent	4	0	1	0	-	-
pAb **GA514**	20	Dako/Agilent	20	0	0	0	100%	100%
pAb **GA514**[4]	3	Dako/Agilent	2	1	0	0	-	-
Total	284		193	61	28	2	-	
Proportion			68%	21%	10%	1%	89%	

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´s semi-automated systems (Autostainer Link/+) but used by laboratories on different platforms (e.g. Ventana Benchmark).
4) RTU system developed for the Dako/Agilent´s full-automated systems (Omnis) but used by laboratories on different platforms (e.g. Ventana Benchmark).
* Discontinued by the vendor

Detailed analysis of PSA, run 49
The following protocol parameters were central to obtain optimal staining.

Concentrated antibodies
mAb **35H9**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using an alkaline buffer as Cell Conditioning 1 (CC1, BenchMark, Ventana) (9/9) *, TRS High pH (3-1) (Dako/Agilent) (4/4), Bond Epitope Retrieval Solution 2 (BERS2, Bond, Leica) (3/5), TRIS-EDTA pH 9 (3/3) or Bond Epitope Retrieval Solution 1 (BERS1, Bond, Leica) (2/3) as retrieval buffer. The mAb was typically diluted in the range of 1:100 – 1:800 depending on the total sensitivity of the protocol employed. Using these protocol settings, 20 of 21 (95%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb **ER-PR8**: Protocols with optimal results were all based on HIER using an alkaline buffer as CC1 (BenchMark, Ventana) (11/19), TRS High pH (3-1) (Dako/Agilent) (5/11), TRS High pH (Dako/Agilent) (1/1), TRIS-EDTA pH 9 (1/1) or BERS1 (Bond, Leica) (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:10 – 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 28 of 34 (82%) laboratories produced a sufficient staining (optimal or good).

mAb **ER-PR8+A67-B/E3**: One protocol with an optimal result was based on HIER using Diva Decloaker pH 6.2 (Biocare Medical) (1/1) as retrieval buffer. The mAb was diluted 1:200 and MACH 1 (Bicare Medical) was used as detection system.

rmAb **EP109**: Protocols with optimal results were all based on HIER using an alkaline buffer as CC1 (BenchMark, Ventana) (2/2), TRIS-EDTA pH 9 (2/2), BERS1 (Bond, Leica) (1/1) or Citrate pH 6 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:25 – 1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 5 of 5 (100%) laboratories produced a sufficient staining (optimal or good).

pAb **0562**: Protocols with optimal results were most frequently based on HIER using an alkaline buffer as CC1 (BenchMark, Ventana) (14/22), TRS High pH (3-1) (Dako/Agilent) (4/8), Cell Conditioning 2 (BenchMark, Ventana) (2/3) or Citrate pH 6 (1/1) as retrieval buffer. Ten protocols based on omission of pretreatment and two protocols based on proteolytic pretreatment also provided optimal results. The mAb was typically diluted in the range of 1:1,000 – 1:10,000 depending on the total sensitivity of the protocol employed. Using these protocol settings, 41 of 48 (86%) laboratories produced a sufficient staining (optimal or good).

Table 3. **Proportion of optimal results for PSA for the most commonly used antibodies as concentrate on the 3 main fully-automated IHC systems***

Concentrated antibodies	**Dako/Agilent Omnis**		**Ventana BenchMark XT / Ultra/GX**		**Leica Bond III / Max**	
	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 **35H9**	3/3 **	-	9/9 (100%)	-	3/3	2/3
mAb clone **ER-PR8**	2/3	-	11/20 (55%)	-	-	1/1
pAb **0562**	0/1	-	14/21 (67%)	2/3	0/1	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)

Table 4. **Proportion of optimal results for PSA for the most commonly used antibodies as concentrate on the 3 main semi-automated IHC systems ***

Concentrated antibodies	**Dako/Agilent Autostainer Link / Classic**		**Thermo/LabVision Autostainer 36/48/72**		**Biocare Medical IntelliPATH**	
	TRS pH 9.0	TRS pH 6.1	HIER buffer H pH 9.0	HIER Buffer L pH 6.0	Borg pH 9.0	Diva pH 6.2
mAb clone **35H9**	-	-	-	-	-	-
mAb clone **ER-PR8**	3/8** (38%)	0/1	-	-	-	-
pAb **0562**	3/6 (50%)	0/1	-	-	-	1/1

