NORDIQC DATA FOR LUNG MARKERS

Antibody selection, protocols and controls

NordiQC Workshop, September 29th – October 1st 2021

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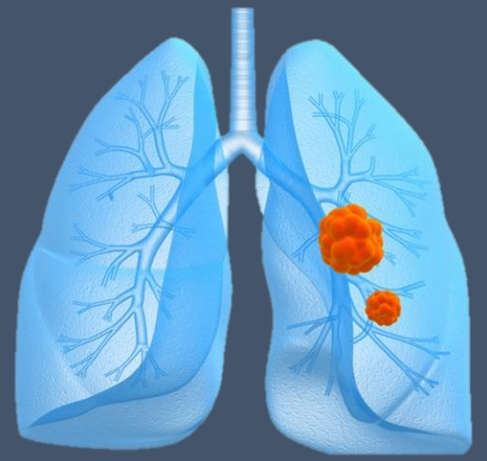
AGENDA

- 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	Lung vs non-lung Adenocarcinoma vs squam.	Run 58, 2020	80%	322
Napsin A	Lung vs non-lung	Run 44, 2015	78%	162
Calretinin	Lung vs mesothelioma	Run 52, 2018	72%	269
WT1	Lung vs mesothelioma	Run 55, 2019	91%	291
EpCAM	Lung vs mesothelioma	Run 56, 2019	57%	256
CGA	NSCLC vs SCLC	Run 53, 2018	76%	296
SYP	NSCLC vs SCLC	Run 52, 2018	75%	308
CD56	NSCLC vs SCLC	Run 61, 2021	62%	324
p40	Adenocarcinoma vs squam.	Run 60, 2020	86%	262
CK5	Adenocarcinoma vs squam.	Run 55, 2019	44%	263
ALK (lung)	Predictive for Crizotinib	Run 57, 2019	84%	201
PD-L1 TPS/CPS	Predictive for Keytruda, Imfinzi, Opdivo……	Run C9, 2021	82%	211

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 (87%), rmAb SP141 (99%)	mAb 8G7G3/1 (20%)
Napsin A	mAbs IP64 (90%) & MRQ-60 (94%)	pAbs (13%)
Calretinin	mAbs DAK-Calret (61%) & CAL6 (77%), rmAb SP65 (90%)	pAbs (35%), rmAb SP13 (42%)
WT1	mAbs 6F-H2 (90%) & WT49 (97%)	-
EpCAM	mAbs BS14 (100%), Ber-EP4 (51%) & MOC-31 (74%)	mAb Ber-EP4 (51%)
CGA	mAb LK2H10 (90%)	mAbs DAK-A3 (13%) & 5H7 (11%)
SYP	mAbs DAK-SYNAP (96%) & 27G12 (67%), rmAbs MRQ-40 (67%) & SP11 (75%)	-
CD56	rmAb MRQ-42 (99%)	mAbs 123C3 (27%) & CD564 (51%)
p40	mAb BC28 (90%)	pAbs (38%)
CK5	mAb XM26 (79%), rmAb SP27 (100%)	mAb D5/16 B4 (23%)
ALK (lung)	mAbs 5A4 (75%) & OTI1A4 (92%), rmAb D5F3 (91%)	mAb ALK1 (0%)
PD-L1 TPS/CPS	mAb 22C3 (56%), rmAb SP263 (86%)	*rmAb SP142 as RTU*

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
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 cytotrhophoblastic 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	Biocare Medical DCS Diagnostics Immunologic Leica/Novocastra Monosan Zytomed Immunologic	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 asessed protocols)
2) Proportion of Optimal Results (≥5 asessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 asessed protocols).

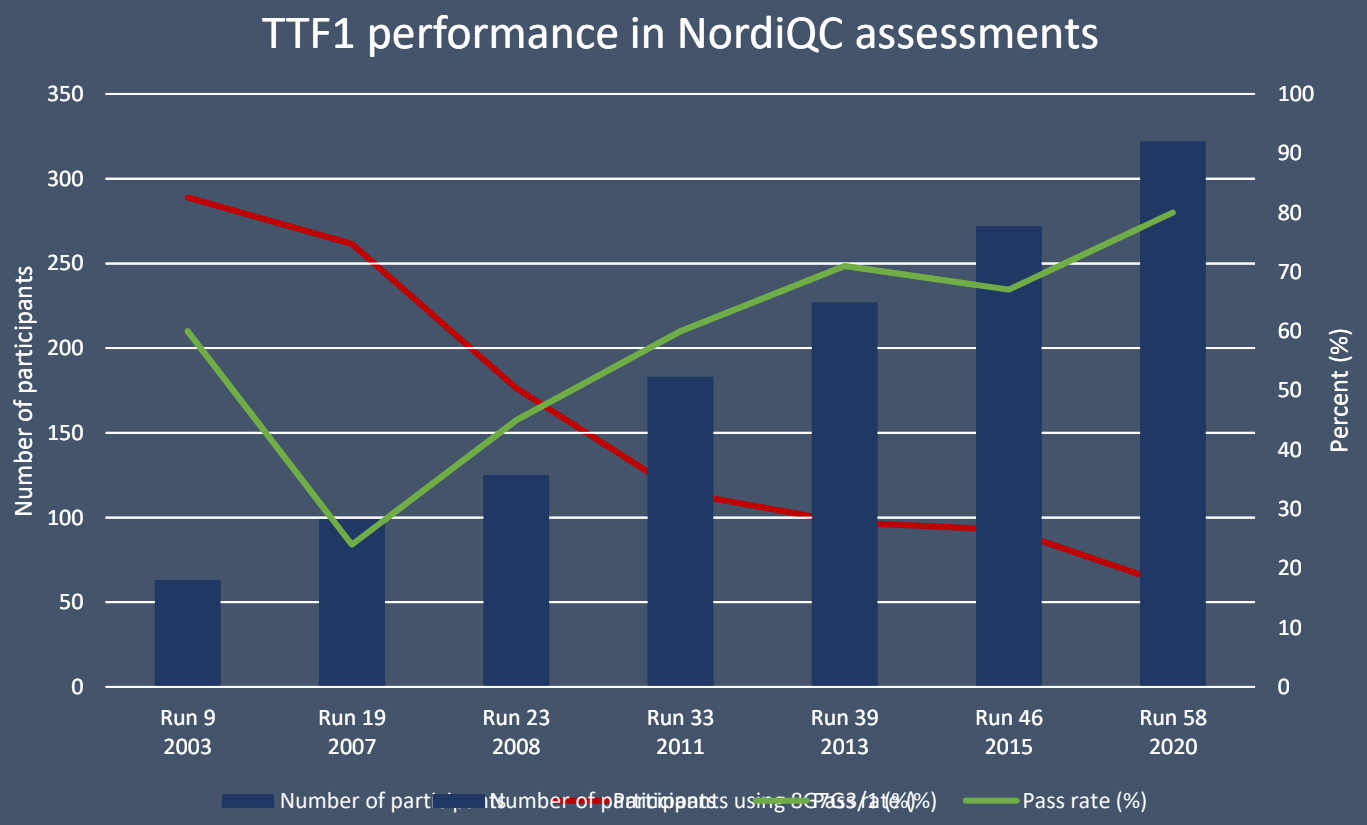


Table 4. **The overall pass rate in the last five runs for the mAb clones SPT24, 8G7G3/1 and the rmAb clone SP141**

	SPT24 All protocol settings		SP141* All protocol settings		8G7G3/1 All protocol settings	
	Sufficient	Optimal	Sufficient	Optimal	Sufficient	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	Biocare Medical DCS Diagnostics Immunologic Leica/Novocastra Monosan Zytomed Immunologic	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%
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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 asessed protocols)
2) Proportion of Optimal Results (≥5 asessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 asessed 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.

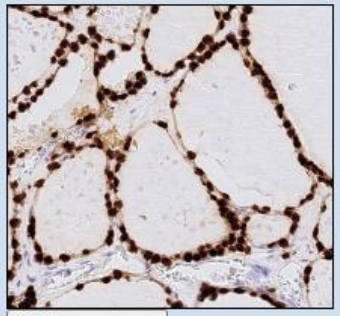
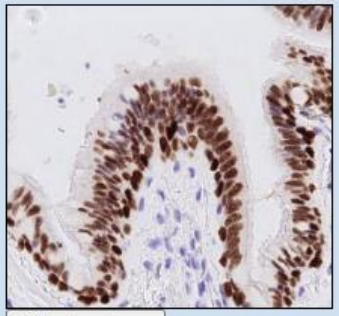
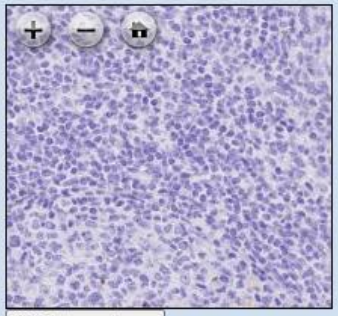
Table 2. **Proportion of optimal results for TTF1 for the mAb clone SPT24 as concentrate on the main IHC systems***

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).

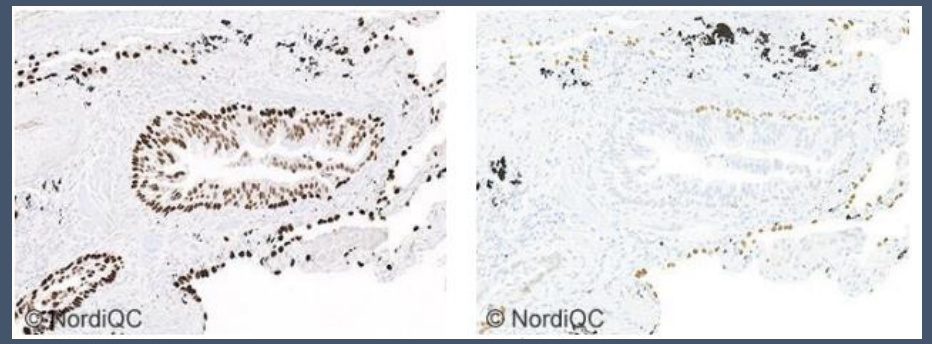
TTF1 – ICAPS

TTF1 - Thyroid transcription factor-1

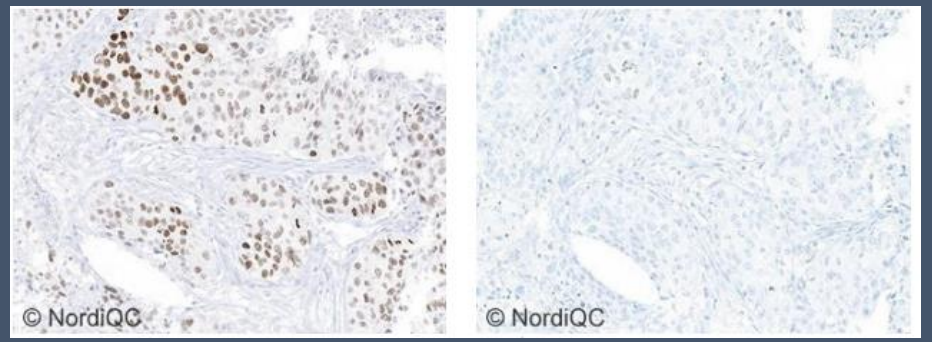
Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Thyroid gland	Lung	Tonsil
Description	Virtually all follicular epithelial cells should display a strong nuclear staining reaction. A weak cytoplasmic staining reaction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

