

NORDIQC DATA FOR LUNG MARKERS

Antibody selection, protocols and controls

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AGENDA

- NordiQC results for selected markers
- Clones - successful vs. less successful
- Tricky markers – pitfalls
- iCAPS



NORDIQC EQA DATA FOR IHC LUNG MARKERS

Marker	Purpose	Last run	Pass rate	No of labs
TTF1	<u>Lung</u> vs non-lung <u>Adenocarcinoma</u> vs squam.	Run 58, 2020	80%	322
Napsin A	<u>Lung</u> vs non-lung	Run 44, 2015	78%	162
Calretinin	Lung vs <u>mesothelioma</u>	Run 64, 2022	76%	350
WT1	Lung vs <u>mesothelioma</u>	Run 55, 2019	91%	291
BAP1	Reactive mesothelioma vs <u>malignant mesothelioma</u>	Run 65, 2022	69%	163
EpCAM	<u>Lung</u> vs mesothelioma	Run 56, 2019	57%	256
CGA	NSCLC vs <u>SCLC</u>	Run 53, 2018	76%	296
SYP	NSCLC vs <u>SCLC</u>	Run 52, 2018	75%	308
CD56	NSCLC vs <u>SCLC</u>	Run 64, 2022	72%	364
p40	Adenocarcinoma vs <u>squam.</u>	Run 60, 2020	86%	262
CK5	Adenocarcinoma vs <u>squam.</u>	Run 65, 2022	71%	311
ALK (lung)	Predictive for Crizotinib	Run 65, 2022	77%	256
PD-L1 TPS/CPS	Predictive for Keytruda, Imfinzi, Opdivo.....	Run C11, 2022	81%	225

Scheduled for
assessment within
the next year



KEY-POINTS FOR BEST PROTOCOLS

- Clone selection
- RTUs – “Plug and Play” or “Play and Plug”?
- Efficient HIER – typically in high pH buffer
- 3 layer detection system
- Use of iCAPS



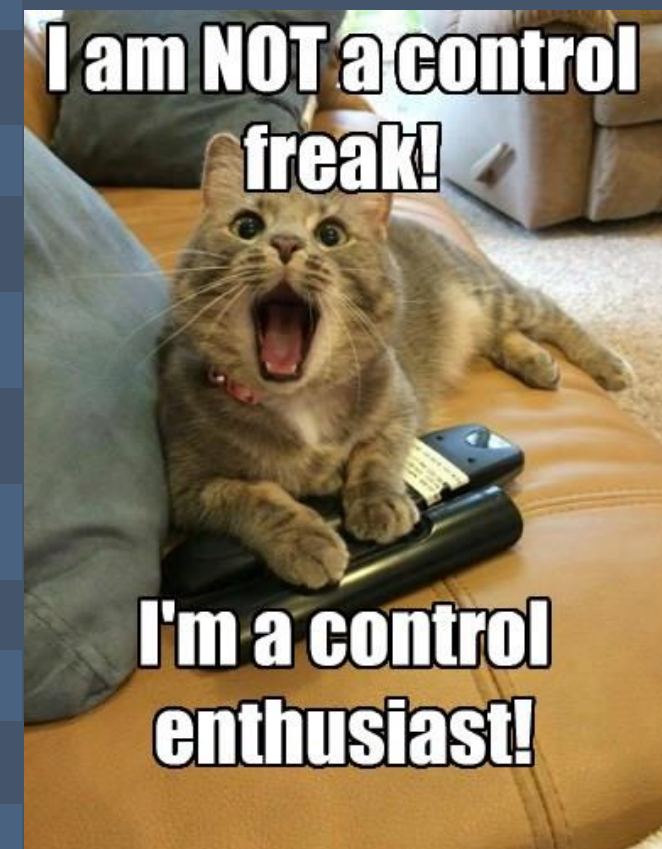
CLONE PERFORMANCE FOR SELECTED LUNG MARKERS

Marker	Successful clones (pass rate)	Less successful clones (pass rate)
TTF1	mAb SPT24, rmAb SP141	mAb 8G7G3/1
Napsin A	mAbs IP64 & MRQ-60	pAbs
Calretinin	mAbs DAK-Calret & CAL6, rmAb SP65	pAbs, rmAb SP13
WT1	mAbs 6F-H2 & WT49	-
BAP1	mAb C-4 & BSB-109, rmAb EPR22826-65	pAb
EpCAM	mAbs BS14, Ber-EP4 & MOC-31	mAb Ber-EP4
CGA	mAb LK2H10	mAbs DAK-A3 & 5H7
SYP	mAbs DAK-SYNAP & 27G12, rmAbs MRQ-40 & SP11	-
CD56	rmAb MRQ-42	mAbs 123C3 & CD564
p40	mAb BC28	pAbs
CK5	mAb XM26, rmAb SP27	mAb D5/16 B4
ALK (lung)	mAbs 5A4 & OTI1A4, rmAb D5F3	mAb ALK1
PD-L1 TPS/CPS	mAb 22C3, rmAb SP263	<i>rmAb SP142</i>



ICAPS FOR SELECTED LUNG MARKERS

Marker	IHC critical assay performance controls Low expression	Negative tissue controls No expression	
TTF1	Lung: Columnar epithelial cells of terminal bronchi.	Tonsil: All cell types.	Link
Napsin A	Kidney: Epithelial cells of proximal tubules.	Appendix/Colon: Epithelial cells and macrophages.	Link
Calretinin	Adrenal gland: Cortical epithelial cells.	Appendix/Colon: Epithelial cells.	Link
WT1	Kidney: Podocytes and parietal epithelial cells of Bowman's capsule.	Kidney: Epithelial cells of the tubules.	Link
BAP1	Tonsil: Mantle zone lymphocytes and germinal centre lymphocytes.	Malignant Mesothelioma: Neoplastic cells	
CGA	Appendix/Colon: Axons and ganglion cells in the nerve plexus.	Appendix/Colon: Epithelial cells and smooth muscle cells.	Link
SYP	Appendix/Colon: Neuroendocrine and scattered goblet cells in epithelial mucosa.	Appendix/Colon: Smooth muscle cells	Link
CD56	Tonsil: NK-cells and scattered T-cells.	Appendix/Colon: Epithelial cells.	Link
p40	Placenta: Dispersed cytotrophoblastic cells.	Tonsil: Lymphocytes.	Link
CK5	Pancreas: Scattered epithelial cells of intercalated ducts.	Liver: All cell types.	Link
ALK (lung)	Appendix/Colon: Dispersed axons of nerve cells.	Tonsil: All cell types.	Link
PD-L1 TPS/CPS	Tonsil: Germinal center macrophages and T-cells.	Tonsil: Stratified normal squamous epithelial cells and vast majority of lymphocytes.	Link



TTF1 – PITFALLS/POINTS OF ATTENTION

Table 1. Antibodies and assessment marks for TTF1, run 58

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 8G7G3/1	2 6 1 8 1 1 1	Biocare Medical Cell Marque CliniSciences Dako/Agilent Diagnostic BioSystems Zytomed Thermo Scientific	0	3	11	6	15%	0%
rmAb clone BSR40	1	Nordic Biosite	0	1	0	0	-	-
mAb clone SPT24	8 1 2 107 9 1 1 1	Biocare Medical DCS Diagnostics Immunologic Leica/Novocastra Monosan Zytomed Immunologic Cell Marque	84	27	13	5	86%	65%
rmAb clone EP229	3	Cell Marque	2	1	0	0	-	-
Ready-To-Use Antibodies							OR²	
mAb clone 8G7G3/1 790-4398 (VRPS)³	1	Ventana/Roche	0	0	0	1	-	-
mAb clone 8G7G3/1 790-4398 (LMPS)⁴	11	Ventana/Roche	0	0	7	4	0%	0%
mAb clone 8G7G3/1 IR056 (VRPS)³	9	Dako/Agilent	0	4	5	0	44%	0%
mAb clone 8G7G3/1 IR056 (LMPS)⁴	14	Dako/Agilent	0	4	5	5	29%	0%
rmAb EP229 343R-17/18	1	Cell Marque	0	0	1	0	-	-
rmAb EP229 8224-C010	1	Sakura Finetek	1	0	0	0	-	-
rmAb clone SP141 790-4756 (VRPS)³	30	Ventana/Roche	25	5	0	0	100%	83%
rmAb clone SP141 790-4756 (LMPS)⁴	75	Ventana/Roche	54	20	1	0	99%	72%
mAb clone SPT24 PA0364 (VRPS)³	6	Leica/Novocastra	5	1	0	0	100%	83%
mAb clone SPT24 PA0364 (LMPS)⁴	16	Leica/Novocastra	10	4	1	1	88%	63%
rmAb clone SP141 AN887	1	Biogenex	0	1	0	0	-	-
mAb clone SPT24 MAD-000486QD	1	Master Diagnostica SL	1	0	0	0	-	-
mAb clone SPT24 API 3126	3	BioCare	0	3	0	0	-	-
Total	322		182	74	44	22	-	
Proportion			56%	23%	14%	7%	80%	

1) Proportion of sufficient stains (optimal or good). For Laboratory Developed (LD) assays (≥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).

TTF1 performance in NordiQC assessments

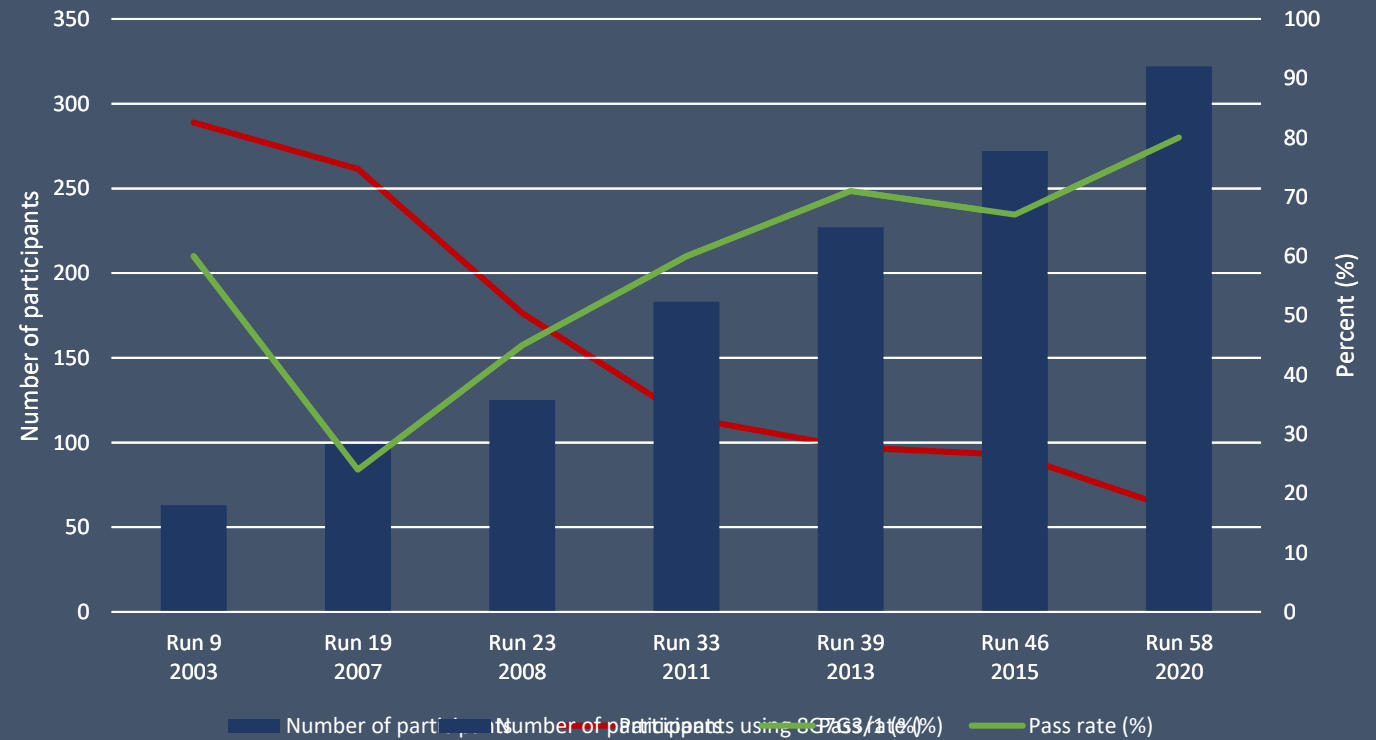


Table 4. The overall pass rate in the last five runs for the mAb clones SPT24, 8G7G3/1 and the rmAb clone SP141