* 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
mAb clone **35H9,** product no. **PA0431,** Leica/Novocastra:
Protocols with optimal results were based on HIER using Bond Epitope Retrieval Solution 1 pH 6 (efficient heating time 10-20 min. at 99-100°C), 15-30 min. incubation of the primary Ab and BOND Refine (DS9800) as detection system. Using these protocol settings, 5 of 5 (100%) laboratories produced a sufficient result.

mAb clone **ER-PR8,** product no. **760-4271,** Ventana Benchmark Ultra/XT/GX:
Protocols with optimal results were typically based on HIER using Cell Conditioning 1 (CC1) (efficient heating time 24-64 min. at 99-100°C) and 16-30 min. incubation of the primary Ab. UltraView (760-500) with or without amplification (760-080) or OptiView (760-700) were used as detection systems. Using these protocol settings, 13 of 15 (87%) laboratories produced a sufficient staining result.

mAb clone **ER-PR8,** product no. **MAD-000532QD,** Master Diagnostica MD-Stainer:
One protocol with an optimal result was based on HIER using a TRIS-EDTA pH 9 based buffer, Master Diagnostica (efficient heating time 20 min. at 100°C) and 45 min. incubation of the primary Ab. Master Polymer Plus was used as detection system.

rmAb clone **EP109,** product no. **PME390,** Biocare Medical IntelliPATH:
One protocol with an optimal result was based on HIER using Diva pH 6.2 as buffer (efficient heating time 15 min. at 110°C, Decloaker) and 30 min. incubation of the primary Ab. MACH4 was used as detection system.

pAb, product no. **760-2506, Ventana** Benchmark XT/Ultra:
Protocols with optimal results were based on HIER using CC1 (efficient heating time 8-64 min. at 95-100°C) and 8-36 min. incubation of the primary Ab. UltraView (760-500) with or without amplification (760-080) or OptiView (760-700) were used as detection systems. Using these protocol settings, 26 of 28 (93%) laboratories produced a sufficient staining result. 12 protocols based on no pre-treatment, one protocol based on HIER in Cell Conditioning 2 and one protocol based on proteolytic pretreatment (Protease 3) also gave an optimal mark.

pAb**,** product no. **IR/IS514,** Dako/Agilent Autostainer+ /Autostainer Link:
Protocols with optimal results were based on HIER using TRS pH 9 (3-1) or TRS pH 6.1 (3-1) (efficient heating time 10-30 min. at 95-97°C), 20-30 min. incubation of the primary Ab and EnVision Flex or Flex+ (K8000/K8002/K8010/K8012) as detection system. Using these protocol settings, 21 of 21 (100%) laboratories produced a sufficient result. One protocol was based on no pre-treatment and two laboratoris obtained an optimal result using same protocol settings as above but performed HIER in a standard TRIS-EDTA buffer pH 9.

pAb**,** product no. **GA514,** Dako/Agilent Omnis:
Protocols with optimal results were based on HIER using TRS pH 9 (3-1) (efficient heating time 20-30 min. at 97°C), 4-13 min. incubation of the primary Ab and EnVision Flex (GV800/GV823) as detection system. Using these protocol settings, 19 of 19 (100%) laboratories produced a sufficient result. One laboratory obtained an optimal result using same protocol settings as above but performed HIER off-board using TRS pH 6 (3-1).

Table 5 summarises 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 significantly. Only protocols performed on the specific IHC stainer device are included, whereas e.g. Dako RTU Ab formats applied on a Ventana stainer device were excluded.

