NORDIQC DATA FOR LUNG MARKERS

Antibody selection, protocols and controls

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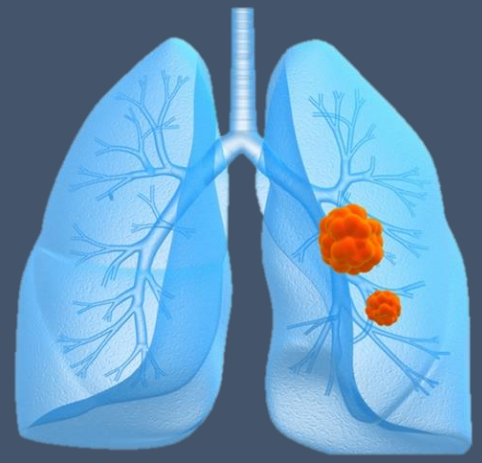
NORDIQC EQA DATA FOR IHC LUNG MARKERS

Marker	Purpose	Last run	Pass rate	No of labs
TTF1	Lung vs non-lung Adenocarcinoma vs squam.	Run 68, 2023	81%	401
Napsin A	Lung vs non-lung	Run 66, 2022	83%	321
Calretinin	Lung vs mesothelioma	Run 64, 2022	76%	350
WT1	Lung vs mesothelioma	Run 55, 2019	91%	291
BAP1	Reactive mesothelioma vs malignant mesothelioma	Run 65, 2022	69%	163
EpCAM	Lung vs mesothelioma	Run 56, 2019	57%	256
CGA	NSCLC vs SCLC	Run 67, 2023	64%	367
SYP	NSCLC vs SCLC	Run 52, 2018	75%	308
CD56	NSCLC vs SCLC	Run 64, 2022	72%	364
p40	Adenocarcinoma vs squam.	Run 67, 2023	85%	344
CK5	Adenocarcinoma vs squam.	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 C13, 2023	81%	225

Scheduled for assessment within the next year



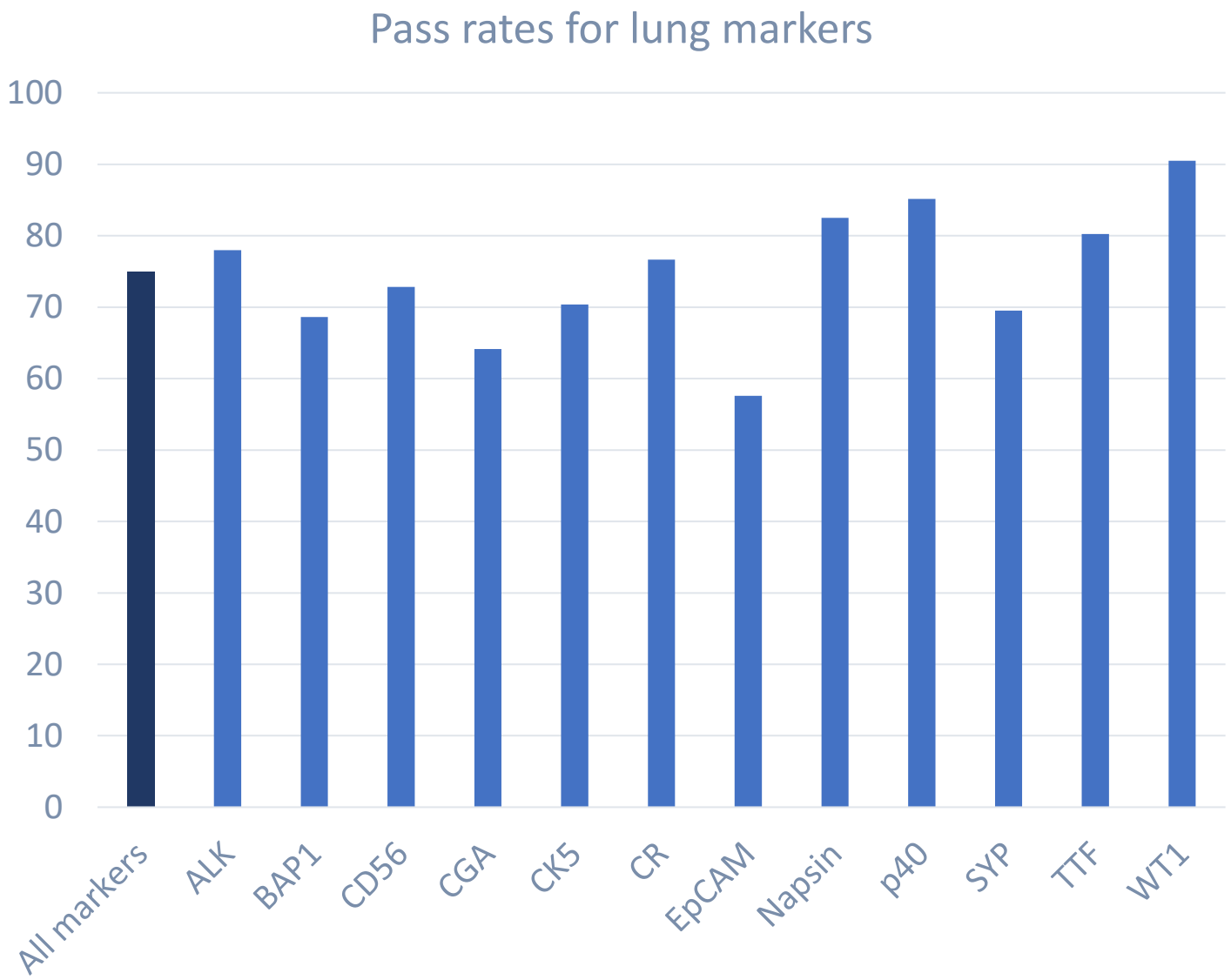
CLONE PERFORMANCE FOR SELECTED LUNG MARKERS