	SPT24		SP141*		8G7G3/1	
	All protocol settings	Optimal	All protocol settings	Optimal	All protocol settings	Optimal
Participants	89% (564/635)	64% (408/635)	97% (164/169)	71% (120/169)	9% (28/314)	0% (0/314)

* Because rmAb clone SP141 is only recently introduced, data represents Run 39, 46 and 58 only

TTF1 – PITFALLS/POINTS OF ATTENTION

Table 1. Antibodies and assessment marks for TTF1, run 58

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 8G7G3/1	2 6 1 8 1 1 1	Biocare Medical Cell Marque CliniSciences Dako/Agilent Diagnostic BioSystems Zytomed Thermo Scientific	0	3	11	6	15%	0%
rmAb clone BSR40	1	Nordic Biosite	0	1	0	0	-	-
mAb clone SPT24	8 1 2 107 9 1 1 1	Biocare Medical DCS Diagnostics Immunologic Leica/Novocastra Monosan Zytomed Immunologic Cell Marque	84	27	13	5	86%	65%
rmAb clone EP229	3	Cell Marque	2	1	0	0	-	-
Ready-To-Use Antibodies							OR ²	
mAb clone 8G7G3/1 790-4398 (VRPS)³	1	Ventana/Roche	0	0	0	1	-	-
mAb clone 8G7G3/1 790-4398 (LMPS)⁴	11	Ventana/Roche	0	0	7	4	0%	0%
mAb clone 8G7G3/1 IR056 (VRPS)³	9	Dako/Agilent	0	4	5	0	44%	0%
mAb clone 8G7G3/1 IR056 (LMPS)⁴	14	Dako/Agilent	0	4	5	5	29%	0%
rmAb EP229 343R-17/18	1	Cell Marque	0	0	1	0	-	-
rmAb EP229 8224-C010	1	Sakura Finetek	1	0	0	0	-	-
rmAb clone SP141 790-4756 (VRPS)³	30	Ventana/Roche	25	5	0	0	100%	83%
rmAb clone SP141 790-4756 (LMPS)⁴	75	Ventana/Roche	54	20	1	0	99%	72%
mAb clone SPT24 PA0364 (VRPS)³	6	Leica/Novocastra	5	1	0	0	100%	83%
mAb clone SPT24 PA0364 (LMPS)⁴	16	Leica/Novocastra	10	4	1	1	88%	63%
rmAb clone SP141 AN887	1	Biogenex	0	1	0	0	-	-
mAb clone SPT24 MAD-000486QD	1	Master Diagnostica SL	1	0	0	0	-	-
mAb clone SPT24 API 3126	3	BioCare	0	3	0	0	-	-
Total	322		182	74	44	22	-	-
Proportion			56%	23%	14%	7%	80%	

1) Proportion of sufficient stains (optimal or good). For Laboratory Developed (LD) assays (≥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).

Table 3. 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
VMS Ultra/XT mAb 8G7G3/1 790-4398	0/1	0/1	0/11 (0%)	0/11 (0%)
Dako AS Link 48+ mAb 8G7G3/1 IR056	4/9 (44%)	0/9 (0%)	3/5 (60%)	0/5 (0%)
VMS Ultra/XT rmAb SP141 790-4756	30/30 (100%)	25/30 (83%)	70/71 (99%)	53/71 (75%)
Leica BOND III/Max mAb SPT24 PA0364	6/6 (100%)	5/6 (83%)	8/8 (100%)	7/8 (88%)

* 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.

RTU assays from Ventana and Leica can be used with the recommended protocol settings.

The concentrated format of mAb SPT24 can provide optimal results on both Dako Autostainer and Omnis.

Table 2. Proportion of optimal results for TTF1 for the mAb clone SPT24 as concentrate on the main IHC systems*

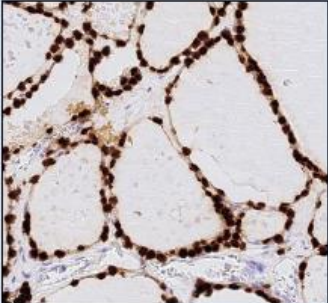
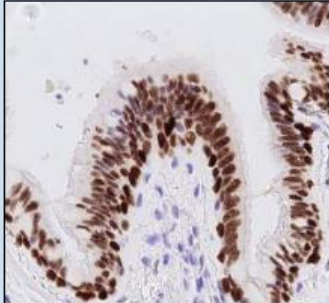
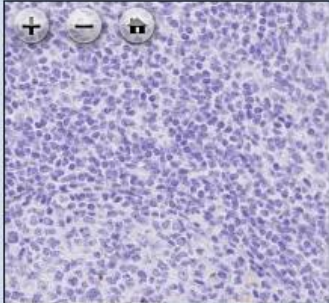
Concentrated antibodies	Dako Autostainer		Dako Omnis		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone SPT24	9/14** (64%)	1/2	19/32 (59%)	1/1	38/52 (73%)	-	14/17 (82%)	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.

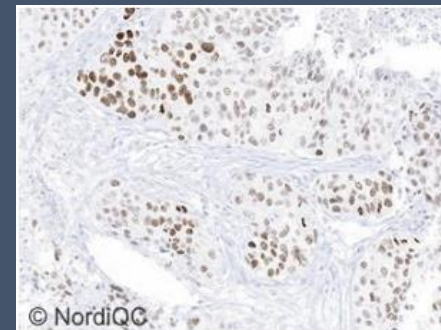
** (number of optimal results/number of laboratories using this buffer).

TTF1 – ICAPS

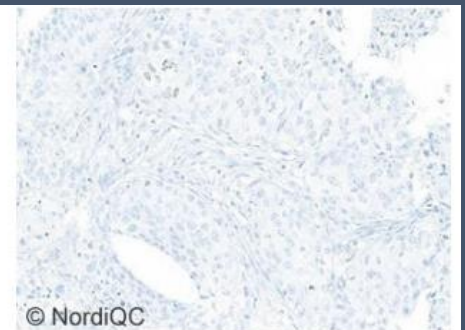
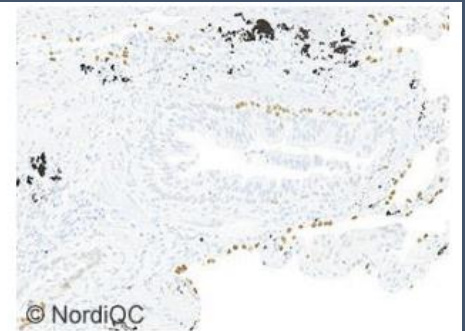
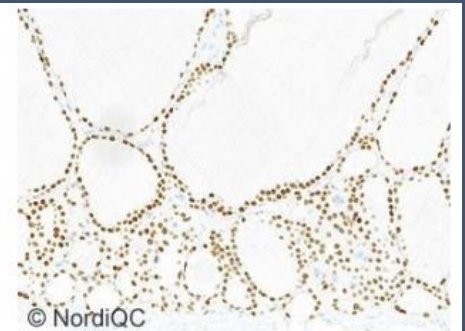
TTF1 - Thyroid transcription factor-1

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Thyroid gland	Lung	Tonsil
Description	<p>Virtually all follicular epithelial cells should display a strong nuclear staining reaction.</p> <p>A weak cytoplasmic staining reaction can be seen in the cytoplasmic compartment and in the extracellular colloids.</p>	<p>The vast majority of columnar luminal epithelial cells of the terminal bronchioles must show an at least weak to moderate, distinct nuclear staining reaction.</p> <p><i>Note, type II pneumocytes will show a strong nuclear staining reaction and cannot be used to evaluate analytical sensitivity.</i></p>	No staining reaction should be seen.
Example	 <p>Click to enlarge</p>	 <p>Click to enlarge</p>	 <p>Click to enlarge</p>

rmAb clone SP141



mAb clone 8G7G3/1



Thyroid gland

Normal lung

Lung
adenocarc.

NAPSIN A – PITFALLS/POINTS OF ATTENTION

Table 1. Antibodies and assessment marks for Napsin A, run 44

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	Suff. OPS ²
mAb clone IP64	86	Leica/Novocastra	39	39	6	2	91%	92%
mAb clone MRQ-60	8	Cell Marque	3	4	1	0	88%	100%
mAb, clone TMU-Ad02	4	Biocare	1	2	4	0	43%	-
	3	IBL						
rmAb clone KCG1.1	2	Zytomed						
	2	Diagnostic Biosystems	1	5	0	0	100%	-
	1	Abcam						
rmAb clone BC15	1	Acris						
	1	Zytomed	1	0	0	0	-	-
mAb, clone BS10	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone EPR6252	1	Abcam	1	0	0	0	-	-
pAb 352A-7x	8	Cell Marque	0	1	1	6	13%	-
Ready-To-Use antibodies								
mAb clone MRQ-60 760-4867	18	Ventana/Cell Marque	1	16	1	0	84%	-
mAb clone MRQ-60 352M-98	3	Cell Marque	0	3	0	0	-	-
mAb clone MRQ-60 MAD-000633QD	3	Master Diagnostica	0	3	0	0	-	-
rmAb clone BC15 API 3043	1	Biocare	0	0	1	0	-	-
mAb clone IP64 AM701-5M	1	BioGenex	0	0	1	0	-	-
mAb clone IP64 ZM-0473	1	ZSGB-BIO	0	1	0	0	-	-
rmAb clone EP205 352R-18	1	Cell Marque	1	0	0	0	-	-
mAb clone MX015 MAB-0704	1	Maixin	0	1	0	0	-	-
pAb 760-4446	12	Ventana/Cell Marque	0	1	0	11	8%	-
pAb PPM428DS	1	Biocare	0	0	0	1	-	-
pAb MP-394-DS6	1	Menapath	0	0	0	1	-	-
pAb RAB-0639	1	Maxim	0	1	0	0	-	-
Total	162		49	77	15	21	-	
Proportion			30%	48%	9%	13%	78%	

1) Proportion of sufficient stains (optimal or good)

2) Proportion of sufficient stains with optimal protocol settings only, see below.

Table 3. Proportion of optimal results for Napsin A using concentrated antibodies on the 3 main IHC systems*

Concentrated antibodies	Dako		Ventana		Leica	
	Autost.	Link / Classic, Omnis	BenchMark XT / Ultra		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 IP64	10/16 (63%)**	1/5 (20%)	17/35 (49%)	1/1	2/8 (25%)	4/12 (33%)
mAb clone MRQ-60	3/4	-	0/1	-	-	-

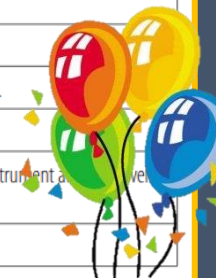
* Antibody concentration applied as listed above, HIER buffers and detection kits used as recommended by the vendors of the respective platforms.

** (number of optimal results/number of laboratories using this buffer)

No RTU for Dako or Leica users. It is possible to achieve optimal results using concentrated formats of mAbs IP64 and MRQ-60.