Table 5. **Comparison of pass rates for vendor recommended and laboratory modified RTU protocols**

RTU systems	**Vendor recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS48 pAb **IR/IS514**	100% (13/13)	100% (13/13)	100% (18/18)	89% (16/18)
Dako Omnis pAb **GA514**	100% (12/12)	100% (12/12)	100% (7/7)	100% (7/7)
VMS Ultra/XT/GX pAb **760-2506**	86% (6/7)	57% (4/7)	89% (39/44)	68% (30/44)
VMS Ultra/XT/GX mAb ER-PR8 **760-4271**	66% (2/3)	33% (1/3)	93% (14/15)	47% (7/15)
BOND MAX/III mAb 35H9 **PA0431**	100% (3/3)	0% (0/3)	100%(8/8)	80% (6/8)

* 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 are included.

Comments

In this assessment for PSA, the prevalent features of an insufficient staining were characterized by a general poor signal-to-noise ratio and/or false positive staining reaction in e.g. the epithelial cells of the kidney and appendix. This pattern was seen in 60% (18 of 30) of the insufficient results. The remaining 40% of insufficient results were characterized by a too weak or completely false negative staining reaction in one or more cores. Virtually all laboratories were able to demonstrate PSA in high-level PSA expressing cells (normal epithelial cells of hyperplastic prostate glands and neoplastic cells of the prostate adenocarcinoma no. 3), whereas low-level PSA expressing cells in the prostate adenocarcinoma no. 4 could only be demonstrated using a carefully calibrated protocol.

Used as concentrates within LD assays, the mAb clone 35H9 (Leica/Novocastra) and the rmAb clone EP109 (Biocare Medical or Cell Marque) provided the highest proportion of sufficient and optimal results (see table 1).
The mAb 35H9 and mAb ER-PR8 used as LD assays could produce optimal results on all three main fully-automated IHC systems from Dako/Agilent, Leica/Novocastra and Roche/Ventana/ (see table 3). Comparing the proportion of optimal results using these primary Abs on the individual IHC systems, the mAb 35H9 produced significantly higher proportion of optimal results than the mAb ER-PR8 on the Ventana Benchmark Ultra/XT/GX platforms, 100 % (9 of 9) and 55% (11 of 20), respectively. Although using identical protocol settings, the mAb 35H9 showed a slightly superior performance compared to the mAb ER-PR8 and was less sensitive to small variations in the staining conditions. Irrespective of the primary Ab applied, efficient HIER preferable in alkaline buffer, careful calibration of the primary Ab and use of a sensitive detection system were the most central parameters for optimal results.

The pAb A0562 was the most widely used primary Ab as concentrated format and provided a reduced number of sufficient and optimal results compared to mAb 35H9 and rmAb EP109. Optimal result could be obtained using either HIER (21 of 44), no pre-treatment (10 of 16) or proteolytic pre-treatment (2 of 2). There was no significant difference in performance if HIER was based on either an alkaline or acidic buffer. In contrast to the results obtained in Run 40, laboratories applying no pre-treatment had a high pass rate of 94% (15 of 16) of which 63% (10 of 16) was optimal. In run 40 the pass rate for omission of pre-treatment was 60% of which 10 % was optimal. The reason for this discrepancy is uncertain, but differences in PSA expression levels in the material circulated and most likely differences for NBF fixation time in these specimens might impact the result. In general, HIER must be preferred as pre-treatment to secure a consistent demonstration of the antigens irrespective of NBF fixation time.
In addition, the titre of pAb A0542 must be carefully calibrated to provide an IHC protocol with an optimal signal-to-noise ratio. The protocol should be able to demonstrate PSA in structures with both low-level and high-level expression, as the different neoplasias included in the circulated material, but also avoid false positive staining of tissue structures lacking the antigen (PSA) as e.g. kidney and appendix. In this assessment, using HIER (any antigen retrieval buffer) in combination with a standard 2- or 3-step multimer/polymer detection system, the Average Dilution Value (ADV) for optimal results was 1:7,125 (range 1:3,000-1:15,000), whereas a ADV of 1:4,813 (range 1:100-1:12,000) was seen in protocols with insufficient results. This clearly indicates that one should focus on both the analytical sensitivity and specificity when calibrating the titre of the primary Ab. Inclusion of negative tissue controls will aid to determine the titre for an optimal signal-to-noise ratio as non-specific staining reactions can be revealed.