rmAb clone SP141 mAb clone 8G7G3/1

Thyroid gland

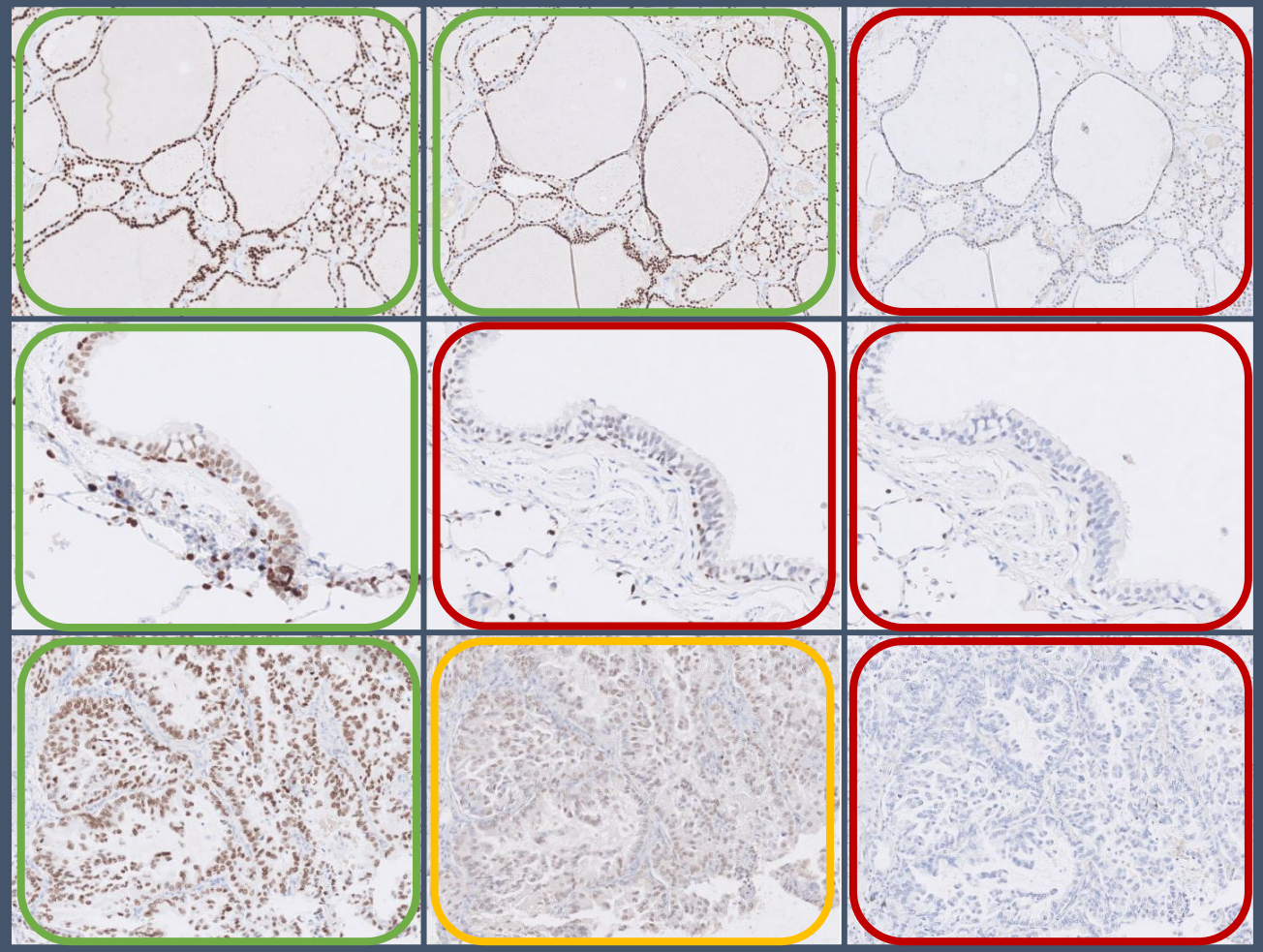


Normal lung



Lung adenocarc.

TTF1 – ICAPS



Optimal protocol | Reduced sensitivity x1 | Reduced sensitivity x2

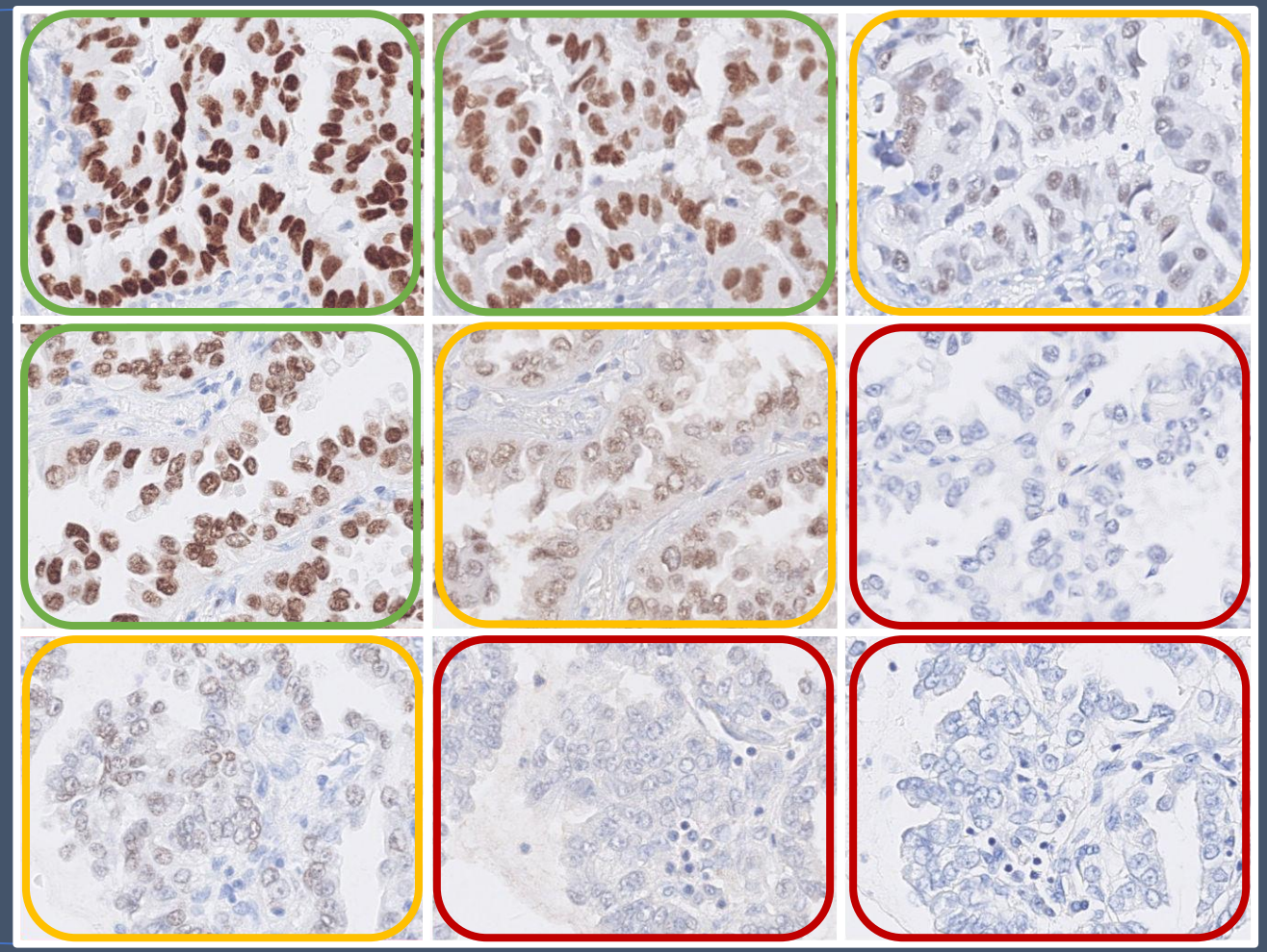
Thyroid gland – High Expressor

Lung, pneumocytes & basal cells of terminal bronchioles – High Expressor

Lung, luminal epithelial cells of terminal bronchioles – Low Expressor

Lung adenocarcinoma – clinical impact

TTF1 – ICAPS


Lung adenocarcinomas
High expression level
Moderate expression level
Low expression level
Optimal protocol
Reduced sensitivity x1
Reduced sensitivity x2

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 3	Biocare IBL	1	2	4	0	43%	-
rmAb clone **KCG1.1**	2 2 1 1	Zytomed Diagnostic Biosystems Abcam Acris	1	5	0	0	100%	-
rmAb clone **BC15**	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 Autost. Link / Classic, Omnis		Ventana BenchMark XT / Ultra		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 **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.

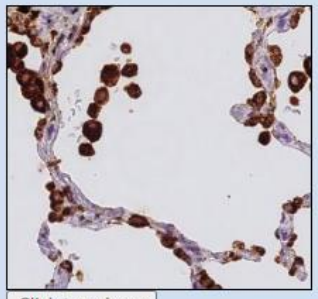
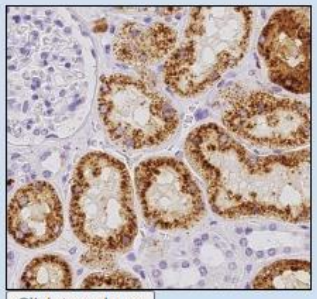
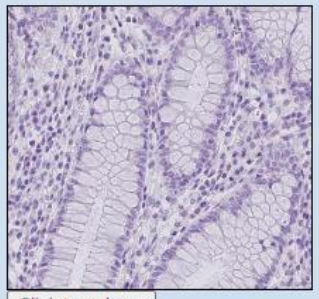
Recommended staining protocol for this antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

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 a… with wash buffer.
7.	Coverslip.

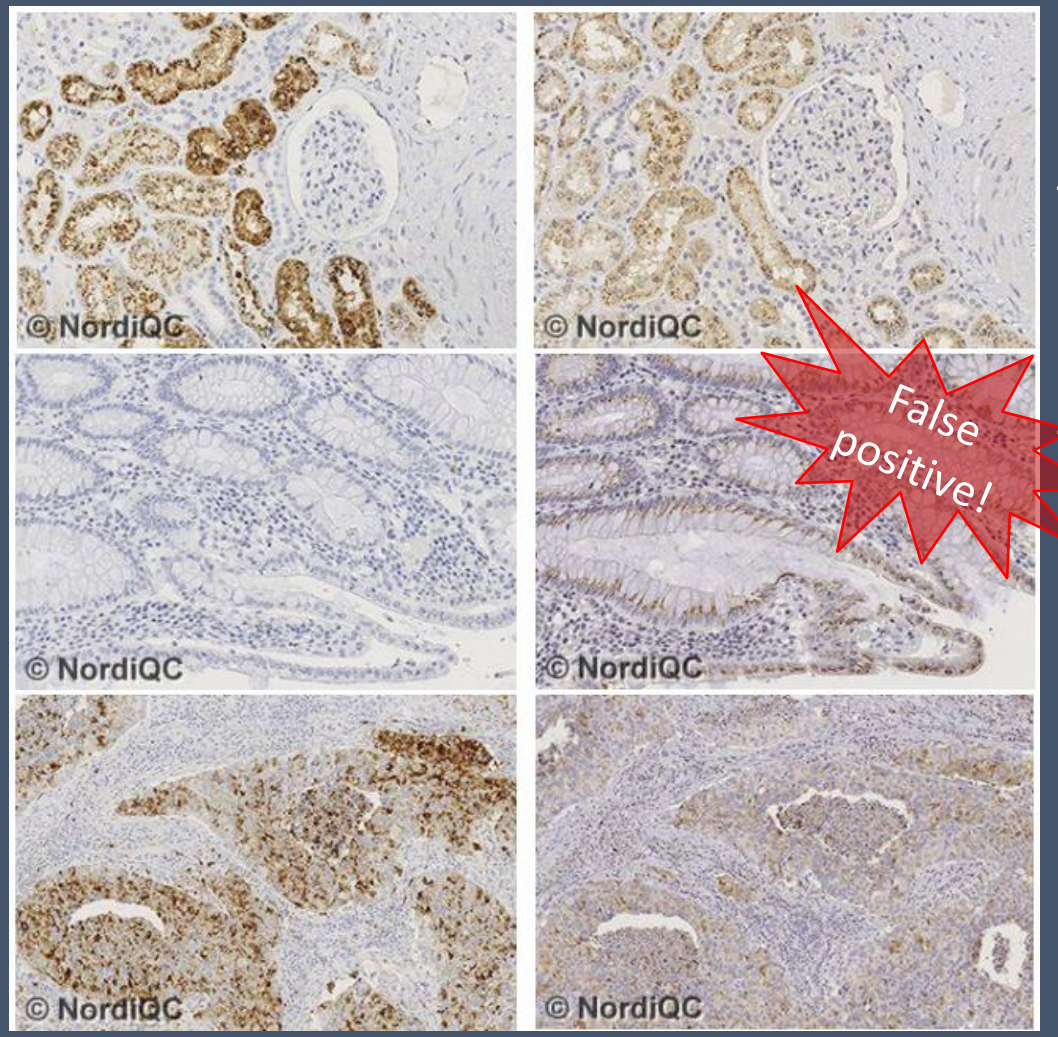
The one optimal protocol used OptiView. Recommended protocol settings in 2015 were based on UltraView. In 2017 the recommended settings changed to also include a protocol for OptiView.