Recommended staining protocol for this antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

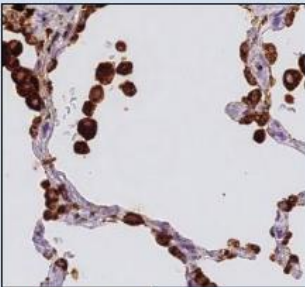
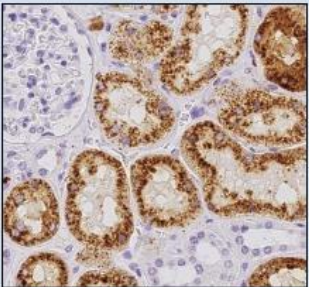
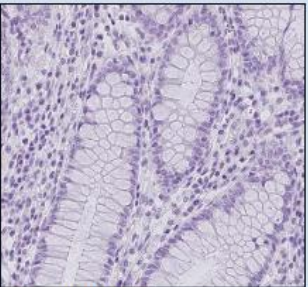
Recommended staining protocol with OptiView	
1.	Load slides, antibody, and detection kit dispensers onto BenchMark® instrument.
2.	Select CC1 32 minutes pretreatment.
3.	Select pre primary peroxidase inhibitor.
4.	Antibody incubation should be set for 8 minutes at 37°C.
5.	Start the run.
6.	When the staining run is complete, move slides from instrument and wash with wash buffer.
7.	Coverslip.



The one optimal protocol used OptiView. Recommended protocol settings in 2015 were based on UltraView. In 2017 the recommended settings changed to also include a protocol for OptiView.

NAPSIN A – ICAPS

Napsin A - Napsin A

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Lung	Kidney	Appendix/colon
Description	Virtually all type II pneumocytes and alveolar macrophages must show a moderate to strong, granular cytoplasmic staining reaction.	Virtually all epithelial cells of the proximal tubules must show an at least moderate, granular cytoplasmic staining reaction. <i>Note, at present no ideal tissue with low level expression has been identified and the combination of using lung and kidney as positive tissue controls and colon/appendix as negative tissue control is suggested.</i>	No staining reaction should be seen in the columnar epithelial cells and macrophages. <i>Note, as no ideal tissue has been identified to evaluate identification of low level Napsin A expression, the protocol should be "as strong as possible" with no staining in colon/appendix as described.</i>
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

mAb IP64

pAb

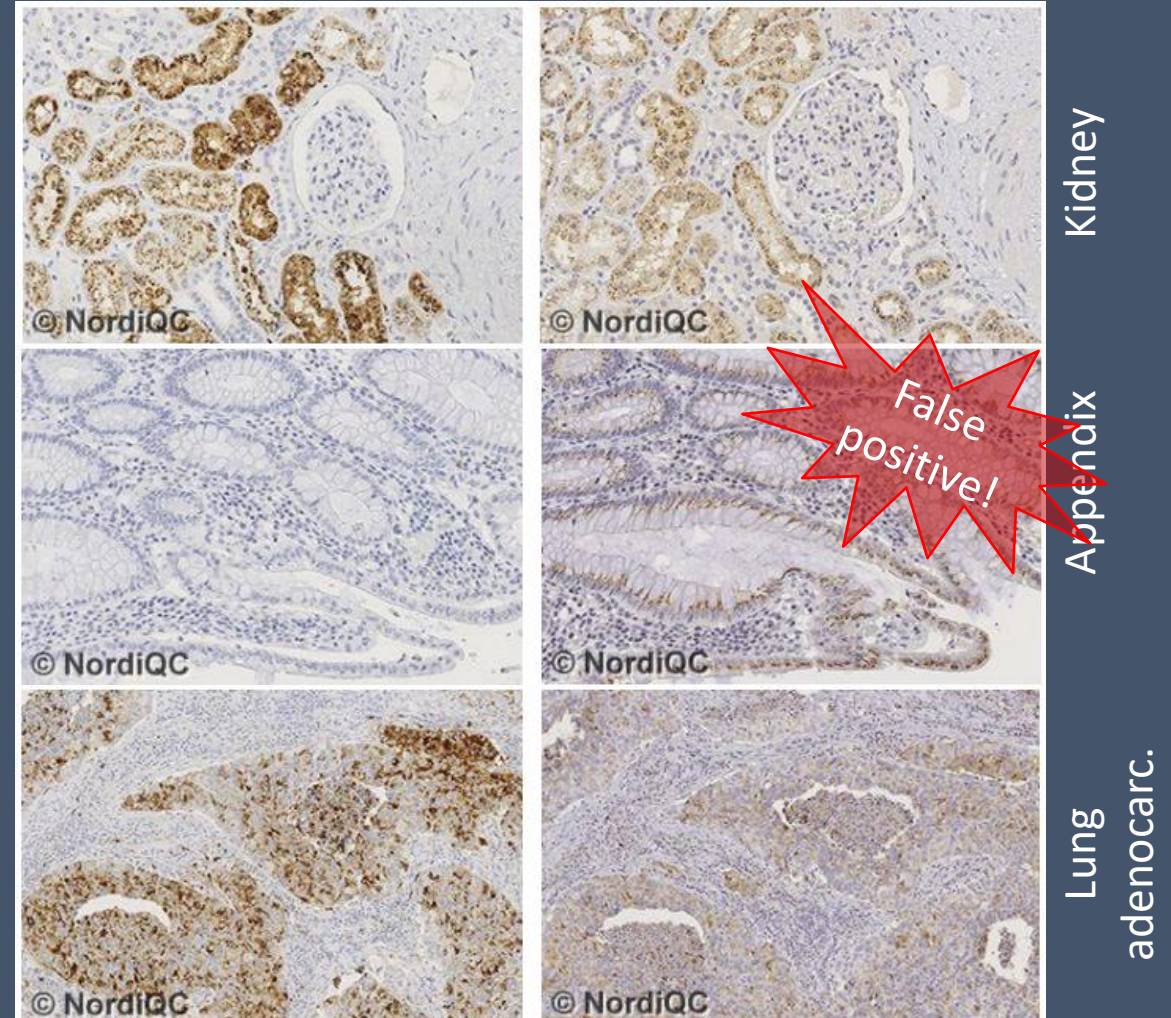


Table 1. Antibodies and assessment marks for CR, run 64

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 2E7	1	BioGenex	0	0	1	0	-	-
mAb clone 5A5	1	Monosan	1	0	0	0	-	-
mAb clone ZM85	1	Zeta Corporation	0	1	0	0	-	-
mAb clone CAL6	19	Leica Biosystems	12	4	1	2	84%	63%
mAb clone DAK-Calret 1	25	Dako/Agilent	6	12	6	2	69%	23%
rmAb clone BSR235	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone SP13	1	Cell Marque	0	2	2	2	33%	-
	1	Zytomed Systems						
	1	Abcam						
	1	Epredia						
	1	Diagnostic Biosystems						
pAb 18-0211	1	Zeta Corporation						
	6	Invitrogen/Thermo S. Zymed	5	1	1	0	86%	71%
pAb 232A	1	Cell Marque	0	0	0	1	-	-
pAb 61-0006	1	Genemed	1	0	0	0	-	-
pAb, CP092C	1	Biocare Medical	0	1	0	0	-	-
pAb RBK003	1	Zytomed Systems	0	1	0	0	-	-
pAb CR7696	1	Swant	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone CAL6 PA0346³	8	Leica Biosystems	4	4	0	0	100%	50%
mAb clone CAL6 PA0346⁴	10	Leica Biosystems	3	3	3	1	60%	30%
mAb clone DAK-Calret 1 IS/IR627³	16	Dako/Agilent	3	5	7	1	50%	19%
mAb clone DAK-Calret 1 IS/IR627⁴	43	Dako/Agilent	5	15	11	12	47%	12%
mAb clone C5G4 CCM-0222	1	Celnovte Biotechnology	1	0	0	0	-	-
mAb clone IHC523 IHC523	1	GenomeMe	1	0	0	0	-	-
rmAb SP13 232R	4	Cell Marque	2	0	1	1	-	-
rmAb SP13 MAD-000315QD	1	Master Diagnostica	0	0	1	0	-	-
rmAb BSR235 MAD-000784QD	2	Master Diagnostica	0	0	1	1	-	-
rmAb RM324 8522-C010	2	Sakura Finetek	2	0	0	0	-	-
rmAb clone SP65 790-4467³	2	Ventana/Roche	2	0	0	0	-	-
rmAb clone SP65 790-4467⁴	177	Ventana/Roche	120	38	18	1	89%	68%
pAb 232A	2	Cell Marque	0	0	1	1	-	-
pAb IP092	1	Biocare Medical	0	0	1	0	-	-
pAb HAP134	1	PathnSitu	0	1	0	0	-	-
pAb 08-1211	1	Invitrogen/Thermo S.	0	0	1	0	-	-
Total	339		169	88	56	26	-	
Proportion			50%	26%	16%	8%	76%	

CALRETININ PITFALLS/POINTS OF ATTENTION



Table 2. Proportion of optimal results for CR for the most commonly used antibodies as concentrates on the 4 main IHC systems*

Concentrated antibodies	Dako Autostainer Link / Classic		Dako Omnis		Ventana BenchMark GX / XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone CAL6	-	-	10/10 ** (100%)	-	-	-	1/1	-
mAb clone DAK-Calret 1	1/1	-	0/4	-	0/2	-	2/4	1/2
pAb 18-0211	1/1	-	2/2	-	1/3	-	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)

Less successful performance on the fully-automated Dako Omnis and Ventana BenchMark platforms for the most widely used conc. Abs

RTU products for Ventana and Leica users

Table 3. Proportion of sufficient and optimal results for CR for the most commonly used RTU IHC systems

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Leica BOND mAb CAL6 PA0346	100% (8/8)	50% (4/8)	63% (5/8)	25% (2/8)
Dako AS mAb DAK-Calret 1 IR/IS627	50% (8/16)	19% (3/16)	75% (6/8)	38% (3/8)
VMS Ultra/XT rmAb SP65 790-4467	(2/2)	(2/2)	89% (154/173)	67% (116/173)

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

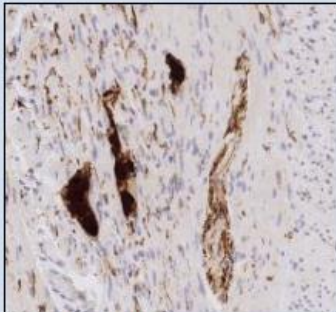
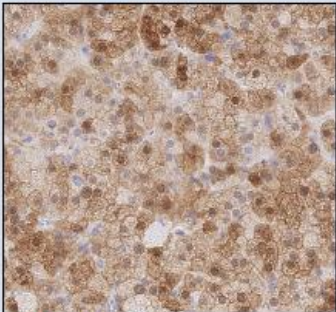
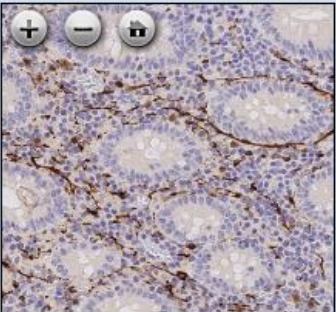
Omnis users cannot use the Autostainer RTU: 36% pass rate (12/33)

UltraView:
88% pass rate (65% optimal)

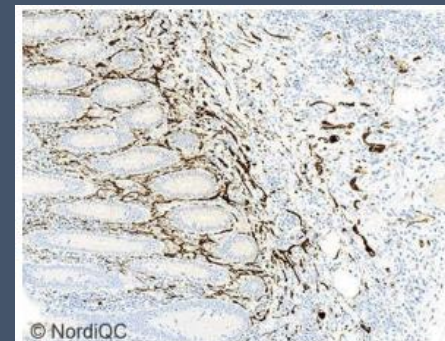
OptiView:
100% pass rate (78% optimal)