All protocols (6) based on the newly launched rmAb clone EP109 were assessed as optimal. This new antibody seems robust as several settings e.g. different HIER buffers (alkaline or acidic) and detection systems (2-step or 3-step) could be used to produce an optimal result.
Ready-To-Use (RTU) antibodies were used by 54% (153 of 284) of the laboratories.
The RTU formats based on the mAb 35H9 (PA0431, Leica/Novocastra) and the pAbs IR/IS514 or GA514 (Agilent/ Dako) showed superior performance as all protocols (64 of 64) were assessed as sufficient. In this assessment the most successful assay was based on the RTU format pAb GA514 (Omnis), as all 100% (20 of 20) of the protocols gave an optimal mark, closely followed by the RTU system pAb IR/IS514 (Autostainer link/classic) where 94% (31 of 33) of the protocols were assessed as optimal. These three RTU systems were used by 23 % (64 of 284) of the laboratories and optimal results could be obtained by using vendor protocol recommendations (only for pAb GA514 or pAb IS/IR514) or by using laboratory modified protocol settings (typically adjusting HIER buffer, HIER time or incubation time of primary Ab).

The Ventana RTU system based on the pAb 760-2506 was the most widely used RTU system applied by 51 laboratories. A relative high overall pass rate of 88% (45 of 51) was seen and 67% (34 of 51) were optimal. Optimal results could be obtained both by use of vendor recommended or laboratory modified protocol settings (see table 5). As observed in run 40, the vendor recommended protocol for the Ventana RTU format prod. no. 760-2506 (no epitope retrieval, 16 min. incubation of the primary Ab and UltraView as detection system) was less successful compared to modified and laboratory validated protocol settings using HIER in CC1 (mild or standard) and 8-32 min. incubation of the primary Ab. A pass rate of 100% (11 of 11) of which 82% (9 of 11) were optimal was seen for participants using laboratory validated protocol settings. Using the vendor recommended protocol, omitting HIER, a pass rate of 86% was seen (6 of 7), 57% optimal.

For the Ventana RTU system 760-4271 based on the mAb ER-PR8, a pass rate of 89% was obtained of which 44% (8 of 18) were optimal. The pass rate was comparable to what could be obtained with the other RTU system 760-2506 (pAb) from Roche/Ventana, but the proportion of optimal results was significantly lower (see table 1). Both vendor protocol recommendations and laboratory modified protocol settings could produce optimal results. As shown in table 5, and although the number of participants following the vendor recommendations was low, there seems to be a tendency towards better results by changing at least some of the key elements in the protocols (typically prolonging incubation time of the primary Ab $\geq$ 24 min., prolonging HIER time $\geq$ 32 min. and using of OptiView as detection system).

This was the fourth NordiQC assessment of PSA. A pass rate of 89% was achieved, which is a significant improvement compared to run 40, 2014 (see table 1). The extended use of RTU products, 54% (153 of 284) in this assessment compared to 43% (102 of 237) in run 34 2012, has increased the overall performance. Grouped together, the RTU formats from the three main suppliers (Leica/Novocastra, Roche/Ventana and Dako/Agilent) provided an overall pass rate of 95% (126 of 133) and 74% (99 of 133) of the protocols were optimal. In addition, the use of robust and high quality primary antibodies as concentrates (e.g. mAb 35H9 and rmAb EP109), also provided a high proportion of sufficient and optimal results (see table 1). From a technical point of view, the mAb ER-PR8, both as concentrate and RTU format, seems more challenging and may require that all key parameters are properly calibrated to obtain an optimal protocol. For all primary Abs, efficient HIER (although optimal results also could be seen in protocols omitting this step), carefully calibration of the primary Ab and use of a sensitive detection system were prerequisites for optimal performance.

Controls
Prostate hyperplasia and kidney/appendix was in this assessment found to be recommendable positive and negative tissue controls for PSA. The epithelial cells of the prostate glands must show an as strong as possible cytoplasmic staining reaction. Due to leakage of the antigen in vicinity of the prostate glands, the stromal cells display a weak to moderate staining reaction. This staining pattern has to be accepted, otherwise the sensitivity of the assay is too low causing a general weak staining reaction of prostate carcinomas. Kidney and appendix can be used as negative tissue controls. No staining reaction should be seen in these tissues. If a positive staining reaction in the epithelial cells and/or a diffuse background staining is seen, the protocol must be recalibrated.