Marker	Successful clones	Less successful clones
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, rmAbs DAK-p40 & ZR8	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	*(rmAb SP142)*

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



KEY-POINTS FOR BEST PROTOCOLS

Lung markers in the General Module:

Overall pass rate: **75%** (2.966/3.954), ranging from 58% for EpCam till 91% for WT1.

KEY-POINTS FOR BEST PROTOCOLS

NordiQC

Lung markers in the General Module:

Overall pass rate: **75%** (2.966/3.954)

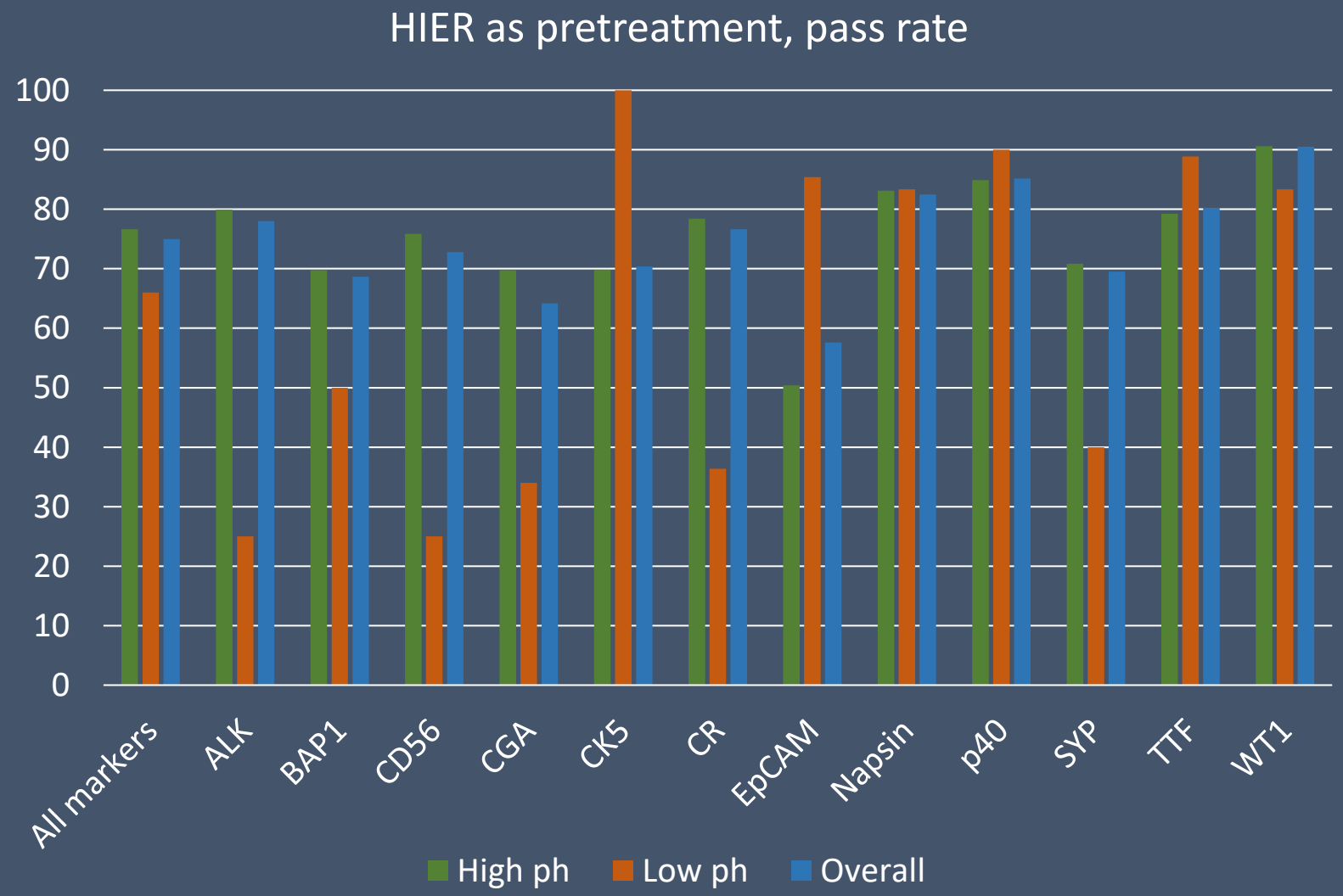
HIER in High pH: **77%** (2.740/3.575)