CALRETININ – ICAPS

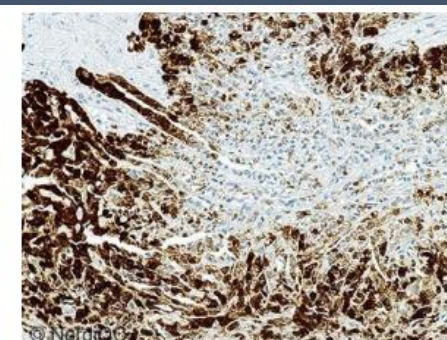
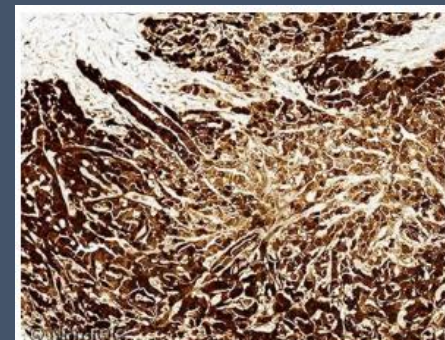
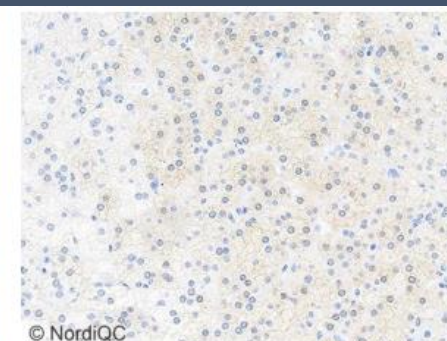
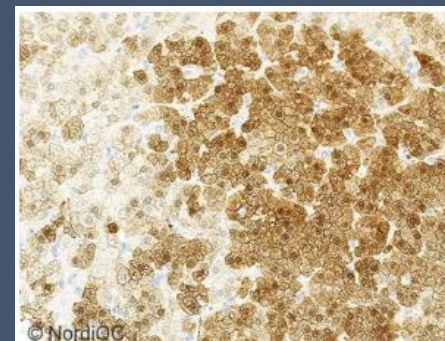
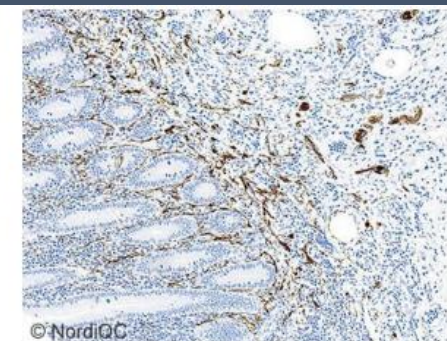
CR - Calretinin

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Appendix/colon	Adrenal gland	Appendix/colon
Description	Virtually all macrophages and peripheral nerves (ganglion cells and axons) must show a moderate to strong, distinct cytoplasmic and nuclear staining reaction.	The majority of cortical epithelial cells must show a at least weak to moderate, distinct cytoplasmic and nuclear staining reaction. <i>Note, nerves will show a moderate to strong staining reaction and cannot be used to evaluate the level of analytical sensitivity.</i>	No staining reaction in the columnar epithelial cells should be seen.
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

Autostainer RTU on
Autostainer



Autostainer RTU on
Omnis



Appendix

Adrenal gland

Mesothelioma

WT1 – PITFALLS/POINTS OF ATTENTION

Table 1. Antibodies and assessment marks for WT1, Run 55

Concentrated Antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	Suff. OPS ²
mAb clone 6F-H2	52 13 2 2 2 2 1	Dako/Agilent Cell Marque BioCare DCS Diagnostic BioSystems Immunologic Zeta	36	31	6	1	91%	92%
mAb clone WT49	13 1	Leica Immunologic	11	2	0	1	93%	100%
rmAb clone D817F	3	Cell Signaling	3	0	0	0	-	-
rmAb clone EP122	3 1	Epitomics Cell Marque	3	1	0	0	-	-
pAb RB-9367-P	1	Neomarkers	0	0	1	0	-	-
Ready-To-Use Antibodies								
mAb clone 6F-H2 760-4397	92	Ventana/Cell Marque	40	37	14	1	84%	94%
mAb clone 6F-H2 IR055/IS055	33	Dako/Agilent	30	3	0	0	100%	100%
mAb clone 6F-H2 IR055/IS055 ³	25	Dako/Agilent	21	3	1	0	96%	-
mAb clone 6F-H2 IR055/IS055 ⁴	9	Dako/Agilent	5	3	1	0	-	-
mAb clone 6F-H2 348M-98 ⁵	14	Cell Marque	5	7	2	0	86%	-
mAb clone 6F-H2 MAD-005671QD	2	Master Diagnostica	2	0	0	0	-	-
mAb clone MX012 MAB-0678	1	Maixin	1	0	0	0	-	-
mAb clone WT49 PA0562	17	Leica	17	0	0	0	100%	100%
mAb clone WT49 PA0562 ⁶	1	Leica	1	0	0	0	-	-
rmAb clone EP122 8340	1	Sakura	1	0	0	0	-	-
Total	291		176	87	25	3	-	-
Proportion			60%	30%	9%	1%	90%	

1) Proportion of sufficient stains (optimal or good)

2) Proportion of sufficient stains with optimal protocol settings only, see below.

3) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer), but used by laboratories on the Dako/Agilent full-automatic platform (Dako Omnis).

4) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer), but used by laboratories on different platforms (e.g. Ventana Benchmark, BioCare IntelliPath and Leica Bond).

5) RTU format not developed for a specific platform, but used by laboratories on the Ventana Benchmark platform.

6) RTU system developed for the Leica Bond system, but used on the Ventana Benchmark platform.

Table 4. Proportion of sufficient and optimal results for WT1 for the most commonly used RTU IHC systems

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Ventana Benchmark mAb clone 6F-H2, 760-4397	80% (20/25)	20% (5/25)	85% (57/67)	52% (35/67)
Dako AS mAb clone 6F-H2, IR055/IS055	100% (21/21)	95% (20/21)	100% (12/12)	83% (10/12)
Leica Bond mAb clone WT49, PA0562	100% (8/8)	100% (8/8)	100% (9/9)	100% (9/9)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.

** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

The most successful modifications were based on combined retrieval and use of OptiView, giving a pass rate of 96% with 66% optimal.

Concentrated Abs can be used on Omnis.

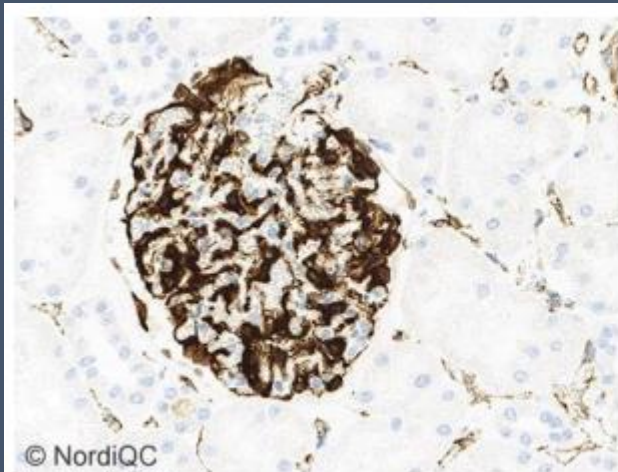
Table 3. Proportion of optimal results for WT1 for the most commonly used antibodies as concentrates on the four main IHC systems*

Concentrated antibodies	Dako Autostainer Link / Classic		Dako Omnis		Ventana BenchMark GX / XT / Ultra			Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC1 pH 8.5 + Protease 3	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone 6F-H2	8/9** 89%	1/1	2/6 33%	-	10/24 42%	4/12 33%	-	8/13 62%	1/2
mAb clone WT49	2/3	-	1/1	-	4/5 80%	-	-	3/4	-

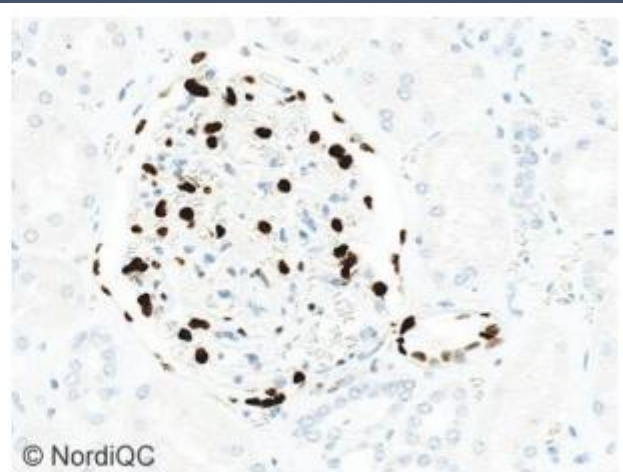
* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.

** Number of optimal results/number of laboratories using this buffer

WT1 – PITFALLS/POINTS OF ATTENTION



If using HIER as single pre-treatment, both a nuclear and cytoplasmic staining reaction is seen.



If using a combined pre-treatment using HIER followed by a weak proteolysis, only a nuclear staining reaction is seen.

mAb clone 6F-H2:

Pre-treatment method determines the outcome.

Depending on the purpose of the test, a combined pre-treatment is making the interpretation easier.

A cytoplasmic cross-reaction can be used for vascular lesions, that will be negative if using the combined pre-treatment.

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Original Article

Diagnostic utility of WT-1 cytoplasmic stain in variety of vascular lesions

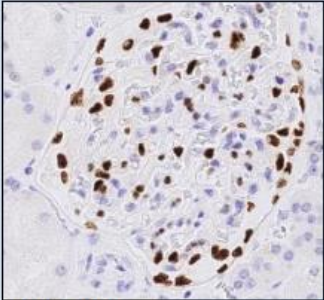
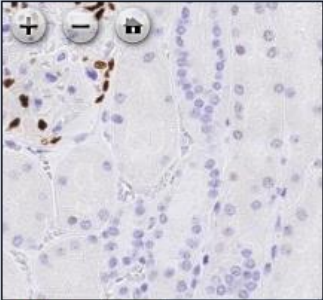
Sarah K Galfione, Jae Y Ro, Alberto G Ayala, Yimin Ge

Department of Pathology and Genomic Medicine, Houston Methodist Hospital, Weill Medical College of Cornell University, Houston, TX, USA

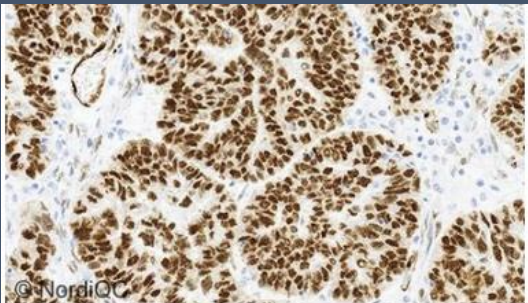
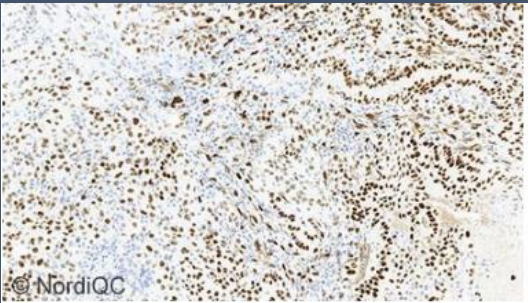
Received February 19, 2014; Accepted April 10, 2014; Epub April 15, 2014; Published May 1, 2014

WT1 - ICAPS

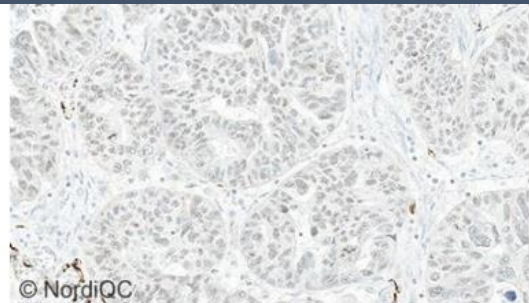
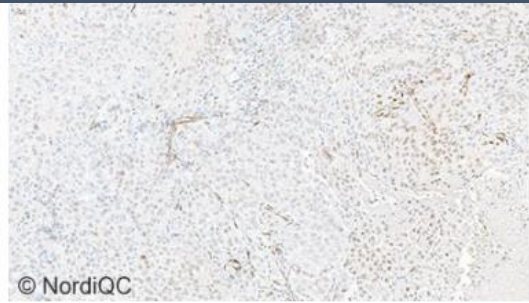
WT1 - Wilms tumour-1 protein