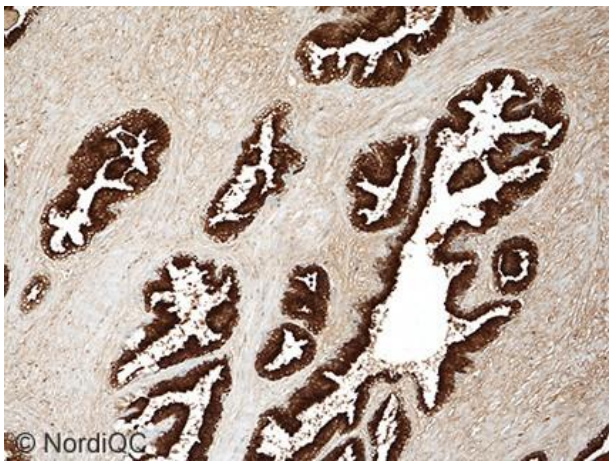


Fig. 1a (x100)
Optimal staining for PSA of the prostate hyperplasia using the pAb 760-2506 (RTU format , Ventana), HIER in an alkaline buffer (CC1) and a multimer based detection system (UltraView, Ventana) - same RTU format used in Figs. 2a - 5a.
The prostate glands show a strong distinct cytoplasmic staining reaction. A weak to moderate stromal reaction is seen (due to leakage of the antigen), which has to be accepted for optimal performance.

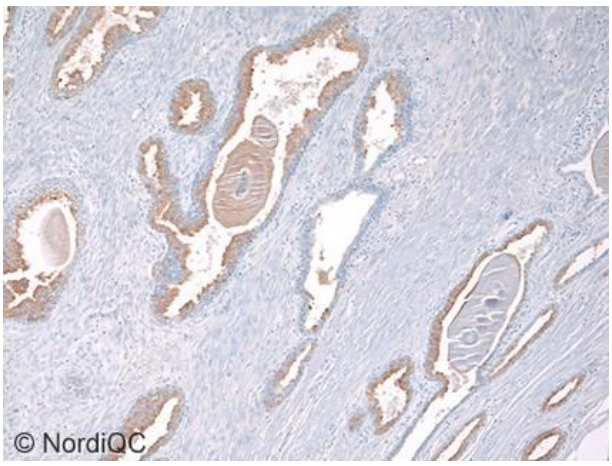


Fig. 1b (x100)
Insufficient staining for PSA of the prostate hyperplasia using the pAb 760-2506 (RTU format , Ventana no pre-treatment and UltraView (Ventana) as the detection system - same protocol used in Figs. 2b – 3b.
The intensity of the staining reaction is significantly reduced and stromal reactivity is absent - compare with Fig. 1a (same field).

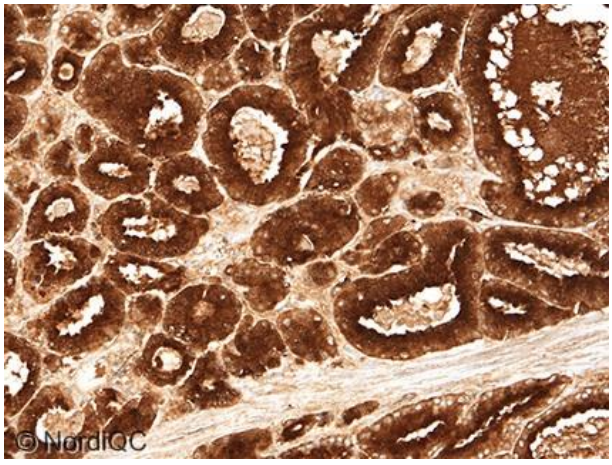


Fig. 2a (x200)
Optimal staining for PSA in the prostate adenocarcinoma, core 3, using same protocol as in Fig. 1a.
All neoplastic cells are strongly stained.