Ranging from 50% for EpCAM till 91% for WT1

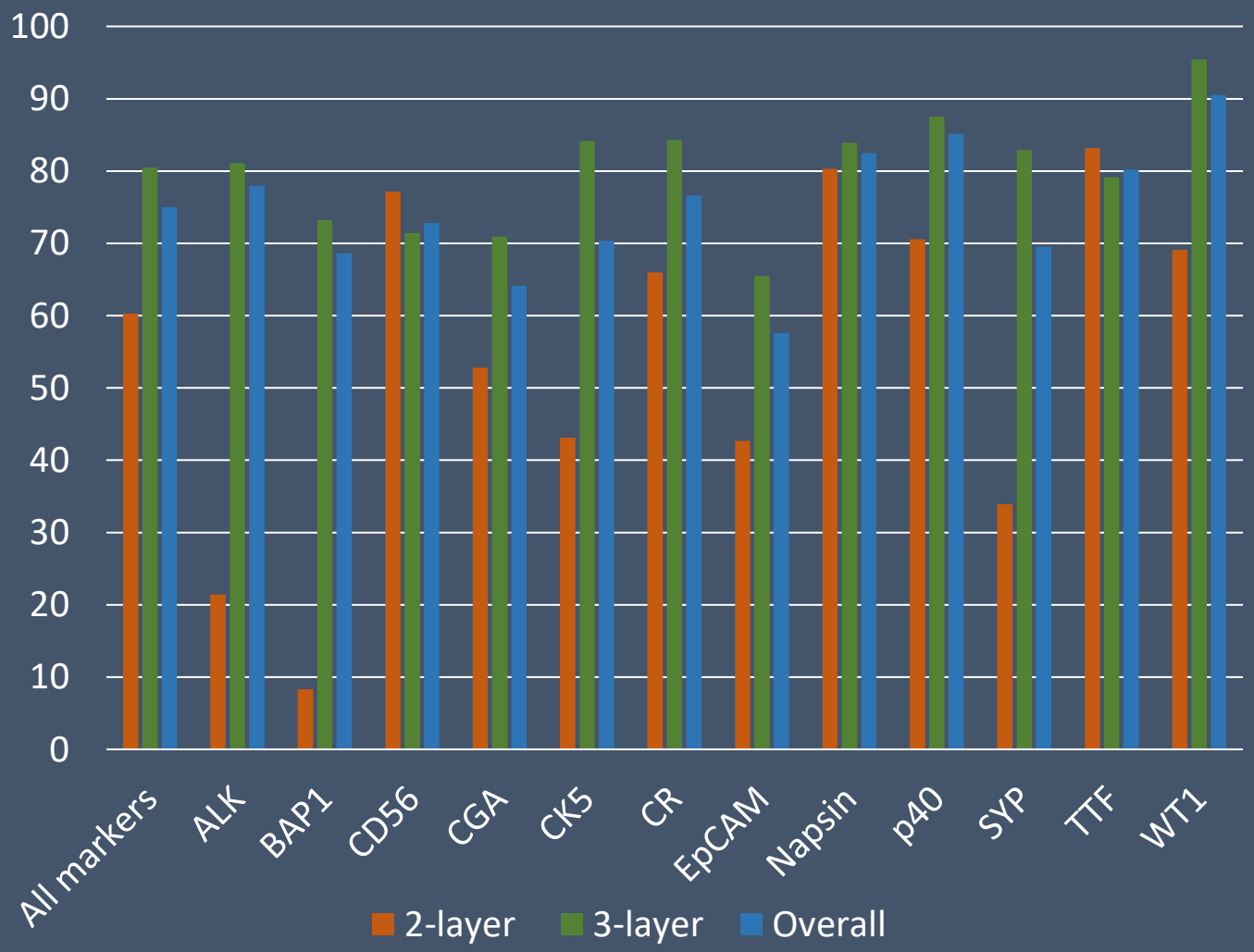
HIER in Low pH: 66% (194/294)

*Ranging from 25% for ALK and CD56 till 100% for CK5**

**9/9 participants used a low pH buffer on a Leica platform.*



KEY-POINTS FOR BEST PROTOCOLS



Lung markers in the General Module:

3-layer detection system: 80% (2.321/2.884)

- OptiView, UltraView DAB + amplification
  - OptiView: 84%
  - UltraView DAB + amp: 77%
- EnVision Flex +, Flex++ (4-layer)
- Bond Refine

2-layer detection system: 60% (645/1.070)