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. *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.*	No staining reaction should be seen in the columnar epithelial cells and macrophages. *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.*
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

mAb IP64 pAb



CALRETININ – PITFALLS/POINTS OF ATTENTION

NordiQC

Table 1. **Antibodies and assessment marks for CR, run 52**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **2E7**	1	Immunologic	1	0	0	0	-	-
mAb clone **5A5**	3 1	Leica/Novocastra Monosan	1	1	2	0	-	-
mAb clone **CAL6**	7	Leica/Novocastra	1	3	0	3	57%	-
mAb clone **DAK-Calret 1**	34	Dako/Agilent	9	8	8	9	50%	81%
rmAb clone **BSR235**	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone **SP13**	3 2 2 2	Cell Marque Immunologic Spring Bioscience Thermo Scientific	1	3	4	1	44%	-
pAb **18-0211**	12	Invitrogen/Thermo	3	3	4	2	50%	100%
pAb, **232A**	2	Cell Marque	0	0	2	0	-	-
pAb **61-0006**	1	Genemed	0	0	1	0	-	-
pAb, **CP092C**	1	Biocare	0	0	1	0	-	-
pAb **RBK003**	1	Zytomed Systems	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone **CAL6 PA0346**	14	Leica/Novocastra	1	11	2	0	86%	92%
mAb clone **CAL6 PA0346**[3]	1	Leica/Novocastra	1	0	0	0	-	-
mAb clone **DAK-Calret 1 IS/IR627**	35	Dako/Agilent	14	19	2	0	94%	97%
mAb clone **DAK-Calret 1 IS/IR627**[4]	20	Dako/Agilent	0	4	11	5	20%	-
mAb clone **MX027 MAB-0716**	1	Maixin	1	0	0	0	-	-
rmAb **SP13 232R**	1	Cell Marque	0	0	1	0	-	-
rmAb **SP13 MAD-000315QD**	1	Master Diagnostica	0	0	1	0	-	-
rmAb **SP13 RMPD010**	1	Diagnostic Biosystems	0	1	0	0	-	-
rmAb clone **SP65 790-4467**	118	Ventana/Roche	86	20	10	2	90%	96%
pAb **232A-78**	2	Cell Marque	0	0	2	0	-	-
pAb **8223-C010**	1	Sakura Finetek	0	1	0	0	-	-
Unknown RTU Ab	1		0	0	1	0	-	-
Total	269		120	74	52	23	-	
Proportion			45%	27%	19%	9%	72%	

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 Leica full-automated system (BOND III/MAX) but used by a laboratory on the Intellipath platform (Biocare).
4) RTU system developed for the Agilent/Dako semi-automatic system (Autostainer) but used by laboratories on different platforms (e.g. Leica BOND III/Max or Dako Omnis).

Table 3. **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**	-	-	1/2 **	-	0/1	-	0/2	0/1
mAb clone **DAK-Calret 1**	3/10 (30%)	-	0/6	-	0/6	-	5/7 (71%)	0/1
rmAb clone **SP13**	-	-	-	-	0/4	-	-	-
pAb **18-0211**	1/2	1/1	-	-	0/6	-	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)

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 Dako Autostainer users

8 of the optimal results added a Linker…

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

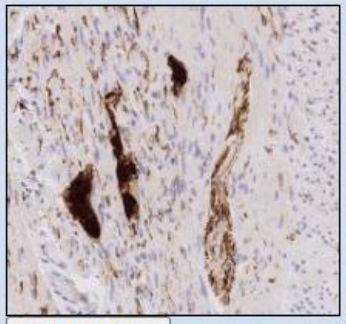
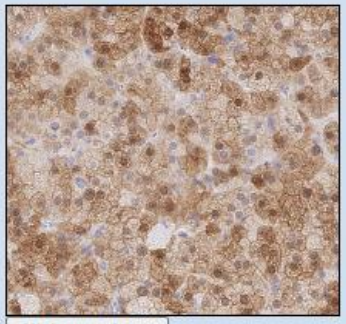
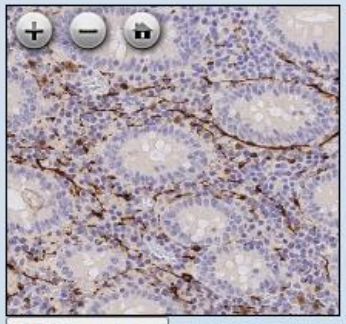
RTU systems	Recommendedprotocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Leica BOND mAb clone **CAL6 PA0346**	80% (4/5)	0% (0/5)	89% (8/9)	11% (1/9)
Dako AS mAb clone **DAK-Calret 1 IS/IR627**	89% (17/19)	26% (4/19)	100% (16/16)	56% (9/16)
VMS Ultra/XT rmAb clone **SP65 790-4467**	100% (19/19)	95% (18/19)	88% (87/99)	69% (68/99)

* 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.

Prolong incubation time of primary Ab may be the best option for the Leica RTU assay.

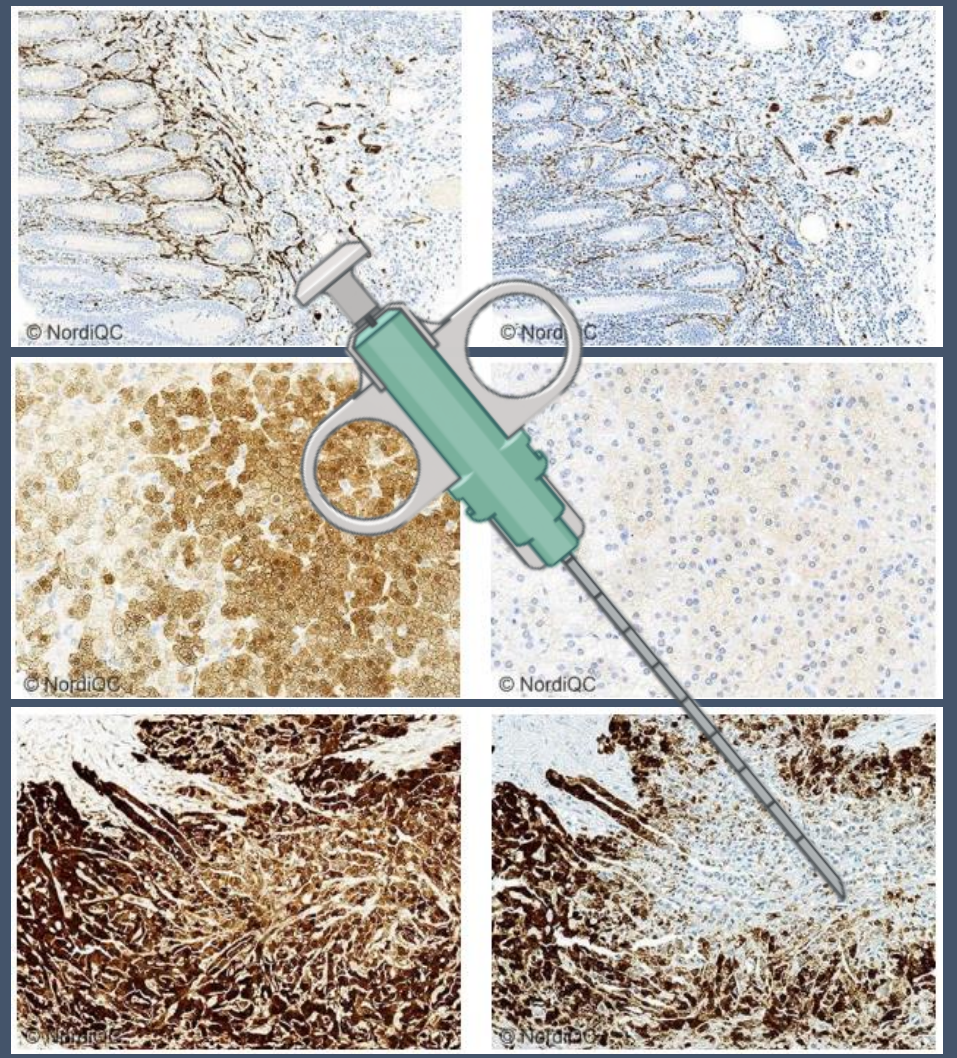
CALRETININ – ICAPS

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. *Note, nerves will show a moderate to strong staining reaction and cannot be used to evaluate the level of analytical sensitivity.*	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



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 **D8I7F**	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

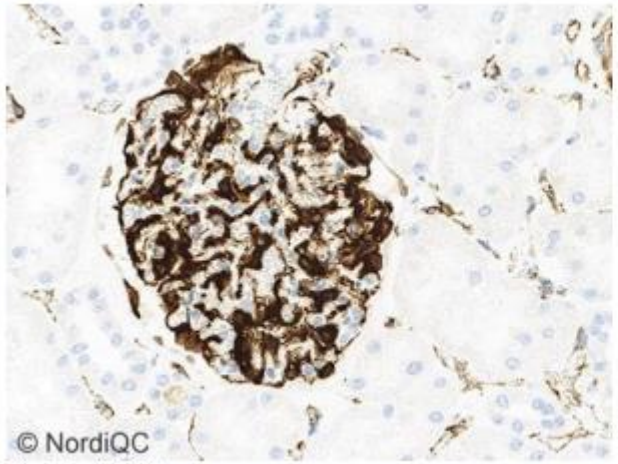


Fig. 7a (x300)