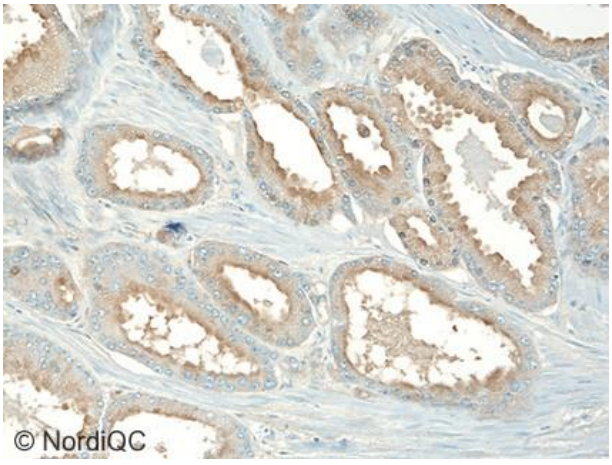


Fig. 2b (x200)
Insufficient staining for PSA in the prostate adenocarcinoma, core 3, same protocol as in Fig 1b.
The intensity of the staining reaction in the neoplastic cells is reduced compared to the result obtained in Fig. 2a.

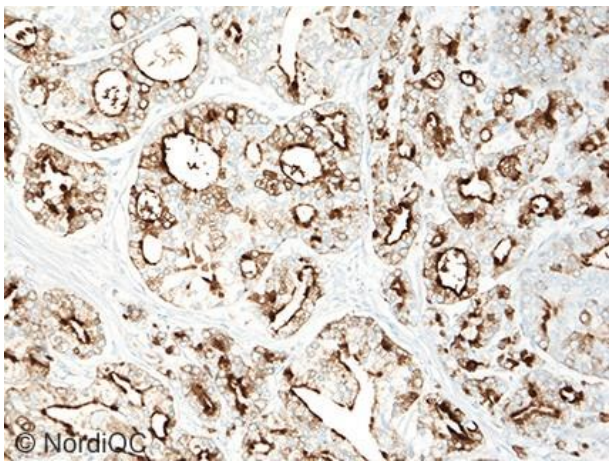


Fig. 3a (x200)
Optimal staining for PSA in the prostate adenocarcinoma, core 4, using same protocol as in Figs. 1a and 2a.
The majority of the neoplastic cells shows a weak to moderate but distinct cytoplasmic staining reaction.

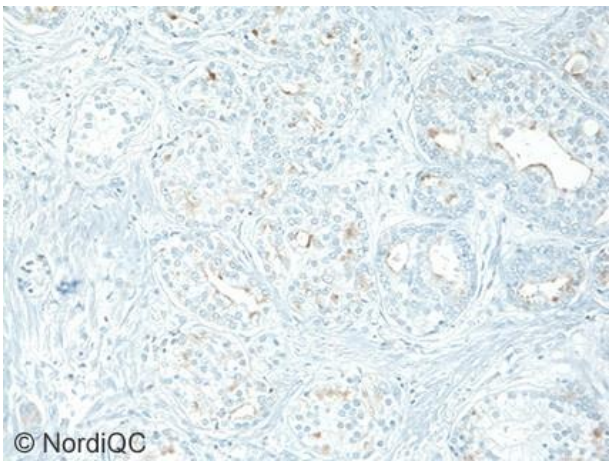


Fig. 3b (x200)
Insufficient staining for PSA in the prostate adenocarcinoma, core 4 using same protocol as in Figs. 1b and 2b.
The intensity of the neoplastic cells is significantly reduced and some glandular structures are completely negative - compare with Fig. 3a.

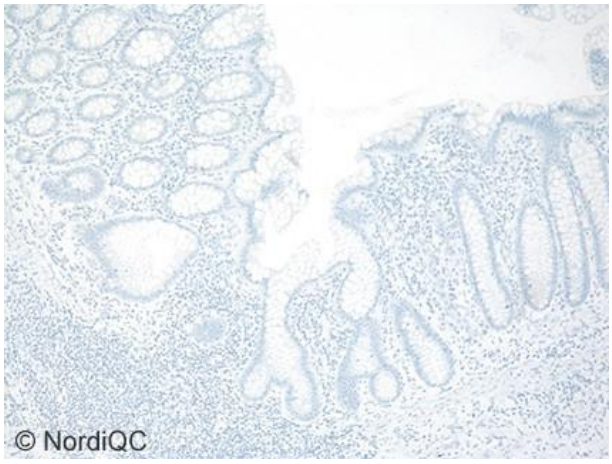


Fig. 4a (x100)
Optimal staining for PSA of the appendix using same protocol as in Figs. 1a - 3a. As expected, no staining reaction is seen of the epithelium and stromal cells.