KEY-POINTS FOR BEST PROTOCOLS

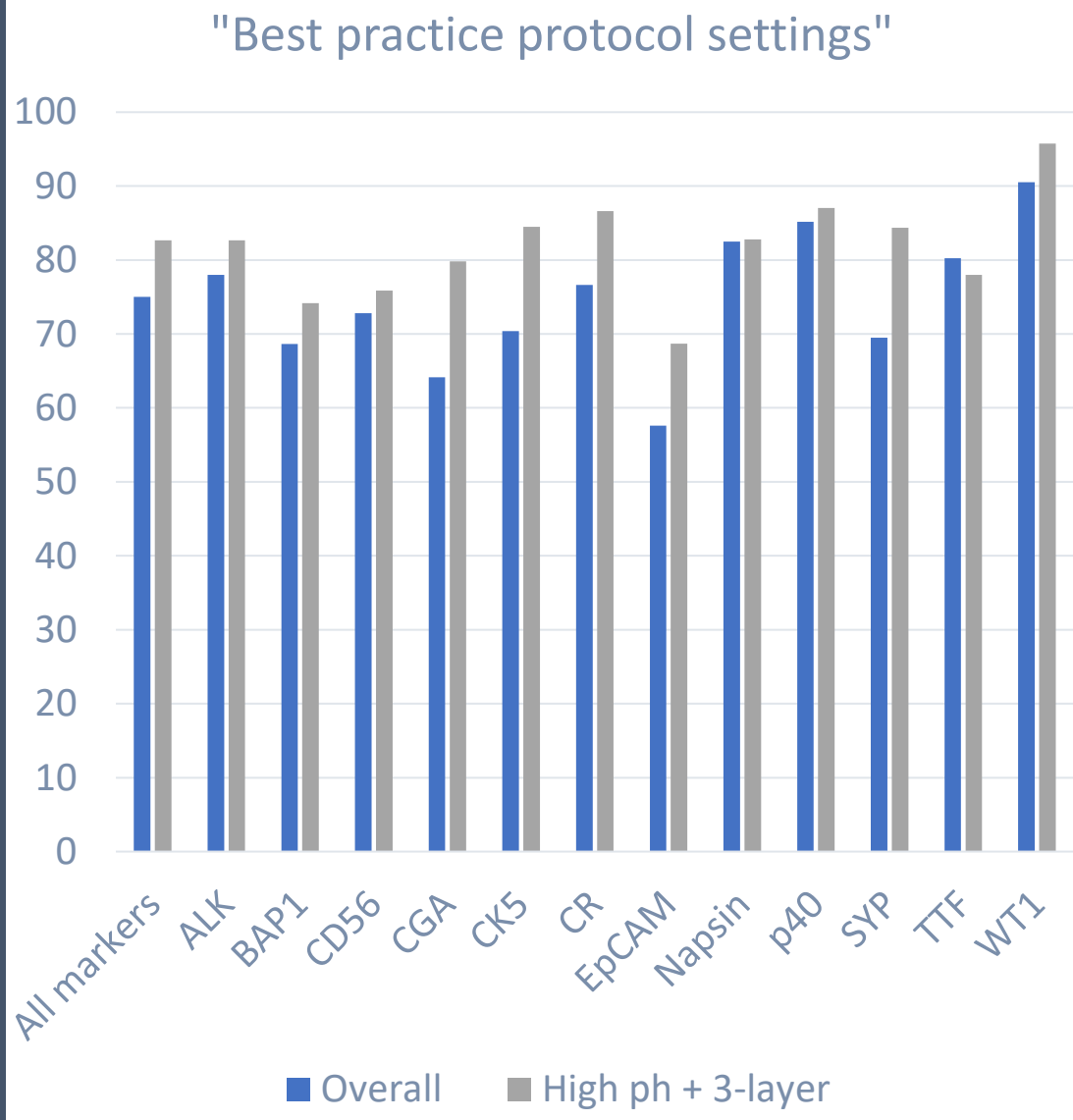
Lung markers in the General Module:

Overall pass rate: **75%** (2.966/3.954)

“Best practice protocol”*: HIER in a high pH buffer AND a 3-layer detection system: **83%** (2.351/2.605)

Ranging from 69% for EpCAM till 96% for WT1

*Clone selection is NOT included.



KEY-POINTS FOR BEST PROTOCOLS

Lung markers in the General Module:

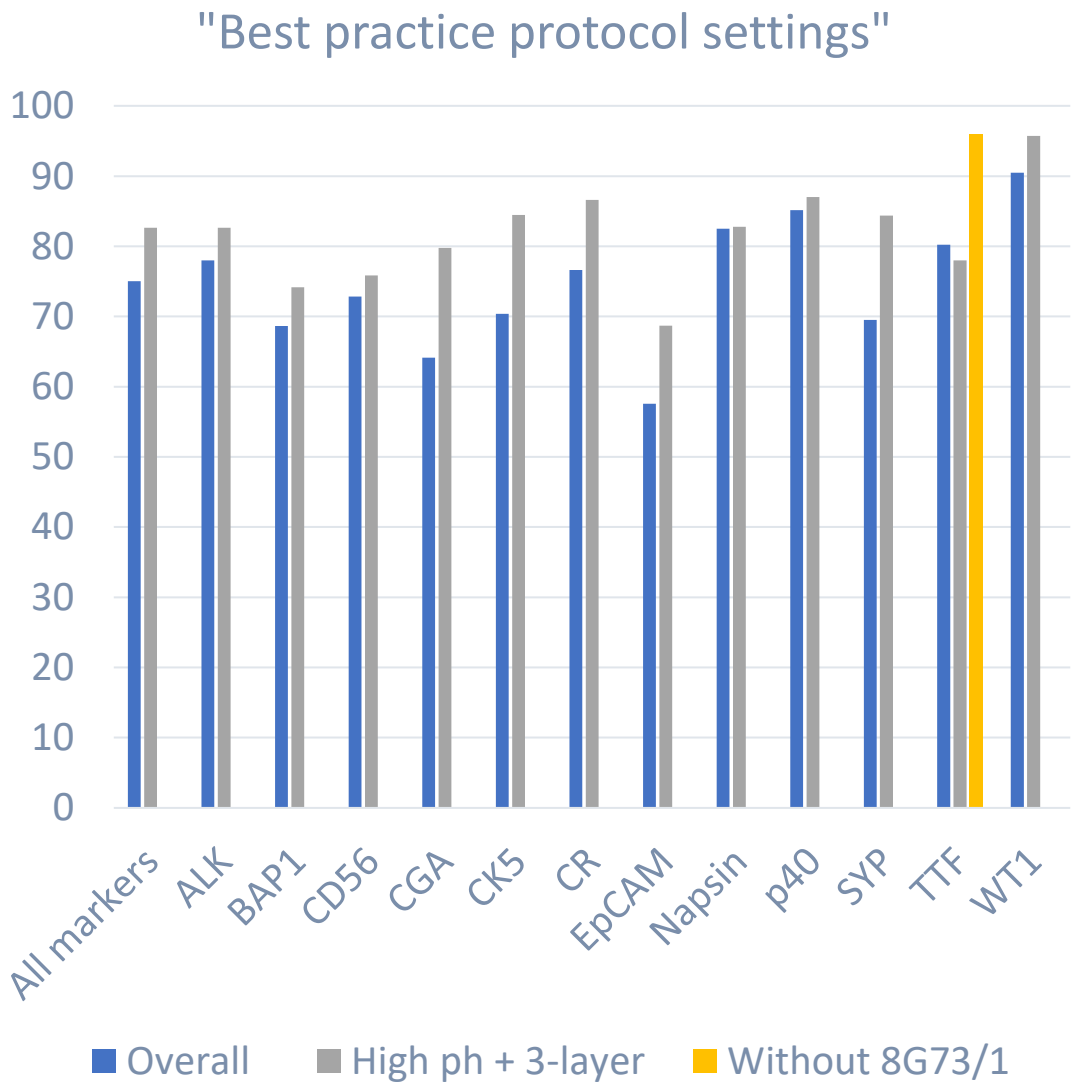
Overall pass rate: **75%** (2.966/3.954)

“Best practice protocol”*: HIER in a high pH buffer AND a 3-layer detection system: **83%** (2.351/2.605)

Ranging from 69% for EpCAM till 96% for WT1

*Clone selection is NOT included.

- E.g. TTF; if not counting participant using the less successful clone 8G7G3/1, the “best practice protocol” pass rate would be **96%** compared to 78% if including the clone.



NOW TIME TO LOOK AT SOME SPECIFIC MARKERS

CALRETININ – PITFALLS

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 1	Dako/Agilent Thermo Scientific	6	12	6	2	69%	23%
rmAb clone **BSR235**	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone **SP13**	1 1 1 1 1 1	Cell Marque Zytomed Systems Abcam Epredia Diagnostic Biosystems Zeta Corporation	0	2	2	2	33%	-
pAb **18-0211**	6 1	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 PA0346**[3]	8	Leica Biosystems	4	4	0	0	100%	50%
mAb clone **CAL6 PA0346**[4]	10	Leica Biosystems	3	3	3	1	60%	30%
mAb clone **DAK-Calret 1 IS/IR627**[3]	16	Dako/Agilent	3	5	7	1	50%	19%
mAb clone **DAK-Calret 1 IS/IR627**[4]	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 790-4467**[3]	2	Ventana/Roche	2	0	0	0	-	-
rmAb clone **SP65 790-4467**[4]	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%	

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

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

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.

UltraView:
88% pass rate (65% optimal)

OptiView:
100% pass rate (78% optimal)

WT1 – PITFALLS

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

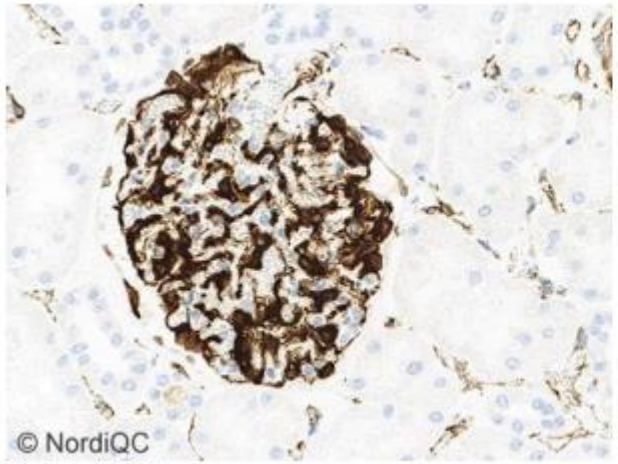


Fig. 7a (x300)