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Fallopian tube	Kidney	Kidney
Description	<p>Virtually all epithelial and smooth muscle cells must show a strong, nuclear staining reaction.</p> <p><i>Note, the mAb 6F-H2 will with HIER as single pretreatment method give a moderate to strong cytoplasmic staining reaction in endothelial cells and smooth muscle cells.</i></p>	<p>Virtually all podocytes and parietal epithelial cells of Bowman's capsule must show an at least moderate nuclear staining reaction.</p> <p><i>Note, the mAb 6F-H2 will with HIER as single pretreatment method give a moderate to strong coexisting cytoplasmic staining reaction challenging the interpretation of the specific nuclear reaction.</i></p>	<p>No staining reaction in the epithelial cells of the tubules should be seen.</p> <p><i>Note, the mAb 6F-H2 will with HIER as single pretreatment method give a moderate to strong cytoplasmic staining reaction in endothelial cells and smooth muscle cells.</i></p>
Example	 <p>Click to enlarge</p>	 <p>Click to enlarge</p>	 <p>Click to enlarge</p>

Optimal protocol settings



Inefficient HIER, 2-layer detection system



Fallopian tube

Mesothelioma

Serous ovarian carcinoma

EP-CAM – PITFALLS/POINTS OF ATTENTION

Table 1. Antibodies and assessment marks for EpCAM, run 56

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	Suff. OPS ²
mAb clone BS14	10	Nordic Biosite	9	1	0	0	100%	100%
mAb clone Ber-Ep4	69	Dako	14	13	21	28	36%	93%
	1	Cell Marque Diagnostic Biosystems						
mAb clone MOC-31	23	Dako	10	10	7	2	69%	71%
	5	Cell Marque Diagnostic Biosystems						
mAb clone VU-1D9	5	Thermo Scientific	9	0	1	0	90%	100%
	3	Merck Millipore						
	1	Immunologic						
	1	Novus Biologicals						
rmAb clone EPR20532-225	1	Abcam	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone Ber-Ep4 760-4383	16	Ventana/Cell Marque	1	6	6	3	44%	100%
mAb clone Ber-Ep4 248M-98	49	Cell Marque	5	13	16	15	37%	-
mAb clone Ber-Ep4 IR/IS637	18	Dako	5	9	3	1	78%	87%
mAb clone Ber-Ep4 IR/IS637³	6	Dako	1	2	2	1	-	-
mAb clone Ber-Ep4 GA637	27	Dako	26	1	0	0	100%	100%
mAb clone Ber-Ep4 GA637³	2	Dako	0	1	1	0	-	-
mAb Ber-Ep4 PM107	1	Biocare	1	0	0	0	-	-
mAb Ber-Ep4 MAD-001709QD	2	Master Diagnostica	0	2	0	0	-	-
mAb clone Ber-Ep4 PDM131	1	Diagnostic Biosystems	0	0	1	0	-	-
mAb clone MOC-31 790-4561	3	Ventana	1	2	0	0	-	-
mAb clone MOC-31 248M-18	2	Cell Marque	2	0	0	0	-	-
mAb clone VU-1D9 8230-C010	2	Sakura FineTek	2	0	0	0	-	-
mAb clone MX066 MAB-0850	1	Maxin	1	0	0	0	-	-
Total	256		87	60	58	51	-	-
Proportion			34%	23%	23%	20%	57%	

1) Proportion of sufficient stains (optimal or good).

2) Proportion of sufficient stains with optimal protocol settings only, see below.

3) Ready-to-use product developed for a specific semi/fully automated platform by a given manufacturer but inappropriately applied by laboratories on other non-validated semi/fully automatic systems or used manually.

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

Concentrated antibodies	Dako Autostainer Link/Classic		Dako Omnis		Ventana BenchMark GX /XT/ Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone Ber-EP4	-	4/7** (57%)	-	3/4	2/16*** (13%)	0/1	-	0/3
mAb clone MOC-31	-	1/1	-	3/5 (60%)	2/11 (18%)	-	-	2/6 (33%)
mAb clone BS14	-	-	2/2	-	4/5*** (80%)	-	-	-
mAb clone VU-1D9	-	-	1/1	-	6/6 (100%)	-	-	-

* 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).

*** Protocols without or combined with proteolytic pre-treatment (see description above).

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

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
BenchMark XT/Ultra mAb Ber-EP4 760-4383	(0/1)	(0/1)	47% (7/15)	7% (1/15)
Autostainer +/-Link mAb Ber-EP4 IS/IR637	80% (8/10)	20% (2/10)	75% (6/8)	38% (3/8)
Omnis mAb Ber-EP4 GA637	100% (23/23)	100% (23/23)	(4/4)	(3/4)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.

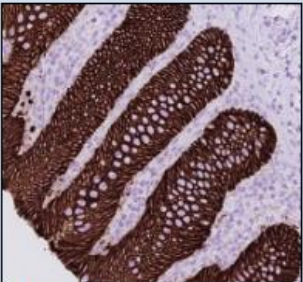
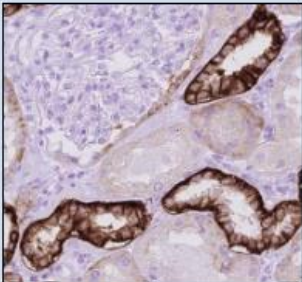
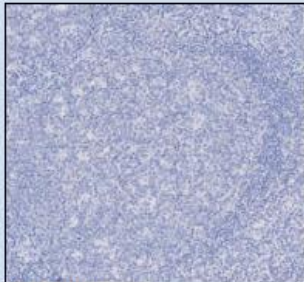
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Less successful performance of the Ventana RTU. Conc. formats of e.g. mAb BS14 and VU-1D9 can be used on BenchMark platforms.

RTUs for both Dako Omnis and Autostainer obtained high pass rates. Use of a 3-layer detection system for IR637 increases optimal results.

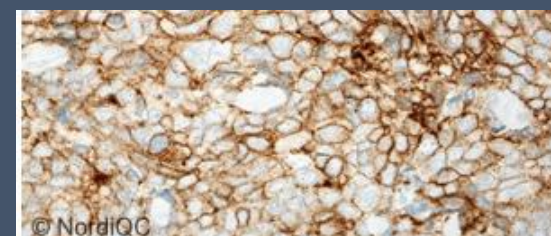
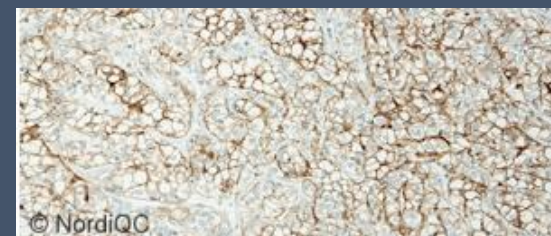
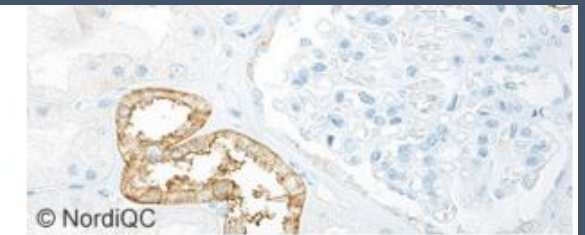
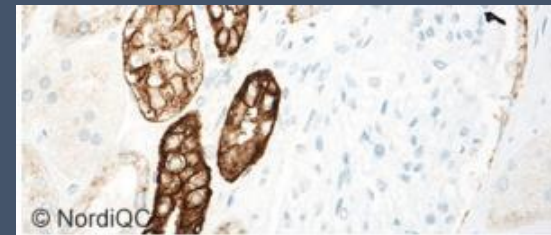
EP-CAM - ICAPS

EpCAM - Epithelial cell-cell adhesion molecule

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Appendix/colon	Kidney	Tonsil
Description	Virtually all columnar epithelial cells must show a moderate to strong and distinct, predominantly membranous staining reaction.	<p>The majority of epithelial cells in the proximal tubules must show an at least weak to moderate, predominantly basolateral staining reaction.</p> <p>Most epithelial cells lining the Bowman capsule must show an at least weak to moderate membranous staining reaction.</p> <p><i>Note, virtually all epithelial cells in the renal distal convoluted tubules will show a strong staining reaction and cannot be used to evaluate the analytical sensitivity.</i></p>	<p>No staining reaction should be seen in lymphocytes, endothelial cells and smooth muscle cells.</p> <p><i>Note, dispersed reactive squamous epithelial can show a distinct membranous staining reaction – the vast majority of squamous epithelial cells are negative.</i></p> <p><i>Mast cells and plasma cells can show a positive cytoplasmic staining reaction.</i></p>
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

Optimal protocol settings

Too diluted Ab + 2-layer detection system



Appendix

Kidney

RCCC

SCLC



HALFWAY THROUGH THE PITFALLS

CGA – PITFALLS/POINTS OF ATTENTION + ICAPS

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

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
VMS GX/XT/Ultra mAb LK2H10 760-2519	6/6 (100%)	4/6 (67%)	91/106 (86%)	68/106 (64%)

* 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 were included.

Typical modifications: prolong incubation time of primary Ab.

Use of OptiView = 84% optimal results

Use of UltraView (with/without amp.) = 49% optimal results

Table 3. Proportion of optimal results for CGA for the most commonly used antibody concentrate on the four main IHC systems*

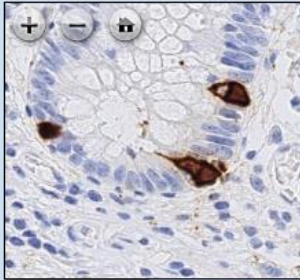
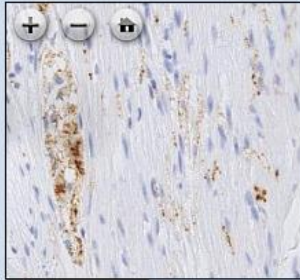
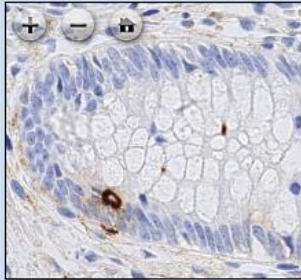
Concentrated antibodies	Dako/Agilent Autostainer		Dako/Agilent Omnis		Ventana/Roche BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone LK2H10	16/18** (89%)	0/4	10/13 (77%)	0/1	19/24 (79%)	0/1	5/6 (83%)	1/6
mAb clones LK2H10+PHE5	0/1	-	2/3	-	7/9 (78%)	-	1/3	1/2

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.

** Number of optimal results/number of laboratories using this buffer.

No RTU for Dako users. The concentrated format of mAb LK2H10 can be used on both Autostainer and Omnis.

CGA - Chromogranin A

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Appendix/colon	Appendix/colon	Appendix/colon
Description	<p>Virtually all neuroendocrine cells in the epithelial mucosa must show a strong intense cytoplasmic staining reaction.</p> <p><i>Note in the vicinity of the specific staining reaction a weak diffuse background reaction can be seen due to leakage of the antigen.</i></p>	Axons and ganglion cells in the nerve plexus (Auerbach's and Meissner's) must show an at least weak to moderate, distinct cytoplasmic staining reaction.	No staining reaction in columnar epithelial cells and smooth muscle cells.
Example	 <p>Click to enlarge</p>	 <p>Click to enlarge</p>	 <p>Click to enlarge</p>

SYP – PITFALLS/POINTS OF ATTENTION

Table 1. Antibodies and assessment marks for SYP, run 52

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	Suff. OPS ²
mAb clone 27G12	64	Leica/Novocastra	13	36	15	3	73%	83%
mAb clone BS15	1	Biocare Medical						
mAb clone DAK-SYNAP	1	Monosan						
mAb clone SNP88	1	KliniPath						
mAb clone SY38 ³	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone MRQ-40	21	Agilent/Dako	12	6	1	2	86%	88%
	7	Biogenex	1	2	4	0	43%	-
	2	Dako	0	0	1	1	-	-
	6	Cell Marque	1	4	1	0	83%	-
rmAb clone SP11	11	Thermo/Neomarkers						
	5	Spring Bioscience	6	5	7	0	61%	64%
	1	Abcam						
	1	Invitrogen						
pAb 336A	1	Cell Marque	0	1	0	0	-	-
pAb RB-1461	1	Thermo/Neomarkers	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone 27G12	13	Leica/Novocastra	0	6	5	2	46%	-
mAb clone PA0299								
mAb clone 27G12	2	Leica/Novocastra	0	0	2	0	-	-
mAb clone PA0299 ⁴								
mAb clone DAK-SYNAP	31	Agilent/Dako	16	15	0	0	100%	100%
mAb clone IR660								
mAb clone DAK-SYNAP	19	Agilent/Dako	8	11	0	0	-	-
mAb clone IR660 ⁴								
mAb clone DAK-SYNAP	5	Agilent/Dako	3	2	0	0	100%	100%
mAb clone GA660								
mAb clone DAK-SYNAP	4	Agilent/Dako	4	0	0	0	-	-
mAb clone GA660 ⁴								
mAb clone BS15	1	Sakura FineTek	1	0	0	0	-	-
mAb clone 8453-C010								
mAb clone SNP88	1	Biogenex	0	0	1	0	-	-
mAb clone AM363-10M ⁴								
mAb clone SY38	1	Dako	0	1	0	0	-	-
mAb clone IR/IS776 ³								
rmAb MRQ-40	43	Ventana/Cell Marque	6	22	13	2	65%	90%
rmAb clone 760-4595								
rmAb clone MRQ-40	12	Cell Marque	2	4	3	3	-	-
rmAb clone 336R								
rmAb clone SP11	48	Ventana	25	14	7	2	81%	96%
rmAb clone 790-4407								
rmAb clone SP11	1	Maixin	1	0	0	0	-	-
rmAb clone KIT-0022								
rmAb clone SP11	1	Diagnostic Biosystem	0	0	1	0	-	-
rmAb clone RMPD018								
rmAb clone EP158	2	Master Diagnostica	0	1	1	0	-	-
rmAb clone MAD-000685QD								
Total	308		100	130	62	16	-	
Proportion			33%	42%	20%	5%	75%	

1) Proportion of sufficient stains (optimal or good).