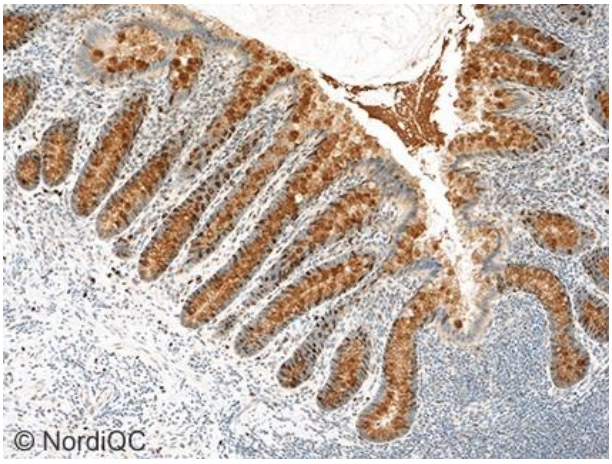


Fig. 4b (x100)
Insufficient staining for PSA of the appendix using the pAb 0562 as concentrate (too high concentration), HIER in alkaline buffer (TRIS-EDTA) and a polymer based detection system (EnVision, Dako) – same protocol used in Fig. 5b.
The epithelial cells and scattered stromal cells are false positive - compare with Fig. 4a.

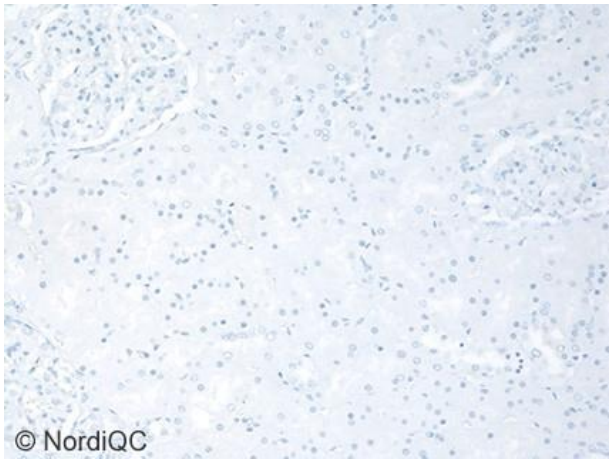


Fig. 5a (x200)
Optimal staining for PSA of the kidney using same protocol as in Figs. 1a - 4a.
The epithelial cells show no staining reaction as expected.

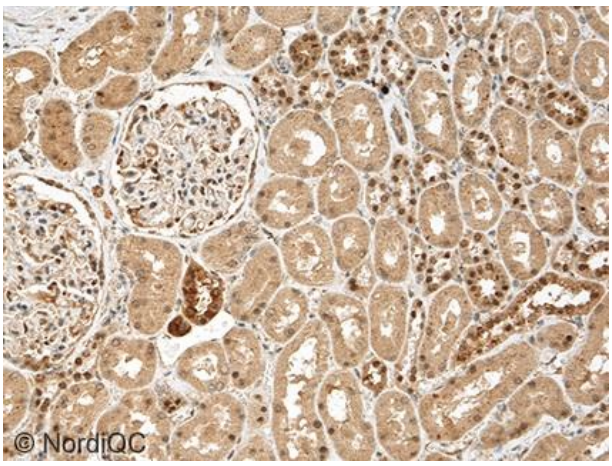


Fig. 5b (x200)
Insufficient staining for PSA using same protocol as in Fig. 4b.
The epithelial cells are false positive and in addition, some of the tubules displayed a moderate to strong unexpected nuclear staining reaction - compare with Fig. 5a.

MB/SN/LE/MV/RR 20.03.2017