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

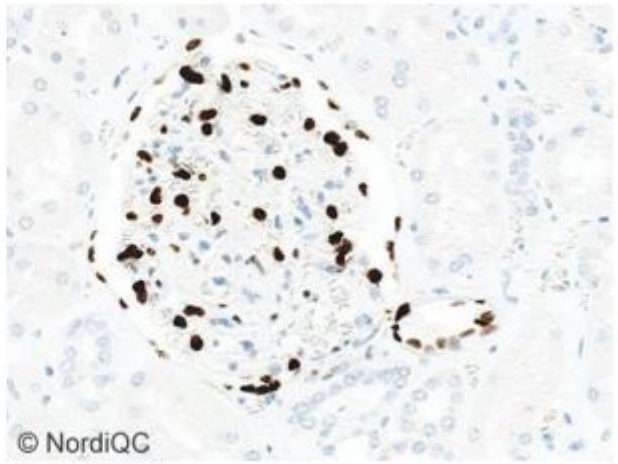


Fig. 7b (x300)

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

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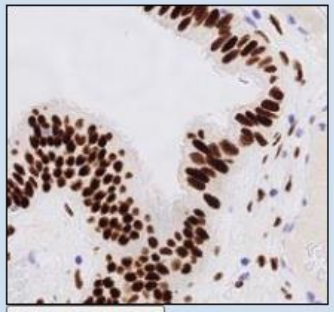
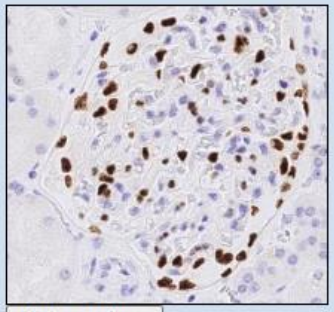
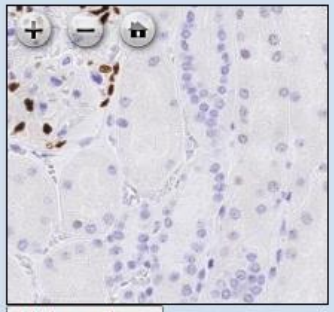
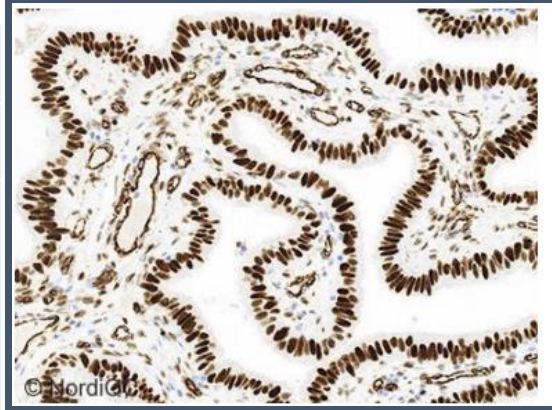
WT1 - ICAPS

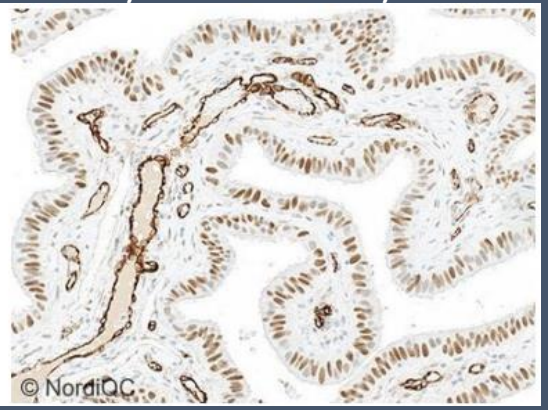
WT1 - Wilms tumour-1 protein

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Fallopian tube	Kidney	Kidney
Description	Virtually all epithelial and smooth muscle cells must show a strong, nuclear staining reaction. *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.*	Virtually all podocytes and parietal epithelial cells of Bowman's capsule must show an at least moderate nuclear staining reaction. *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.*	No staining reaction in the epithelial cells of the tubules should be seen. *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.*
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

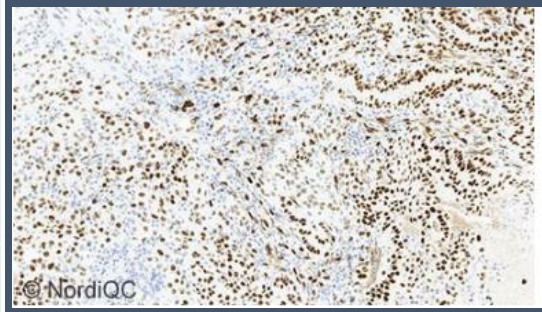
Optimal protocol settings

Inefficient HIER, 2-layer detection system

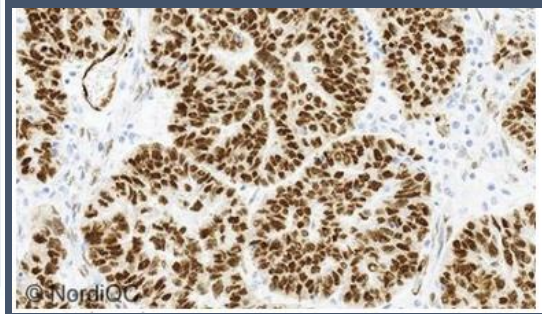


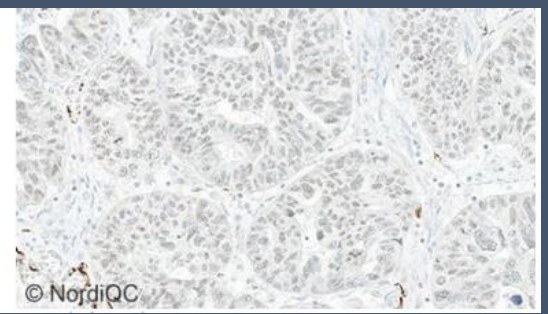


Fallopian tube



Mesothelioma





Serous ovarian carcinoma

EP-CAM – PITFALLS/POINTS OF ATTENTION

NordiQC

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 6 1	Dako Cell Marque Diagnostic Biosystems	14	13	21	28	36%	93%
mAb clone **MOC-31**	23 5 1	Dako Cell Marque Diagnostic Biosystems	10	10	7	2	69%	71%
mAb clone **VU-1D9**	5 3 1 1	Thermo Scientific Merck Millipore Immunologic Novus Biologicals	9	0	1	0	90%	100%
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/IS637**[3]	6	Dako	1	2	2	1	-	-
mAb clone **Ber-Ep4 GA637**	27	Dako	26	1	0	0	100%	100%
mAb clone **Ber-Ep4 GA637**[3]	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)

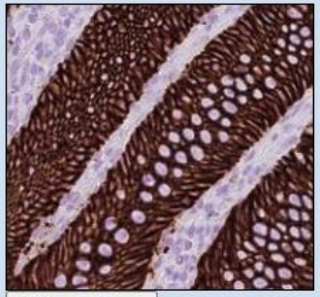
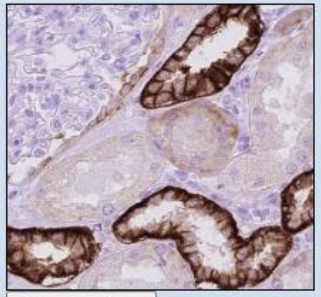
* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor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

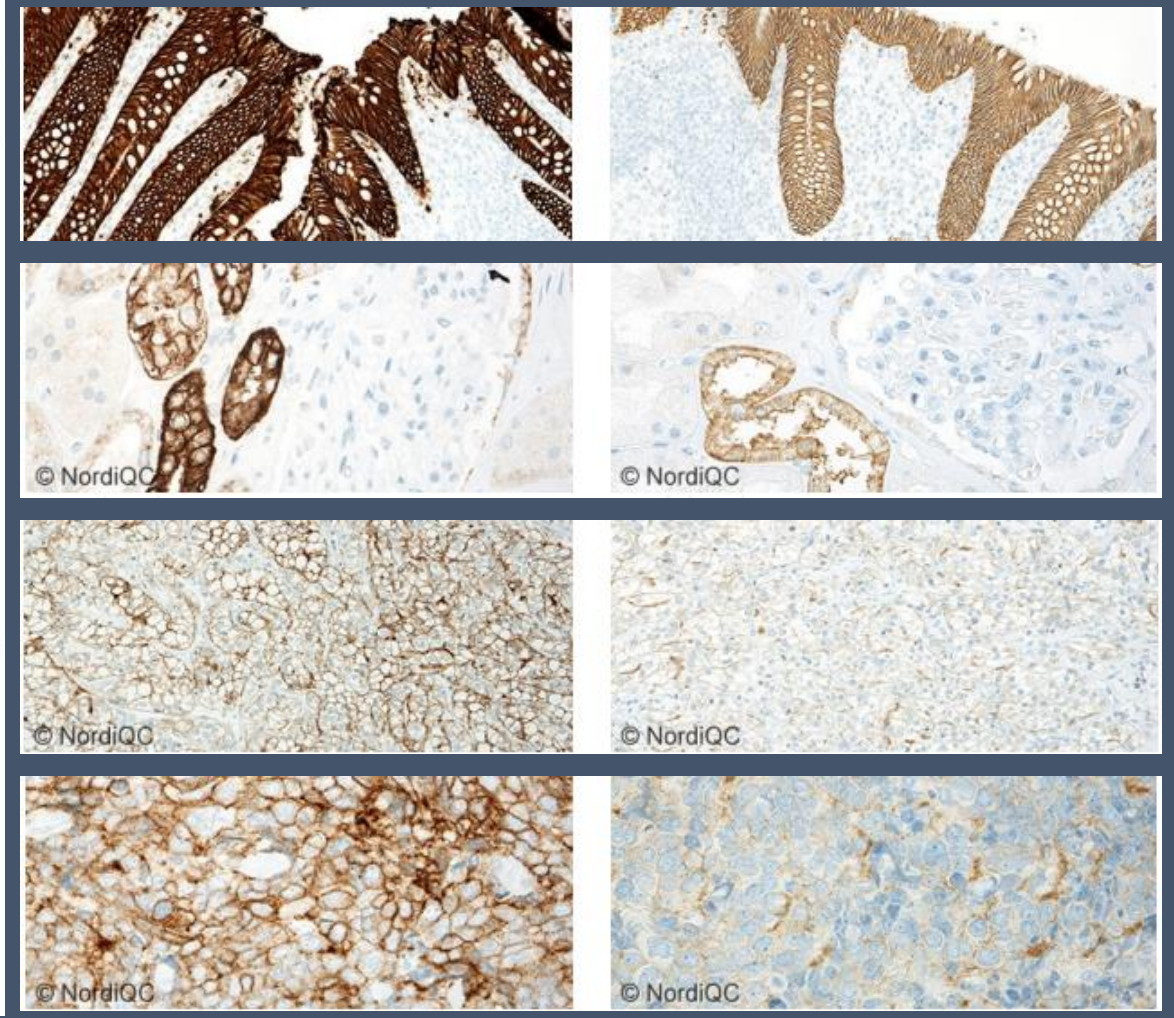
EP-CAM - ICAPS

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.	The majority of epithelial cells in the proximal tubules must show an at least weak to moderate, predominantly basolateral staining reaction. Most epithelial cells lining the Bowman capsule must show an at least weak to moderate membranous staining reaction. *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.*	No staining reaction should be seen in lymphocytes, endothelial cells and smooth muscle cells. *Note, dispersed reactive squamous epithelial can show a distinct membranous staining reaction – the vast majority of squamous epithelial cells are negative.* *Mast cells and plasma cells can show a positive cytoplasmic staining reaction.*
Example	 Click to enlarge	 Click to enlarge	 Work in progress