2) Proportion of sufficient stains with optimal protocol settings only, see below.

3) Product discontinued.

4) Ready-to-use product developed for a specific semi/fully automated platform by a given manufacturer but inappropriately applied by laboratories on other non-validated semi/fully automatic systems or used manually.

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

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Leica BOND MAX/III mAb 27G12 PA0299	40% (2/5)	0% (0/5)	50% (4/8)	0% (0/8)
Dako AS mAb DAK-SYNAP IR660	100% (14/14)	36% (5/14)	100% (17/17)	65% (11/17)
Dako Omnis mAb DAK-SYNAP GA660	3/3	3/3	2/2	0/2
VMS Ultra/XT/GX rmAb MRQ-40 760-4595	0/3	0/3	69% (27/39)	15% (6/39)
VMS Ultra/XT/GX rmAb SP11 790-4407	0/4	0/4	89% (39/44)	57% (25/44)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.

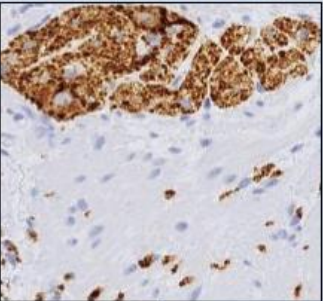
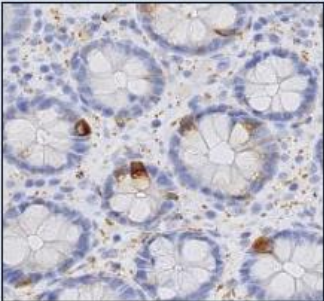
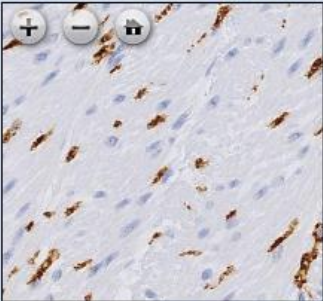
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer were included.

Modified protocol settings typically based on EnVision Flex+ as detection system, increases optimal results till 65% from 36% if using recommended EnVision Flex.

Protocols based on UltraView as detection system obtained a pass rate of 29% and 38%.
If using UltraView + amplification or OptiView as detection system, pass rates of 90% and 96% were obtained.

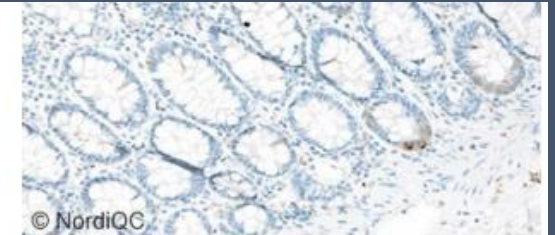
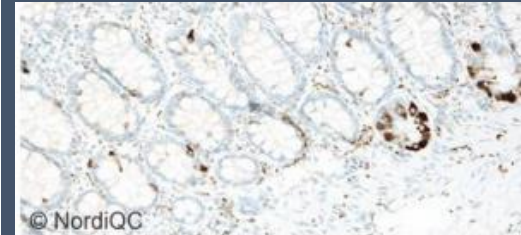
SYP – ICAPS

SYP - Synaptophysin

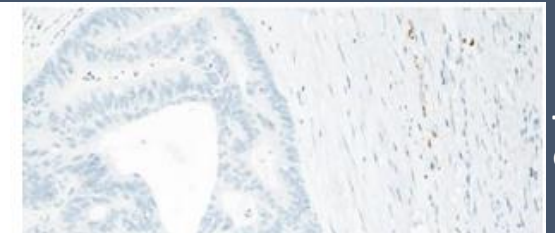
Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Appendix/colon	Appendix/colon	Appendix/colon
Description	Virtually all axons and ganglion cells in the nerve plexus (Auerbach's and Meissner's) must show a moderate to strong, distinct cytoplasmic staining reaction.	Neuroendocrine and scattered goblet cells in the epithelial mucosa must show an at least weak to moderate, distinct cytoplasmic staining reaction.	No staining reaction in smooth muscle cells.
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

Optimal protocol settings

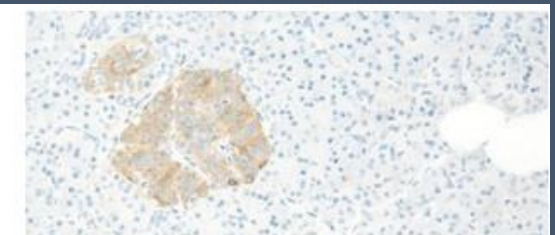
2-layer detection system



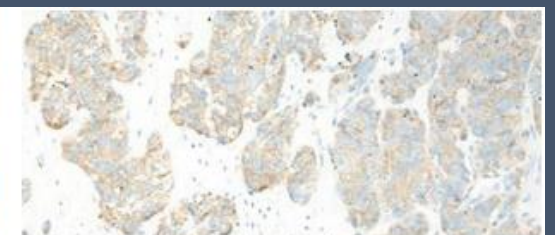
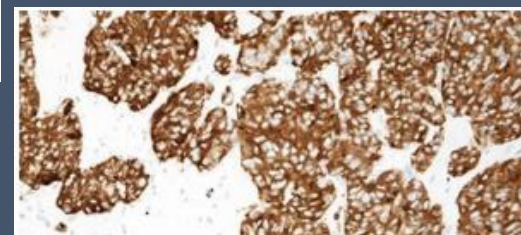
Appendix



Colon
adenocarc.



Pancreas



SCLC

Table 1. Antibodies and assessment marks for CK5, run 65								
Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff ¹	OR ²
mAb clone D5/16 B4*	35	Dako/Agilent	6	15	19	1	51%	15%
	2	Cell Marque						
	1	Millipore						
	1	Epredia						
	2	Zytomed						
mAb clone XM26	2	Abcam	56	11	4	1	93%	78%
	3	Diagnostic BioSystems						
	64	Leica Biosystems						
	3	Monosan						
mAb clone IHC556*	1	GenomeMe	0	0	1	0	-	-
mAb clone ZM186	1	Zeta Corporation	0	0	1	0	-	-
rmAb clone BSR55	2	Nordic Biosite	1	0	1	0	-	-
rmAb clone EP1601Y	3	Cell Marque	0	1	2	0	-	-
rmAb clone EP24/EP67*	2	Cell Marque	0	2	0	0	-	-
rmAb clone EP24	1	Epitomics	0	1	0	0	-	-
rmAb clone EP42	1	Epitomics	1	0	0	0	-	-
rmAb clone SP27	1	Immunologic	1	0	0	0	-	-
rmAb clone QR027	1	Quartett	0	1	0	0	-	-
mAb clone XM26/SF13**	1	DCS Innovative Diagnostik-Systeme	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone D5/16 B4*	6	Ventana/Roche	0	3	3	0	50%	0%
	790-4554 ³							
	mAb clone D5/16 B4*							
	790-4554 ⁴							
mAb clone D5/16 B4*	13	Dako/Agilent						
	GA780 ³							
mAb clone D5/16 B4*	26	Dako/Agilent						
	GA780 ⁴							
mAb clone D5/16 B4*	4	Dako/Agilent	0	1	2	1	-	-
	IR/IS780 ³		1	1	4	3	22%	11%
mAb clone D5/16 B4*	9	Dako/Agilent						
	IR/IS780 ⁴							
mAb clone D5/16 B4*	1	Sakura Finetek	1	0	0	0	-	-
	8295-C010		0	1	0	0	-	-
rmAb clone RM226	1	Sakura Finetek						
	8408-C010							
mAb clone XM26	7	Leica Biosystems	2	4	1	0	86%	29%
	PA0468 ³		8	1	0	0	100%	89%
mAb clone XM26	9	Leica Biosystems						
	PA0468 ⁴							
mAb clone XM26	3	Biocare Medical	2	1	0	0	-	-
	PM234		0	2	2	0	-	-
rmAb clone EP1601Y	4	Cell Marque						
	305R-17/18							
rmAb clone EP42	1	BioGenex						
	AN853-10M		1	0	0	0	-	-
rmAb clone EP24/EP67*	1	Master Diagnostica						
	MAD-000651QD		0	1	0	0	-	-
rmAb clone EP24/EP67*	1	PathnSitu						
	MRH1159		21	0	0	0	100%	100%
rmAb clone SP27	21	Ventana/Roche						
	760-4935 ³		26	3	0	0	100%	90%
rmAb clone SP27	29	Ventana/Roche						
	760-4935 ⁴		0	0	1	0	-	-
rmAb clone C9E33	1	Celnovte						
	CCR-0973		0	0	1	0	-	-
mAb clone 150A8C1	1	Abcarta						
	PA018		136	84	81	10		
Total	311							
Proportion			44%	27%	26%	3%	71%	

CK5

PITFALLS/POINTS OF ATTENTION



Table 2. Proportion of optimal results for CK5 for the most commonly used antibodies as concentrates on the four main IHC systems*

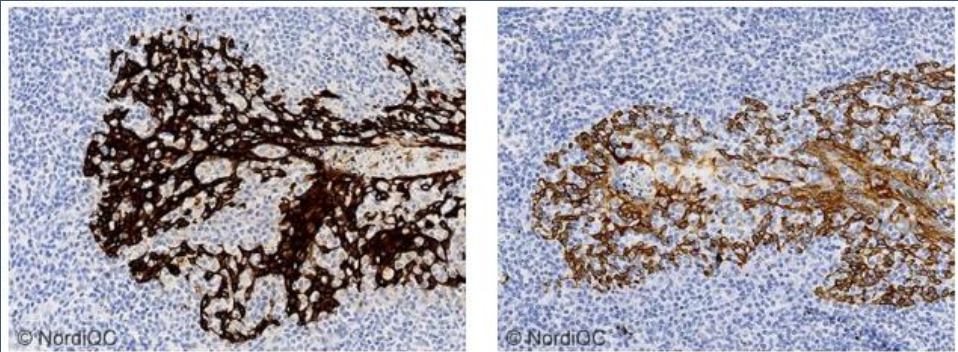
Concentrated antibodies	Dako Autostainer Link / Classic		Dako Omnis		Ventana BenchMark GX / XT / Ultra			Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC1 pH 8.5 + Protease 3	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone D5/16 B4	0/2	-	0/2	-	5/12 (42%)	1/1	-	0/5 (0%)	0/2
mAb clone XM26	1/4	-	24/26 (92%)	-	17/24 (71%)	1/1	-	12/12 (100%)	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).