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

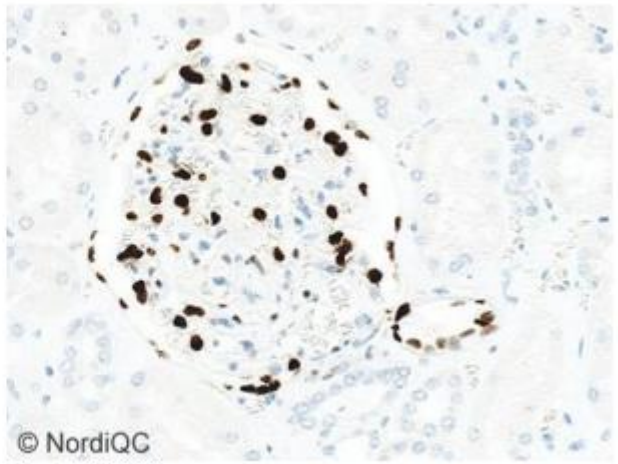


Fig. 7b (x300)

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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EP-CAM – PITFALLS

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.

CK5 – PITFALLS

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 2 1 1 2	Dako/Agilent Cell Marque Millipore Epredia Zytomed	6	15	19	1	51%	15%
mAb clone **XM26**	2 3 64 3	Abcam Diagnostic BioSystems Leica Biosystems Monosan	56	11	4	1	93%	78%
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* 790-4554**[3]	6	Ventana/Roche	0	3	3	0	50%	0%
mAb clone **D5/16 B4* 790-4554**[4]	46	Ventana/Roche	9	23	11	3	70%	20%
mAb **D5/16 B4* GA780**[3]	13	Dako/Agilent	0	1	12	0	8%	0%
mAb **D5/16 B4* GA780**[4]	26	Dako/Agilent	0	9	16	1	35%	0%
mAb clone **D5/16 B4* IR/IS780**[3]	4	Dako/Agilent	0	1	2	1	-	-
mAb clone **D5/16 B4* IR/IS780**[4]	9	Dako/Agilent	1	1	4	3	22%	11%
mAb clone **D5/16 B4* 8295-C010**	1	Sakura Finetek	1	0	0	0	-	-
rmAb clone **RM226 8408-C010**	1	Sakura Finetek	0	1	0	0	-	-
mAb clone **XM26 PA0468**[3]	7	Leica Biosystems	2	4	1	0	86%	29%
mAb clone **XM26 PA0468**[4]	9	Leica Biosystems	8	1	0	0	100%	89%
mAb clone **XM26 PM234**	3	Biocare Medical	2	1	0	0	-	-
rmAb clone **EP1601Y 305R-17/18**	4	Cell Marque	0	2	2	0	-	-
rmAb clone **EP42 AN853-10M**	1	BioGenex	0	1	0	0	-	-
rmAb clone **EP24/EP67* MAD-000651QD**	1	Master Diagnostica	1	0	0	0	-	-
rmAb clone **EP24/EP67* MRH1159**	1	PathnSitu	0	1	0	0	-	-
rmAb clone **SP27 760-4935**[3]	21	Ventana/Roche	21	0	0	0	100%	100%
rmAb clone **SP27 760-4935**[4]	29	Ventana/Roche	26	3	0	0	100%	90%
rmAb clone **C9E33 CCR-0973**	1	Celnovte	0	0	1	0	-	-
mAb clone **150A8C1 PA018**	1	Abcarta	0	0	1	0	-	-
Total	311		136	84	81	10		
Proportion			44%	27%	26%	3%	71%	

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

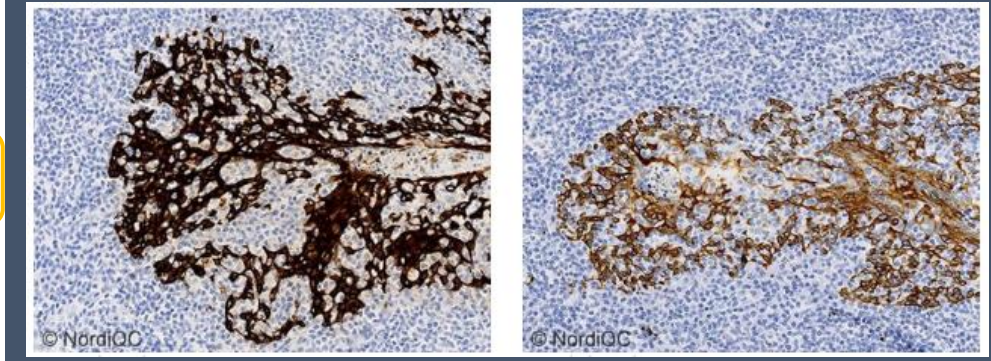
Concentrated antibodies	Dako Autostainer Link / Classic		Dako Omnis		Ventana BenchMark GX / XT / Ultra			Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC1 pH 8.5 + Protease 3	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **D5/16 B4**	0/2	-	0/2	-	5/12 (42%)	1/1	-	0/5 (0%)	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 Røge, MD,*‡ and Mogens Vyberg, MD*‡*