Optimal protocol settings

Too diluted Ab +
2-layer detection system



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

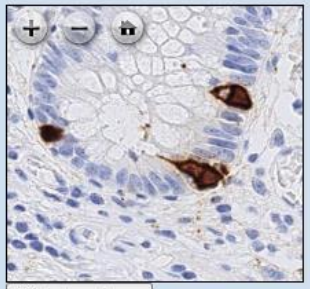
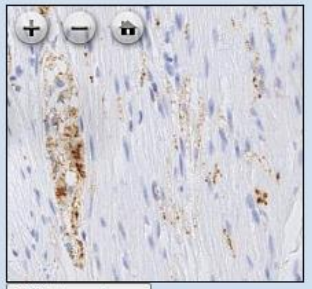
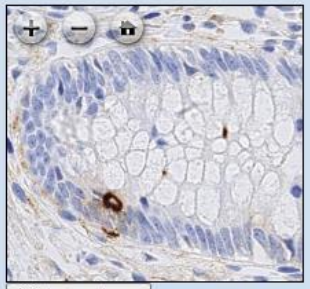
Table 3. **Proportion of optimal results for CGA for the most commonly used antibody concentrate on the four main IHC systems***

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	Virtually all neuroendodocrine cells in the epithelial mucosa must show a strong intense cytoplasmic staining reaction. *Note in the vicinity of the specific staining reaction a weak diffuse background reaction can be seen due to leakage of the antigen.*	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	 Click to enlarge	 Click to enlarge	 Click to enlarge

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 1 1 1	Leica/Novocastra Biocare Medical Monosan KliniPath	13	36	15	3	73%	83%
mAb clone **BS15**	1	Nordic Biosite	1	0	0	0	-	-
mAb clone **DAK-SYNAP**	21	Agilent/Dako	12	6	1	2	86%	88%
mAb clone **SNP88**	7	Biogenex	1	2	4	0	43%	-
mAb clone **SY38**[3]	2	Dako	0	0	1	1	-	-
rmAb clone **MRQ-40**	6	Cell Marque	1	4	1	0	83%	-
rmAb clone **SP11**	11 5 1 1	Thermo/Neomarkers Spring Bioscience Abcam Invitrogen	6	5	7	0	61%	64%
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 PA0299**	13	Leica/Novocastra	0	6	5	2	46%	-
mAb clone **27G12 PA0299**[4]	2	Leica/Novocastra	0	0	2	0	-	-
mAb clone **DAK-SYNAP IR660**	31	Agilent/Dako	16	15	0	0	100%	100%
mAb clone **DAK-SYNAP IR660**[4]	19	Agilent/Dako	8	11	0	0	-	-
mAb clone **DAK-SYNAP GA660**	5	Agilent/Dako	3	2	0	0	100%	100%
mAb clone **DAK-SYNAP GA660**[4]	4	Agilent/Dako	4	0	0	0	-	-
mAb clone **BS15 8453-C010**	1	Sakura FineTek	1	0	0	0	-	-
mAb clone **SNP88 AM363-10M**[4]	1	Biogenex	0	0	1	0	-	-
mAb clone **SY38 IR/IS776**[3]	1	Dako	0	1	0	0	-	-
rmAb **MRQ-40 760-4595**	43	Ventana/Cell Marque	6	22	13	2	65%	90%
rmAb clone **MRQ-40 336R**	12	Cell Marque	2	4	3	3	-	-
rmAb clone **SP11 790-4407**	48	Ventana	25	14	7	2	81%	96%
rmAb clone **SP11 KIT-0022**	1	Maixin	1	0	0	0	-	-
rmAb clone **SP11 RMPD018**	1	Diagnostic Biosystem	0	0	1	0	-	-
rmAb clone **EP158 MAD-000685QD**	2	Master Diagnostica	0	1	1	0	-	-
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)

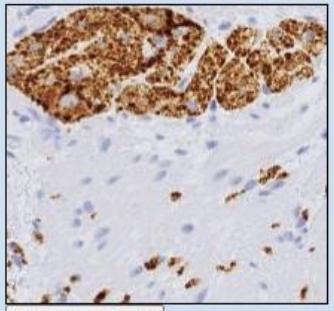
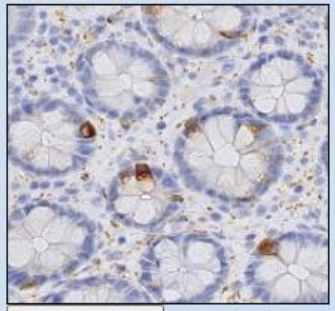
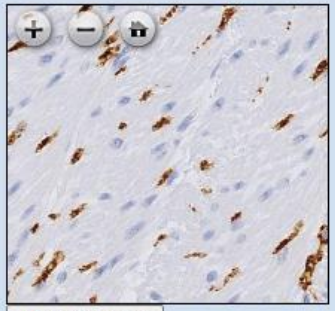
* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor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

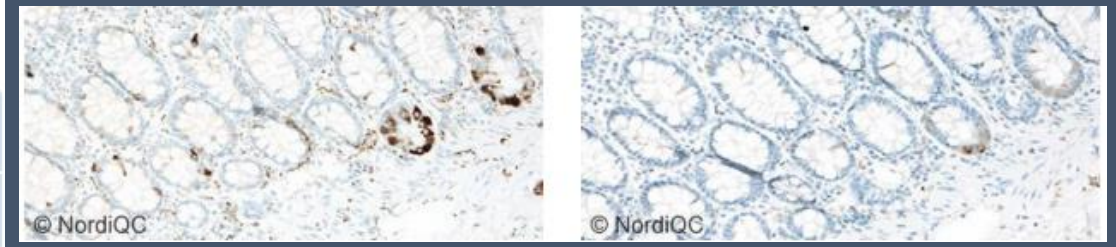
SYP – ICAPS

SYP - Synaptophysin

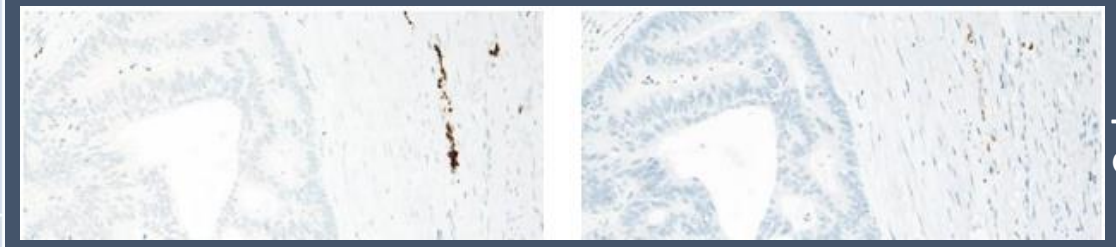
Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Appendix/colon	Appendix/colon	Appendix/colon
Description	Virtually all axons and ganglion cells in the nerve plexus (Auerbach's and Meissner's) must show a moderate 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

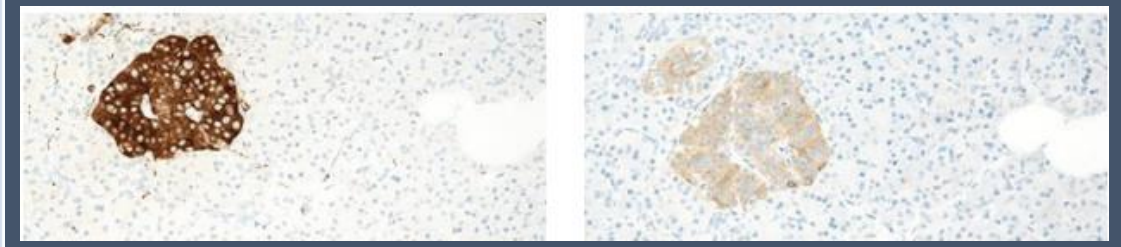
2-layer detection system



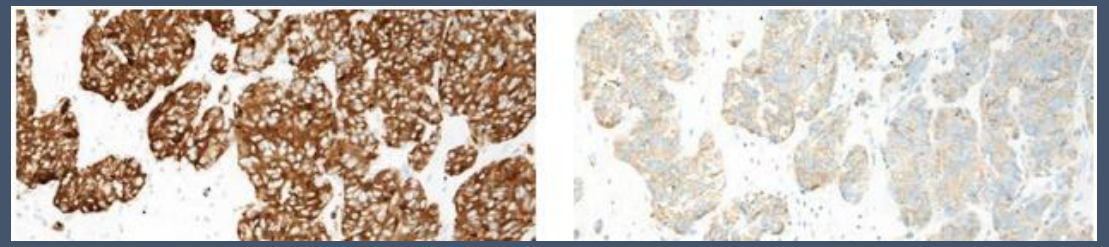
Appendix



Colon adenocarc.



Pancreas



SCLC

CD56 - PITFALLS/POINTS OF ATTENTION

Table 2. **Proportion of optimal results for CD56 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 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 **123C3**	0/2**	0/1	2/4	-	0/5 (0%)	-	-	1/1
rmAb clone **MRQ-42**	1/1	-	5/5 (100%)	1/1	24/28 (86%)	-	2/3	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).

rmAb MRQ-42 as conc. format obtained optimal results on the four main platforms.

All RTU products based on rmAb MRQ-42 from Ventana, Cell Marque and Sakura obtained optimal results and an overall pass rate of 100%.

Table 3. **Proportion of sufficient and optimal results for CD56 for the most commonly used RTU IHC systems**

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb 123C3 **IR/IS628**	47% (7/15)	0% (0/15)	44% (8/18)	0% (0/18)
VMS Ultra/XT/GX mAb 123C3 **790-4465**	0/2	0/2	20% (5/25)	0% (0/25)
Leica Bond III/MAX mAb CD564 **PA0191**	67% (4/6)	0% (0/6)	62% (8/13)	8% (1/13)
VMS Ultra/XT/GX rmAb MRQ-42 **760-4596**	100% (12/12)	67% (8/12)	100% (56/56)	73% (41/56)

* 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 integrated.

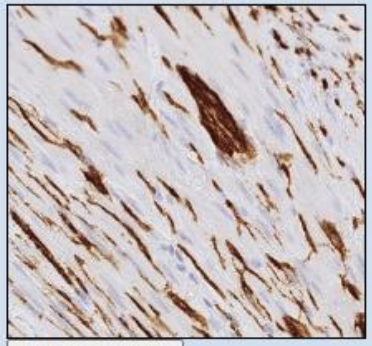
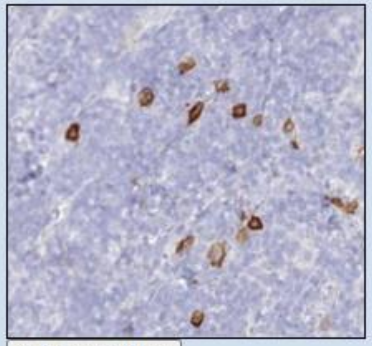
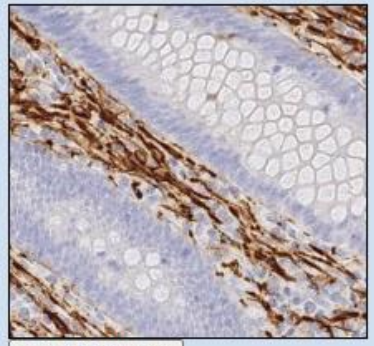
37 laboratories applied the IR/IS628 developed for Autostainer on the Dako Omnis platform (not shown in Table 3). None produced a sufficient result.