Table 3. Proportion of sufficient and optimal results for CK5 for the most commonly used RTU IHC systems

RTU systems	Vendor recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Ventana Benchmark mAb clone D5/16 B4, 790-4554	50% (3/6)	0% (0/6)	70% (32/46)	20% (9/46)
Dako Omnis mAb clone D5/16 B4, GA780	8% (1/13)	0% (0/13)	36% (9/25)	0% (0/25)
Dako Autostainer mAb clone D5/16 B4, IR/IS780	(1/4)	(0/4)	0% (0/6)	0% (0/6)
Leica Bond mAb clone XM26, PA0468	86% (6/7)	29% (2/7)	100% (9/9)	89% (8/9)
Ventana Benchmark rmAb clone SP27, 760-4935	100% (21/21)	100% (21/21)	100% (27/27)	89% (24/27)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
 ** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.



Less successful performance of the mAb D5/16 B4 both as RTU and Conc.

mAb XM26 obtained optimal results on the main systems.

rmAb SP27 with a pass rate of 100%. However, the specificity is reduced compared to e.g. XM26...

OPEN

NordiQC Assessments of Keratin 5 Immunoassays

Christian Thomsen, MD,* Ole Nielsen, HT,† Søren Nielsen, HT,* Rasmus Roge, MD,*‡ and Mogens Vyberg, MD*‡

Left: XM26 // Right: D5/16 B4

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 5A4	26	Leica Biosystems						
	2	Monosan						
	1	Abcam						
	1	DBS	8	9	14	4	49%	23%
	2	Biocare Medical						
mAb clone OTI1A4*	2	Zytomed Systems						
	2	Invitrogen						
	19	Origene						
	1	Nordic Biosite	16	6	0	0	100%	73%
mAb clone IHC509	1	Cell Signaling						
rmAb clone D5F3	1	Zeta Corporation						
rmAb clone ALK1	3	Dako/Agilent	0	0	0	4	-	-
rmAb clone QR017	1	Cell Marque						
rmAb clone SP8	1	Quartett	0	1	0	0	-	-
rmAb clone ZR305	1	BioGenex	0	0	0	1	-	-
rmAb clone ZR305	1	Zeta Corporation	0	0	1	0	-	-

Ready-To-Use antibodies								
mAb clone 5A4 PA0306**/PA0831 (VRPS) ³	2	Leica Biosystems	1	1	0	0	-	-
mAb clone 5A4 PA0306*/PA0831 (LMPS) ⁴	10	Leica Biosystems	4	3	2	1	70%	40%
mAb clone 5A4 API3041	1	BioCare	0	0	1	0	-	-
mAb clone 5A4 CAM-0170	1	Celnovte	0	1	0	0	-	-
mAb clone 5A4 MAD-0017200D	1	Master Diagnostica	0	0	1	0	-	-
mAb clone ALK1 GA641	3	Dako/Agilent	0	0	0	3	-	-
mAb clone ALK1 IR641	4	Dako/Agilent	0	0	0	4	-	-
mAb clone ALK1 790/800-2918 (LMPS) ⁴	10	Ventana/Roche	1	0	1	8	10%	10%
mAb clone 137E9E8 PA132	1	Abcarta	0	0	0	1	-	-
mAb clone OTI1A4 / 1A4 8344-C010	1	Sakura Finetek	1	0	0	0	-	-
mAb clone OTI1A4 / 1A4 GA785 (VRPS) ³	12	Dako/Agilent	12	0	0	0	100%	100%
mAb clone OTI1A4 / 1A4 GA785 (LMPS) ⁴	4	Dako/Agilent	4	0	0	0	-	-
rmAb clone D5F3 790-4794 (VRPS) ³	73	Ventana/Roche	62	7	1	3	95%	85%
rmAb clone D5F3 790-4794 (LMPS) ⁴	48	Ventana/Roche	36	9	3	0	94%	75%
rmAb clone SP8 RMPD007	1	Diagnostic BioSystems	0	0	0	1	-	-
Total	256		152	46	28	30		
Proportion			59%	18%	11%	12%	77%	

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).

*) OTI1A4 is called 1A4 by some vendors

**) Product no. PA0306 has been terminated and replaced by PA0831.

ALK-LUNG – PITFALLS/POINTS OF ATTENTION

rmAb clone ALK1 is not "fit for purpose" for lung diagnostic!

- Be sure to order the right product as both Dako and Ventana have different clones on the market!

Table 4. Proportion of sufficient and optimal results for ALK (lung) for the most commonly used RTU IHC systems

RTU-systems	Recommended protocol settings [*]		Laboratory modified protocol settings ^{**}	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/XT rmAb D5F3 790-4794	95% (69/73)	85% (62/73)	93% (41/44)	80% (35/44)
Dako Omnis mAb OTI1A4 GA785	100% (12/12)	100% (12/12)	(4/4)	(4/4)
Leica BOND mAb 5A4 PA0306/PA0831	(2/2)	(1/2)	75% (6/8)	50% (4/8)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.

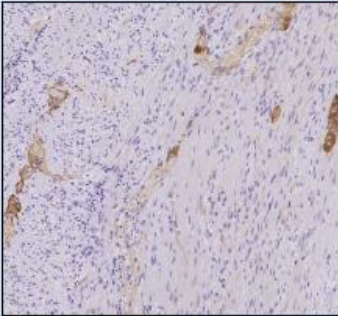
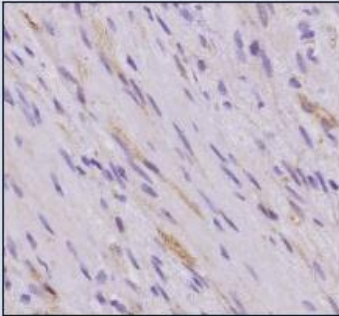
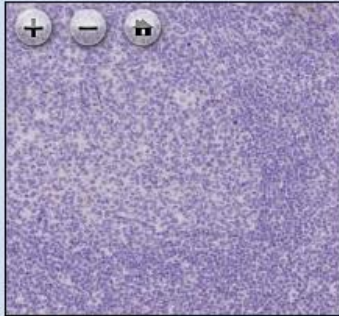
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

RTU products for
the automated
systems, working
as plug-and-play

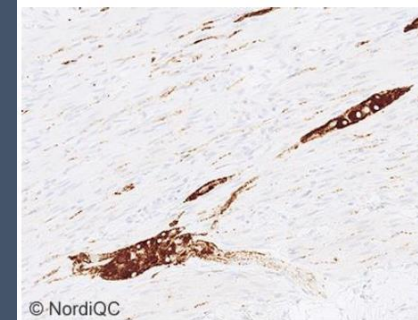


ALK-LUNG – ICAPS

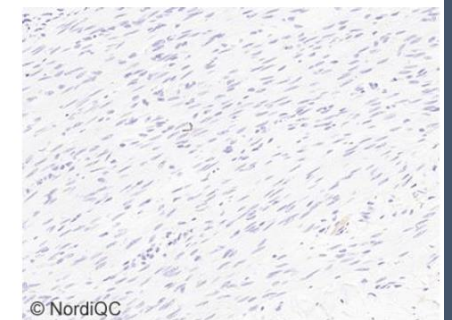
ALK (lung) - Anaplastic lymphoma kinase

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Appendix/colon	Appendix/colon	Tonsil
Description	Most ganglion cells of the myenteric plexus must show a moderate to strong distinct cytoplasmic staining reaction.	At least dispersed axons of nerve cells must show a weak to moderate staining reaction. <i>Note, IHC assays based on tyramide amplification (e.g. OptiView with amplification kit, Ventana) typically will provide a strong intensity in axons.</i>	No staining reaction should be seen. <i>Note, mast cells and plasma cells can show a weak to strong cytoplasmic staining reaction.</i>
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

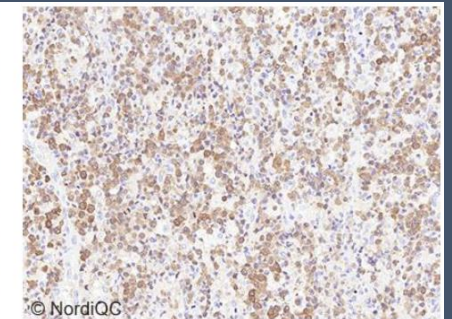
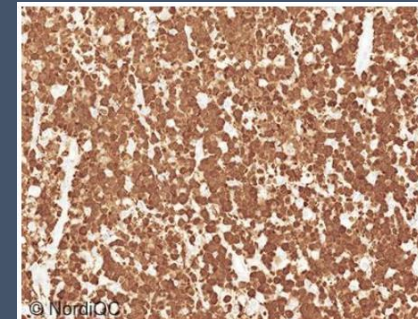
rmAb D5F3 (RTU)



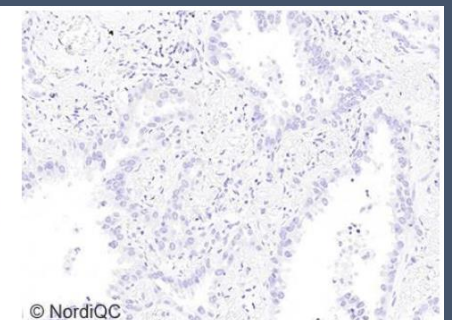
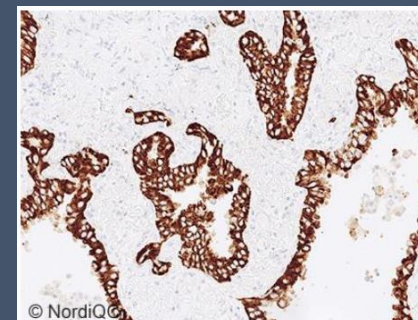
rmAb ALK1 (RTU)



Appendix



ALCL



Lung
adenocarc.

PD-L1 – PITTFALLS/POINTS OF ATTENTION

Table 2. Assessment marks for IHC assays and antibodies run C11, PD-L1 TPS/CPS (KEYTRUDA®)

CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
rmAb clone SP263, 741-4905 (VRPS) ³	50	Ventana/Roche	9	24	16	1	66%	18%
rmAb clone SP263, 741-4905 (LPMS) ⁴	1	Ventana/Roche	-	-	-	1	-	-
rmAb clone SP263, 740-4907 (VRPS) ³	11	Ventana/Roche	3	5	1	2	73%	27%
rmAb clone SP142, 741-4860 (VRPS) ³	1	Ventana/Roche	-	-	-	1	-	-
mAb clone 22C3 pharmDX, SK006 (VRPS) ³	24	Dako/Agilent	14	6	4	-	83%	58%
mAb clone 22C3 pharmDX, SK006 (LMPS) ⁴	13	Dako/Agilent	4	5	3	1	69%	31%
mAb clone 22C3 pharmDX, GE006 (VRPS) ³	31	Dako/Agilent	25	5	1	-	97%	81%
mAb clone 22C3 pharmDX, GE006 (LMPS) ⁴	9	Dako/Agilent	4	5	-	-	100%	44%
rmAb clone 28-8 pharmDX, SK005 (VRPS) ³	2	Dako/Agilent	-	2	-	-	-	-
Antibodies ⁵ for laboratory developed PD-L1 assays, concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 22C3	39	Dako/Agilent	19	18	2	-	95%	49%
rmAb CAL10	4 2	Zytomed Systems Biocare Medical	1	3	2	-	67%	17%
rmAb clone E1L3N	5	Cell Signaling	3	-	2	-	60%	60%
rmAb clone QR1	1 1	Quartett Diagomics	-	2	-	-	-	-
Ready-To-Use antibodies ⁶	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone C9C9 CPM-0278	1	Celnovte	-	1	-	-	-	-
rmAb clone SP263, 790-4905 ⁵ (VRPS) ³	17	Ventana/Roche	3	13	1	-	94%	18%
rmAb clone SP263, 790-4905 ⁵ (LMPS) ⁴	9	Ventana/Roche	-	7	2	-	78%	-
rmAb clone AC37 AD80167	1	Abcarta	-	-	1	-	-	-
rmAb clone QR1 2-PR292-13	2	Diagomics	-	1	-	1	-	-
rmAb clone RM320 8263-C010	1	Sakura Finetek	1	-	-	-	-	-
Total	225		85	98	35	7		
Proportion			38%	43%	16%	3%	81%	

1) Proportion of sufficient stains (optimal or good).