Left: XM26 // Right: D5/16 B4

Table 1. **Antibodies and assessment marks for ALK (lung), run 65**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **5A4**	26 2 1 1 2 2 1	Leica Biosystems Monosan Abcam DBS Biocare Medical Zytomed Systems Invitrogen	8	9	14	4	49%	23%
mAb clone **OTI1A4***	19 1 1 1	Origene Nordic Biosite Cell Signaling Zeta Corporation	16	6	0	0	100%	73%
mAb clone **IHC509**	1	GenomeMe	0	0	1	0	-	-
rmAb clone **D5F3**	19	Cell Signaling	7	9	3	0	84%	36%
rmAb clone **ALK1**	3 1	Dako/Agilent Cell Marque	0	0	0	4	-	-
rmAb clone **QR017**	1	Quartett	0	1	0	0	-	-
rmAb clone **SP8**	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

NordiQC

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)

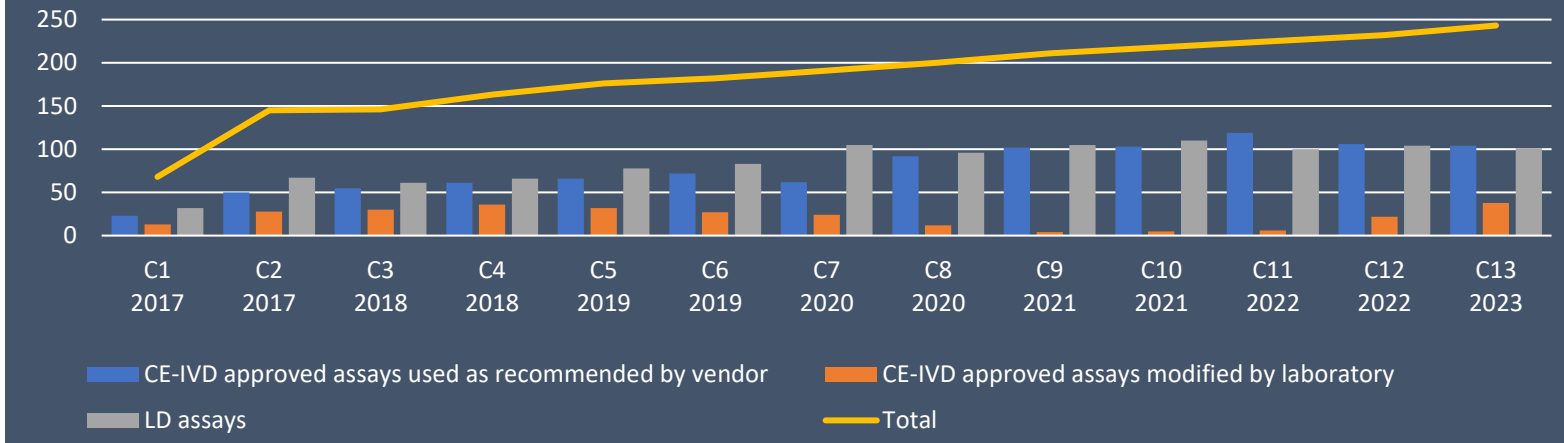
* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor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

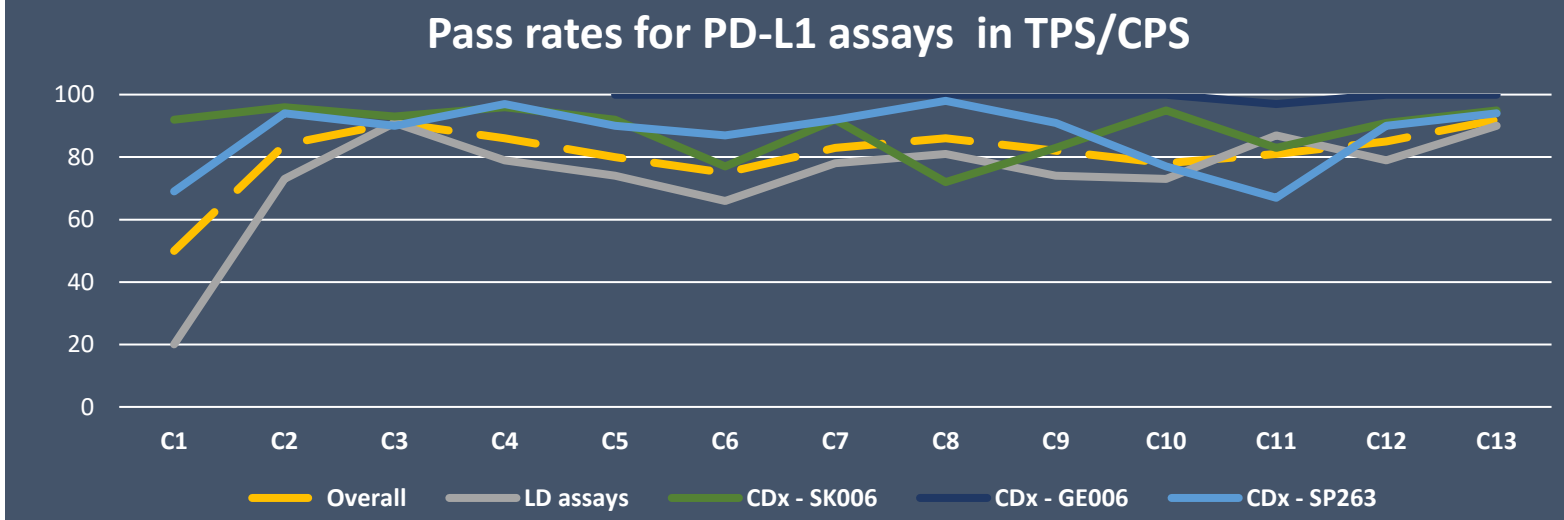
RTU products for the automated systems, working as plug-and-play