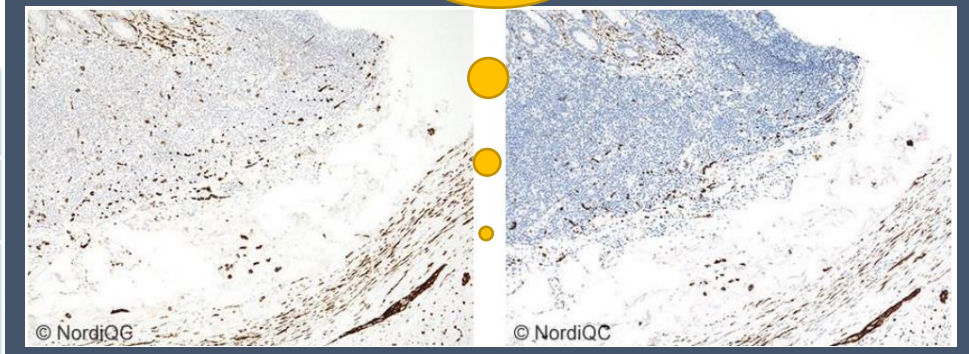
Dako Omnis users can use the conc. formats of e.g. rmAb MRQ-42 or mAb 123C3.

CD56 - ICAPS

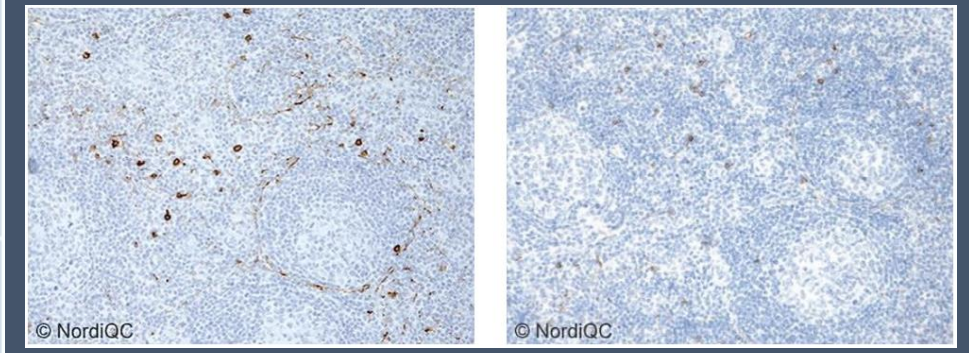
3-layer vs. 2-layer detection system

CD56 - CD56

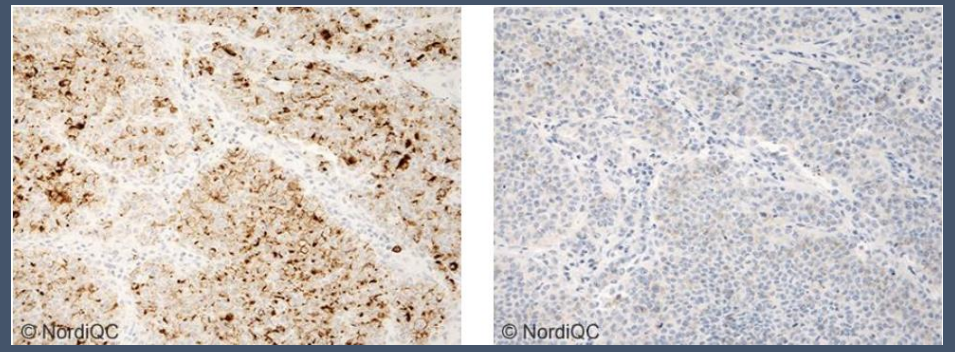
Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Appendix/colon	Tonsil	Appendix/colon
Description	Virtually all axons and ganglion cells in the nerve plexus (Auerbach's and Meissner's) must show a moderate to strong, distinct predominantly membranous staining reaction.	NK-cells and scattered T-cells (double hit CD4 and CD8 positive) must show an at least weak to moderate, distinct predominantly membranous staining reaction. Note, nerve fibres e.g. in the vicinity of germinal centres might be demonstrated.	No staining reaction in the columnar epithelial cells should be seen.
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge



Appendix



Tonsil



Neuroendocrine

CK5 - PITFALLS/POINTS OF ATTENTION

Table 1. **Antibodies and assessment marks for CK5, run 55**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **CK5/6.007**	1	Biocare	0	1	0	0	-	-
mAb clone **D5/16 B4**	45 7 1 1 1	Dako/Agilent Cell Marque Millipore Thermo Scientific Zytomed	4	10	31	10	25%	26%
mAb clone **XM26**	49 1 1 1 1	Leica/Novocastra Biocare Diagnostic BioSystems Histols Reagents Monosan	32	9	10	2	77%	81%
mAb clone **XM26/LL002**	1 1 1	Biocare Diagnostic BioSystems Zytomed	1	1	1	0	-	-
rmAb clone **BSR55**	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone **EP1601Y**	5 1	Cell Marque Biocare	0	1	5	0	-	-
rmAb clone **EP24**	1	Cell Marque	1	0	0	0	-	-
rmAb clone **SP27**	1	Immunologic	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **D5/16 B4 790-4554**	56	Ventana/Cell Marque	4	14	34	4	32%	82%
mAb **D5/16 B4 GA780**	21	Dako/Agilent	0	1	20	0	5%	-
mAb **D5/16 B4 GA780** [3]	1	Dako/Agilent	0	0	0	1	-	-
mAb clone **D5/16 B4 IR/IS780**	16	Dako/Agilent	0	0	12	4	0%	-
mAb clone **D5/16 B4 IR/IS780** [4]	9	Dako/Agilent	1	2	4	2	-	-
mAb clone **D5/16 B4 356M-10** [5]	2	Cell Marque	0	0	2	0	-	-
mAb clone **GM028 8294**	1	Sakura	0	0	1	0	-	-
mAb clone **XM26 PA0468**	7	Leica/Novocastra	4	2	1	0	-	-
mAb clone **XM26 PA0468** [6]	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **XM26 PM234**	1	Biocare	0	1	0	0	-	-
mAb clone **XM26/LL002 MSG106**	1	Zytomed	0	1	0	0	-	-
rmAb/mAb clone **EP1601Y/LL002 905H-8**	1	Cell Marque	0	0	1	0	-	-
rmAb clone **EP1601Y**	4	Cell Marque	0	3	1	0	-	-
305R-18								
rmAb clone **EP24 RMA-0846**	1	Maixin	1	0	0	0	-	-
rmAb clone **EP24/EP67 MAD-000651QD**	2	Master Diagnostica	0	1	1	0	-	-
rmAb clone **SP27 760-4935**	18	Ventana /Cell Marque	15	3	0	0	100%	100%
Total	263		65	51	124	23	-	
Proportion			25%	19%	47%	9%	44%	

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

Table 3. **Proportion of optimal results for CK5 for the most commonly used antibodies as concentrates on the four main IHC systems***

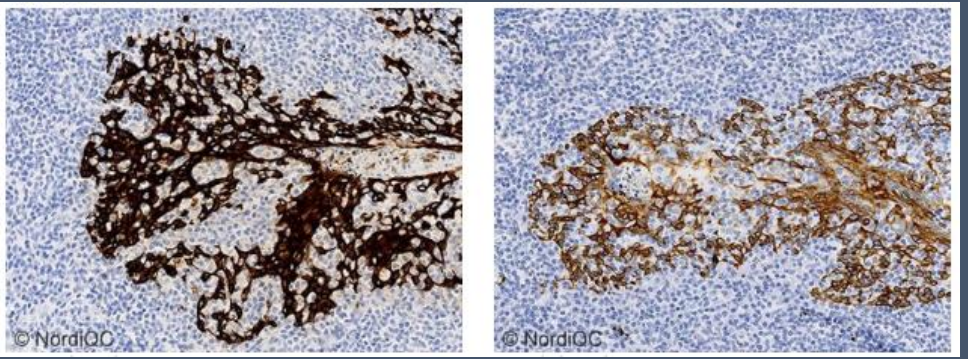
Concentrated antibodies	Dako Autostainer Link / Classic		Dako Omnis		Ventana BenchMark GX / XT / Ultra			Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC1 pH 8.5 + Protease 3	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **D5/16 B4**	0/3**	-	0/4	-	1/34 (3%)	0/1	-	3/9 (33%)	-
mAb clone **XM26**	5/6 (83%)	-	3/7 (43%)	-	9/20 (45%)	2/3	-	7/10 (70%)	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 sufficient and optimal results for CK5 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 **D5/16 B4, 790-4554**	0% (0/14)	0% (0/14)	43% (18/42)	10% (4/42)
Dako Omnis mAb clone **D5/16 B4, GA780**	6% (1/17)	0% (0/17)	0% (0/4)	0% (0/4)
Dako Autostainer mAb clone **D5/16 B4, IR/IS780**	0% (0/5)	0% (0/5)	0% (0/11)	0% (0/11)
Leica Bond mAb clone **XM26, PA0468**	86% (6/7)	57% (4/7)	(0/0)	(0/0)
Ventana Benchmark rmAb clone **SP27, 760-4935**	100% (7/7)	100% (7/7)	100% (11/11)	73% (8/11)

* 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.



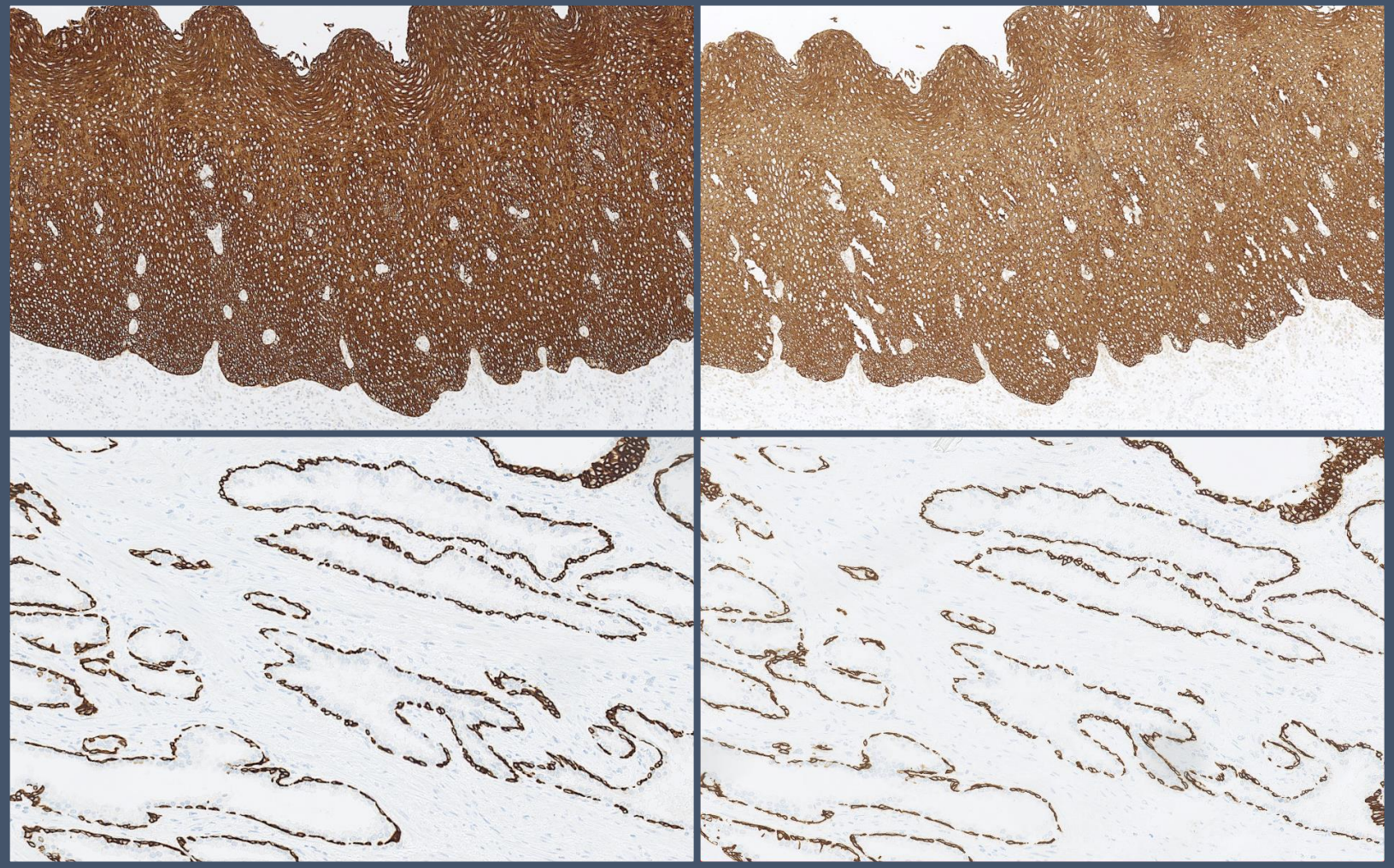
Less successful performance of the mAb D5/16 B4 both as RTU and Conc.