2) Proportion of optimal results.

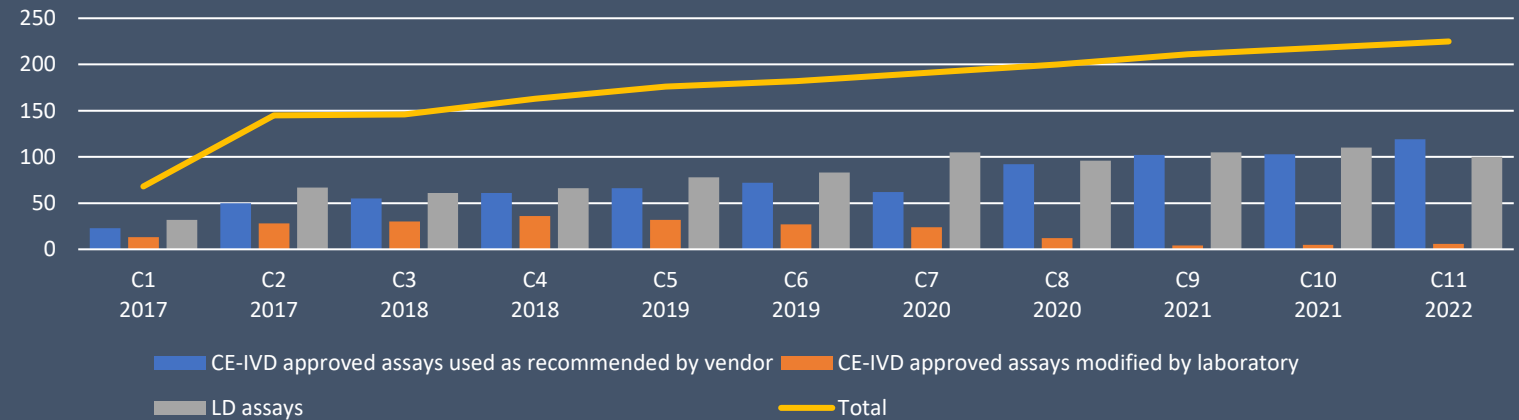
3) Vendor recommended protocol settings – RTU product used in compliance to protocol settings, platform and package insert.

4) Laboratory modified protocol settings for a RTU product applied either on the vendor recommended platform(s) or other platforms.

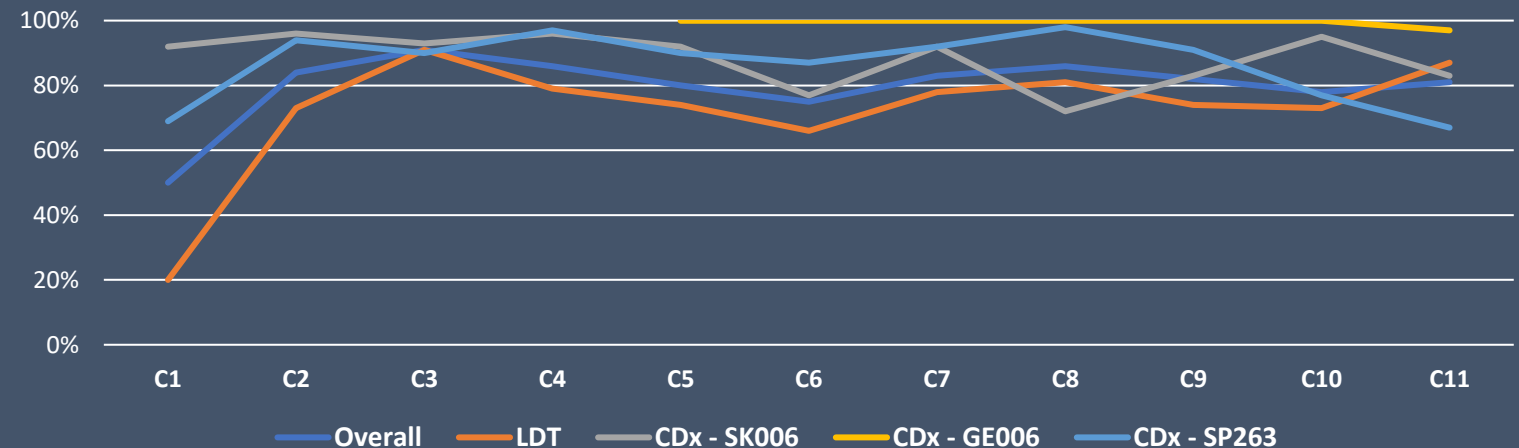
5) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody.

6) Ready-To-Use antibodies without predictive claim.

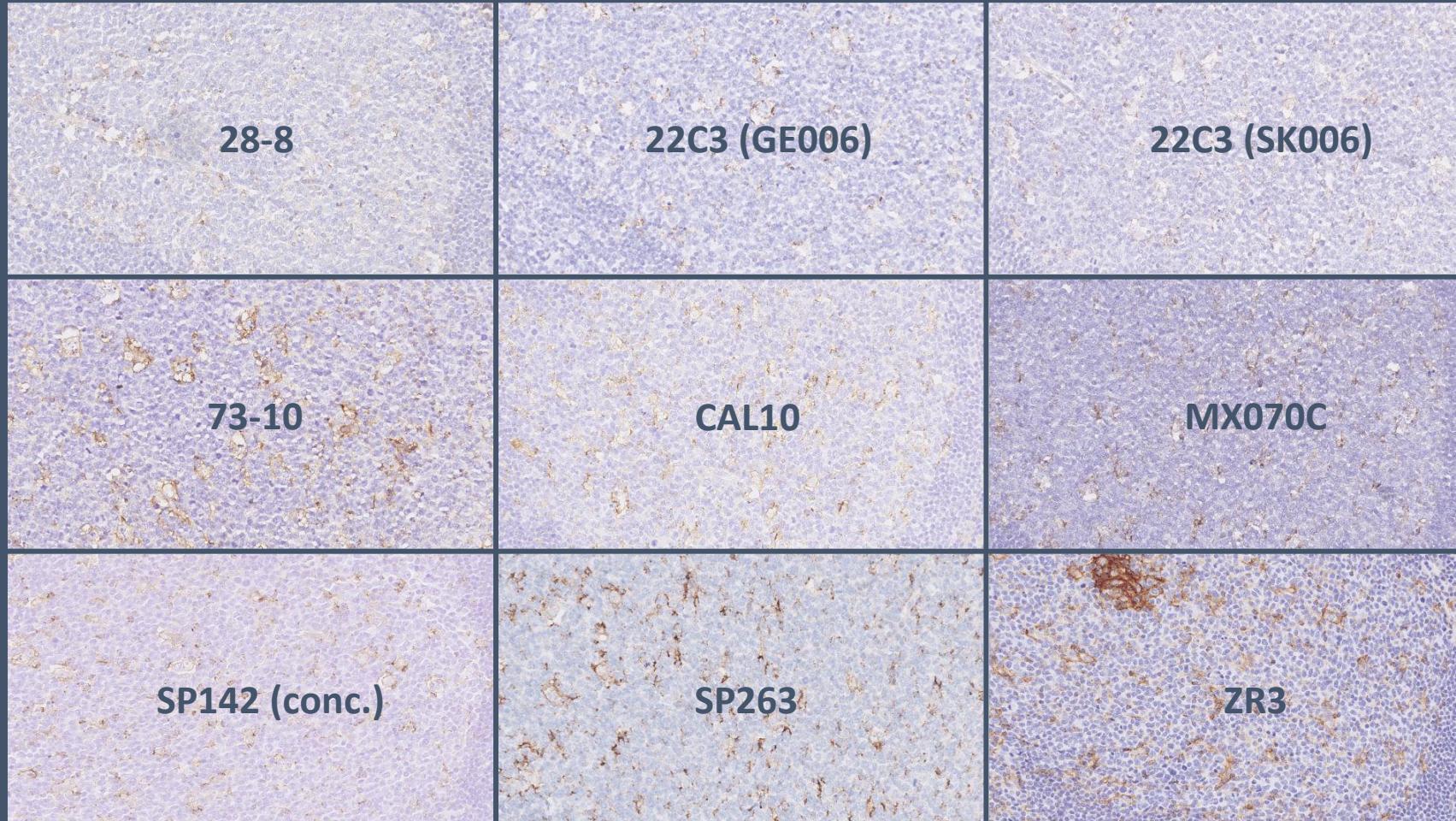
Use of IHC assays in PD-L1 TPS/CPS



Pass rate - PD-L1 assays for TPS/CPS



PD-L1 - ICAPS - TONSIL



In tonsil, a weak to moderate staining reaction in germinal center macrophages should be seen.



Different assays → different staining patterns.

All 9 assays achieved an optimal score for PD-L1 TPS/CPS.

THANK YOU FOR YOUR ATTENTION!



BONUS – ROS1

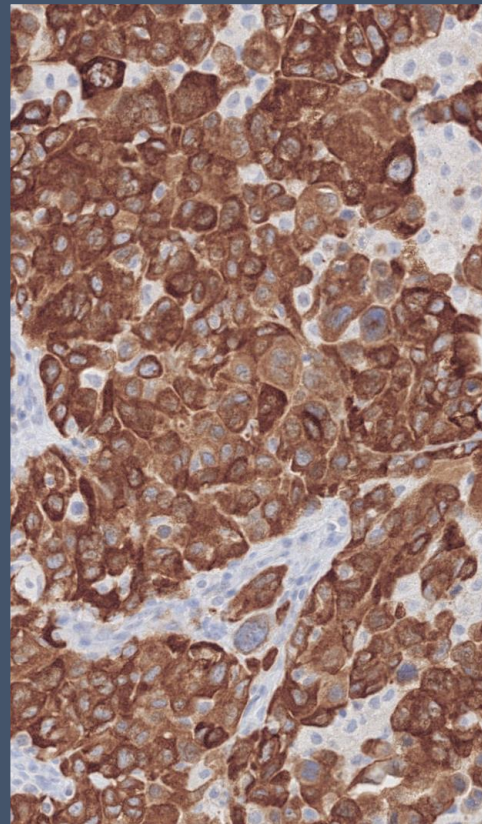
No NordiQC data available for ROS1.

For these stains, the Ventana RTU based on rmAb SP384 is used.

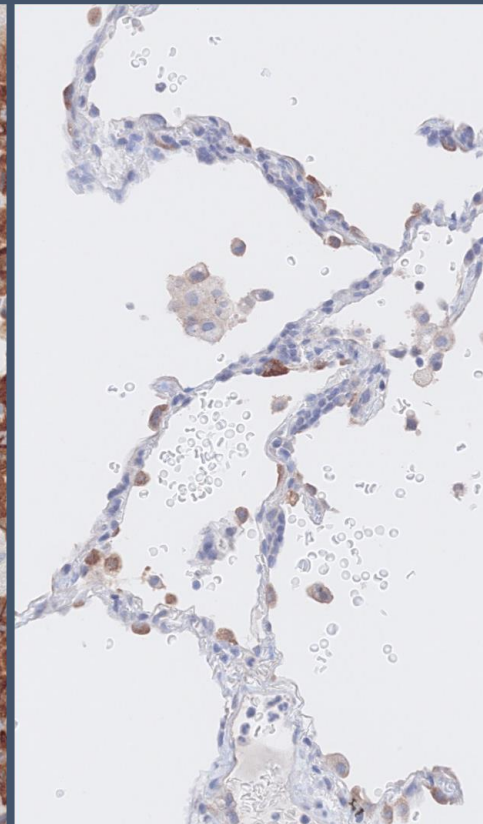
Positive controls:
Tumor with known ROS1-translocation
Type II-pneumocytes in normal lung

Negative control:
Appendix

Tumor with ROS1-
translocation
(lung adenocarc.)



Normal lung



Appendix