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…

Left: XM26 // Right: D5/16 B4

CK5 - PITFALLS/POINTS OF ATTENTION



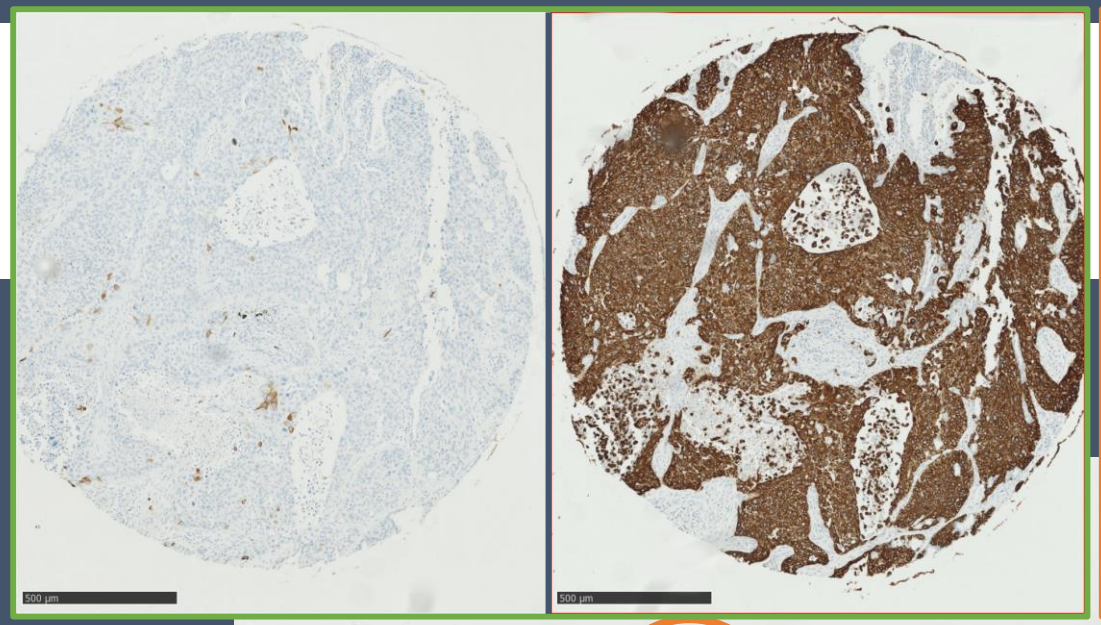
mAb XM26

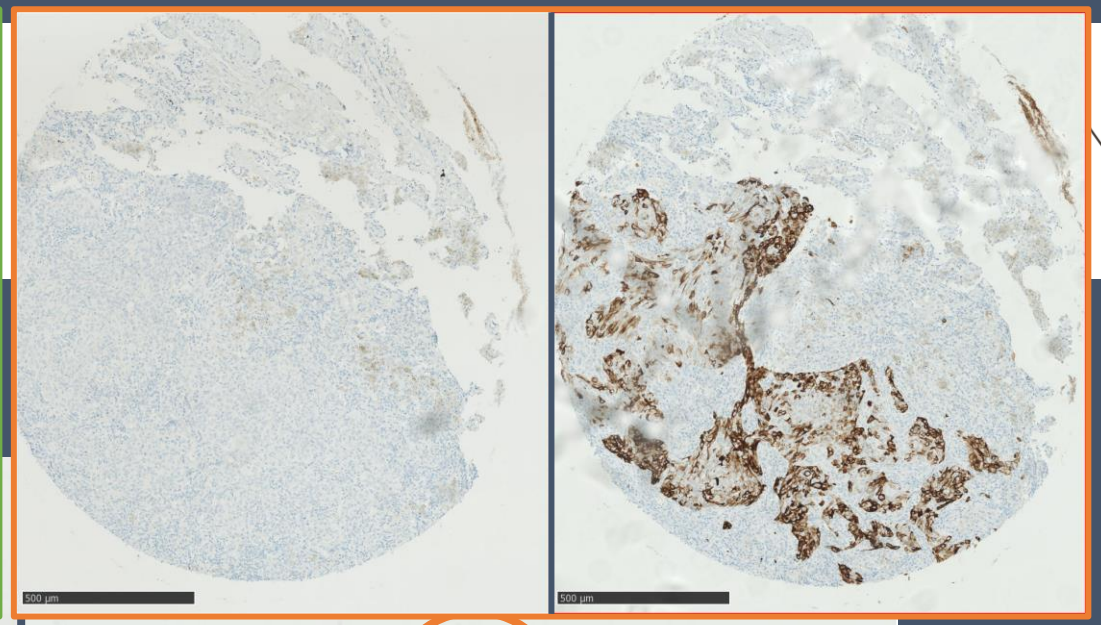
rmAb SP27

Esophagus

Controls are OK

Prostate





Lung adenocarc.

Internal NordiQC data[1]	p40 (BC28)	CK5 (XM26)	CK5 (SP27)
Lung adenocarcinoma	0/62 (0%)	0/62 (0%)	14/62 (23%)

1) Thomsen C, Nielsen O, Nielsen S, Røge R, Vyberg M. NordiQC Assessments of Keratin 5 Immunoassays. Appl Immunohistochem Mol Morphol. 2020 Aug;28(7):566-570. doi: 10.1097/PAI.0000000000000855. PMID: 32243261.

uamous cell carc.

rge cell carc.

mAb XM26

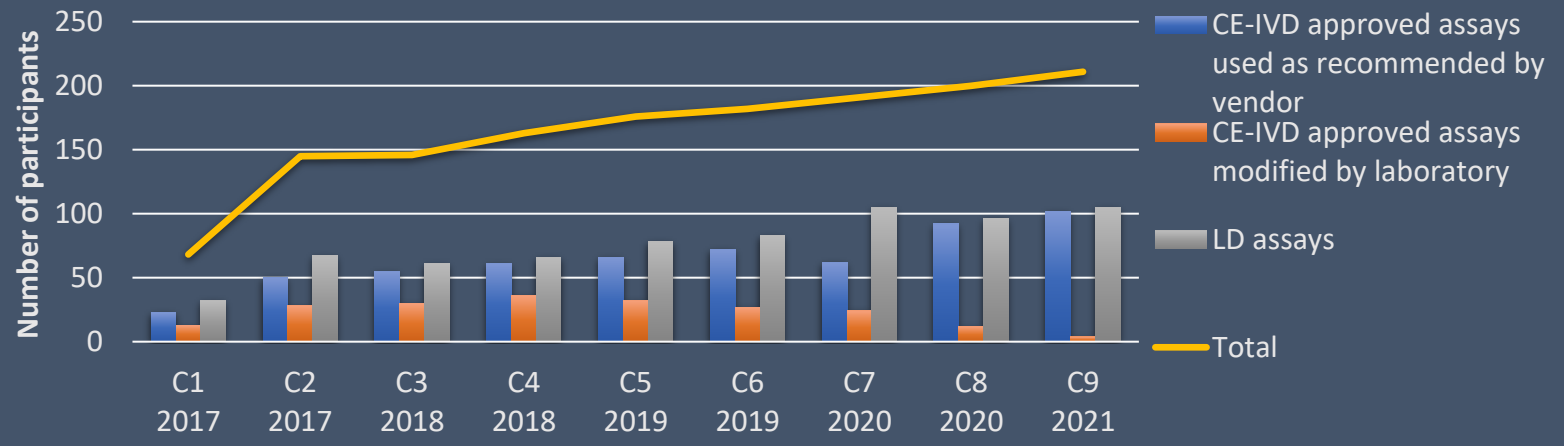
rmAb SP27

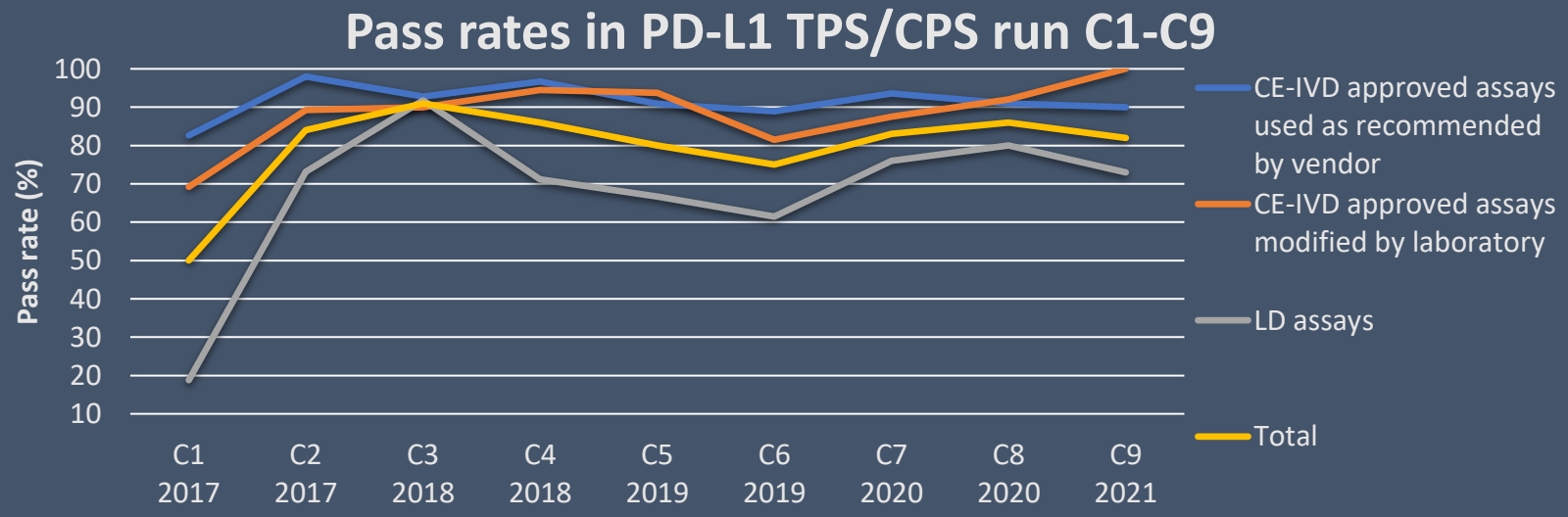
PD-L1 – PITTFALLS/POINTS OF ATTENTION

Table 2. **Assessment marks for IHC assays and antibodies run C9, PD-L1 TPS/CPS (KEYTRUDA®)**

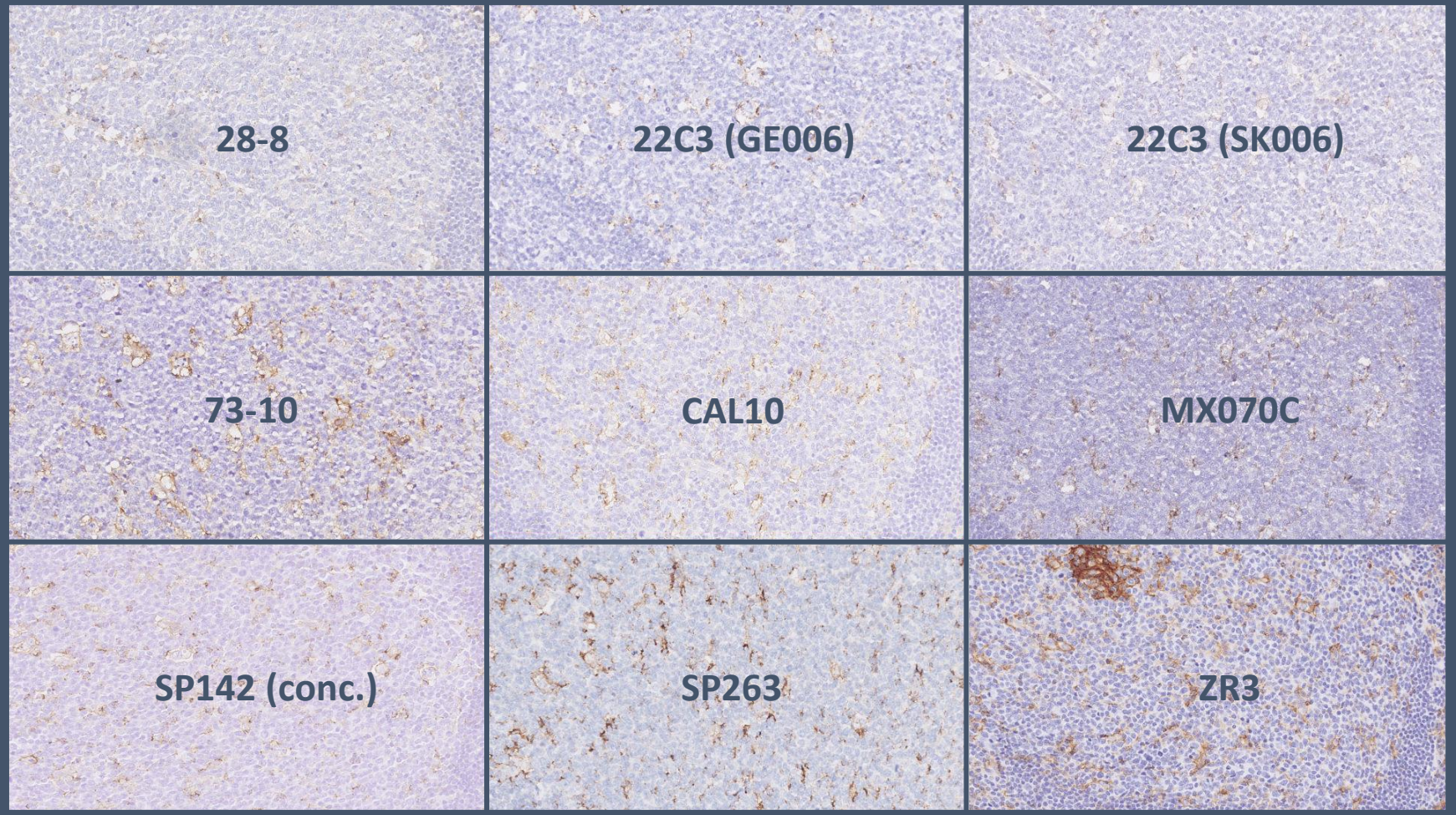
CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone SP263, **741-4905 (VRPS)**[3]	42	Ventana/Roche	29	9	4	-	91%	69%
rmAb clone SP263, **741-4905 (LPMS)**[4]	2	Ventana/Roche	-	-	1	1	-	-
rmAb clone SP263, **740-4907 (VRPS)**[3]	13	Ventana/Roche	8	4	1	-	92%	62%
rmAb clone SP142, **740-4859 (VRPS)**[3]	1	Ventana/Roche	-	-	-	1	-	-
mAb clone 22C3 pharmDX, **SK006 (VRPS)**[3]	23	Dako/Agilent	5	14	4	-	83%	22%
mAb clone 22C3 pharmDX, **SK006 (LMPS)**[4]	9	Dako/Agilent	2	3	2	2	56%	22%
mAb clone 22C3 pharmDX, **GE006 (VRPS)**[3]	21	Dako/Agilent	17	4	-	-	100%	81%
mAb clone 22C3 pharmDX, **GE006 (LMPS)**[4]	7	Dako/Agilent	2	3	2	-	71%	29%
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**	38	Dako/Agilent	10	21	5	2	82%	26%
mAb clone **E1L3N**	4	Cell Signaling	-	1	3	-	-	-
rmAb clone **28-8**	1	Abcam	-	1	-	-	-	-
rmAb clone **BSR90**	1	Nordic Biosite	-	1	-	-	-	-
rmAb **CAL10**	3 1	Biocare Zytomed Systems	3	1	-	-	-	-
rmAb clone **QR1**	1	Biocyc	-	-	1	-	-	-
rmAb clone **SP142**	1	Abcam	-	1	-	-	-	-
rmAb clone **ZR3**	1 1	Zeta Corporation Zytomed systems	-	-	2	-	-	-
Ready-To-Use antibodies[6]	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone SP263, **790-4905[6] (VRPS)**[3]	13	Ventana/Roche	8	3	1	1	85%	62%
rmAb clone SP263, **790-4905[6] (LMPS)**[4]	20	Ventana/Roche	12	6	1	1	90%	60%
mAb **405-9A11 PDM572**	1	Diagnostic Biosystems	-	-	1	-	-	-
mAb **IHC441 IHC441-7**	1	GenomeMe	-	-	1	-	-	-
rmAb clone 73-10, **PA0832 (VRPS)**[3]	1	Leica Biosystems	-	1	-	-	-	-
rmAb clone MX070C, **MAB-0854**	2	Maixin	2	-	-	-	-	-
rmAb clone **ZR3 GT228002**	1	Gene Tech	-	-	1	-	-	-
Total	211		100	73	30	8		
Proportion			47%	35%	14%	4%	82%	

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.





PD-L1 - ICAPS - TONSIL



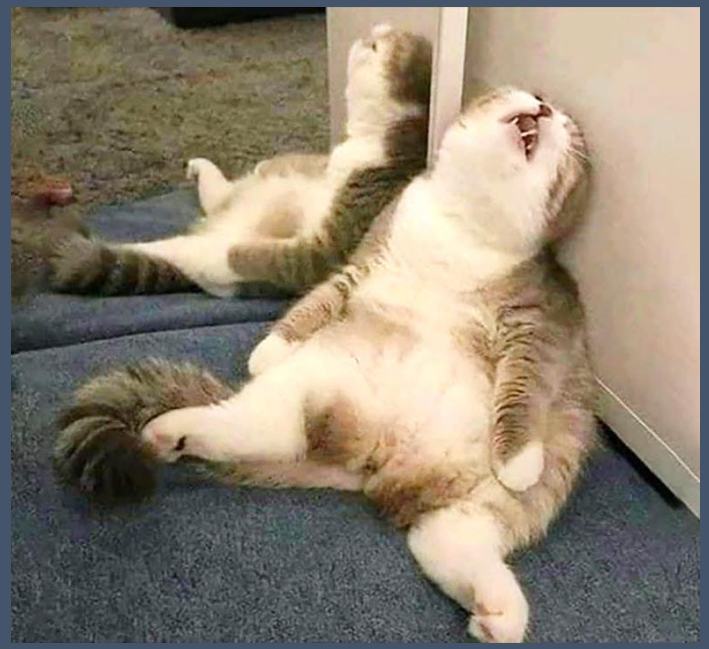
In tonsil, a weak to moderate staining reaction in germinal center macrophages should be seen.

Different assays → different staining patterns.

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

THANK YOU FOR YOUR ATTENTION!

NordiQC



BONUS – ROS1

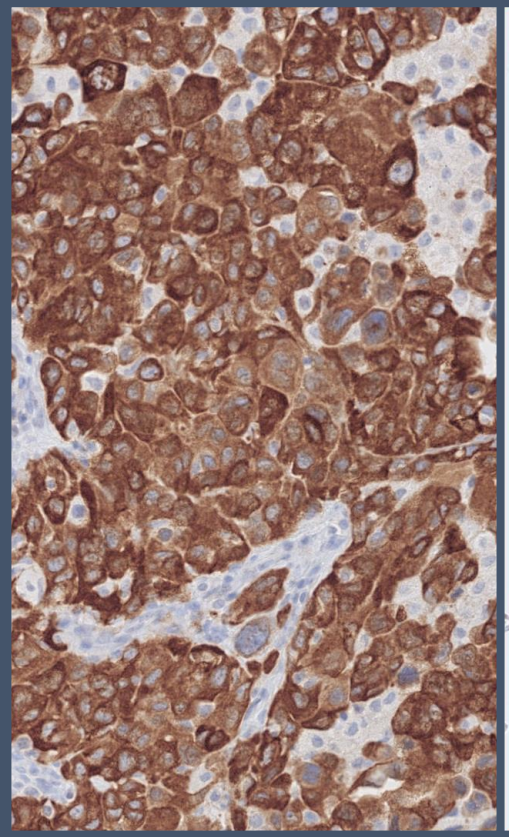
No NordiQC data available for ROS1.

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

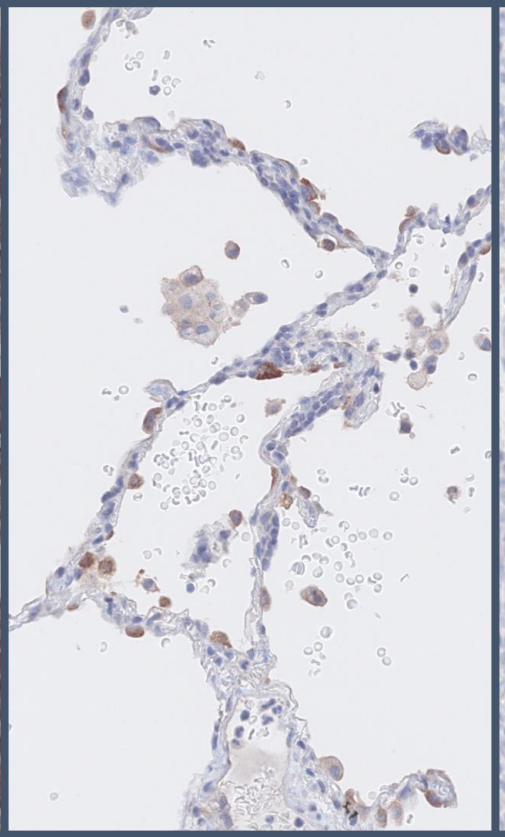
Positive controls:
Tumor with known ROS1-translocation
Type II-pneumocytes in normal lung

Negative control:
Appendix

Tumor with ROS1-translocation (lung adenocarc.)



Normal lung



Appendix