PD-L1

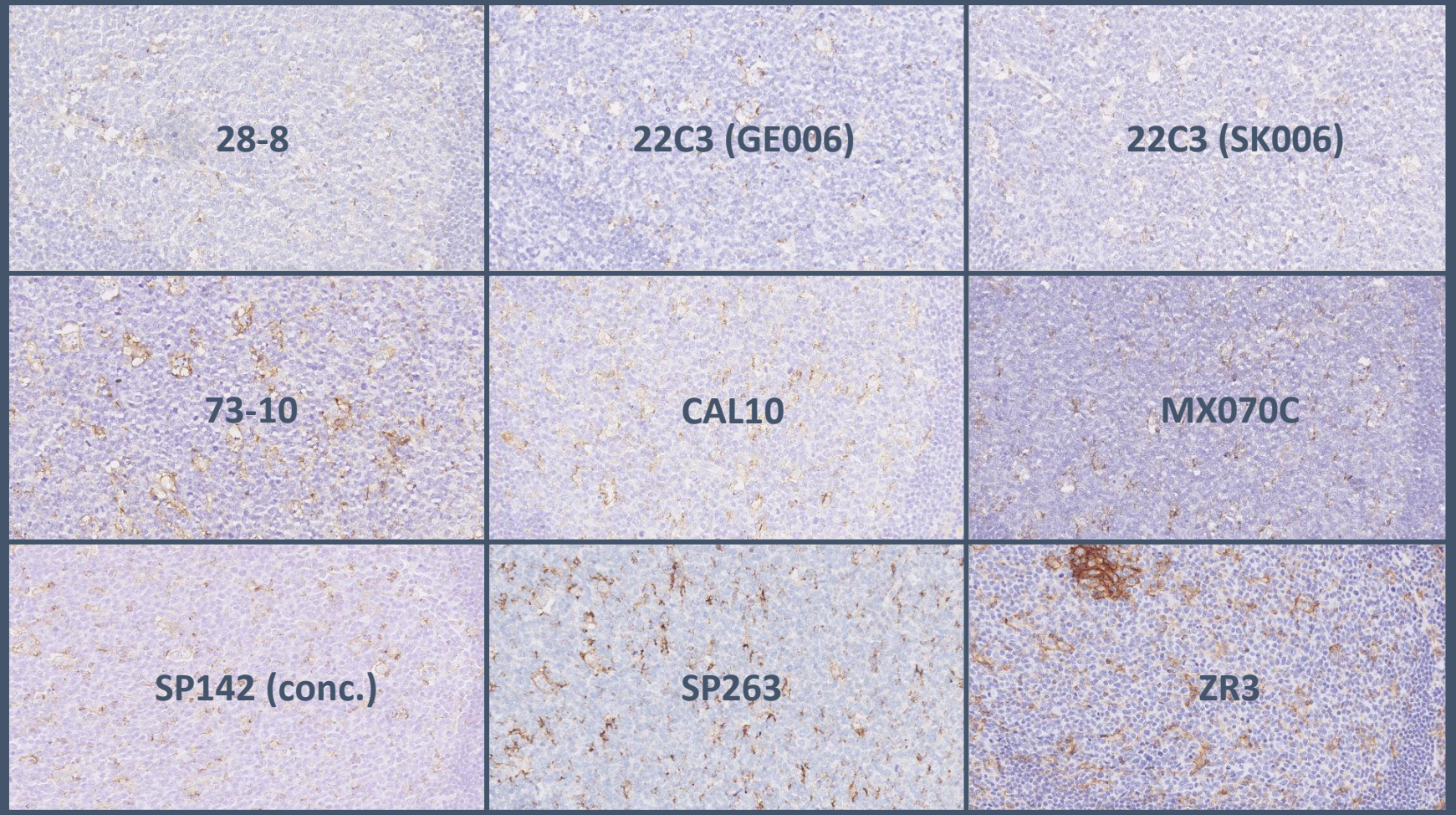
Table 2. **Assessment marks for IHC assays and antibodies run C13, PD-L1 TPS/CPS (KEYTRUDA®)**

CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone SP263, **741-4905 (VRPS)**[3]	41	Ventana/Roche	5	33	3	-	93%	12%
rmAb clone SP263, **741-4905 (LPMS)**[4]	2	Ventana/Roche	-	1	1	-	-	-
rmAb clone SP263, **740-4907 (VRPS)**[3]	12	Ventana/Roche	3	9	-	-	100%	25%
mAb clone 22C3 pharmDX, **SK006 (VRPS)**[3]	19	Dako/Agilent	14	4	-	1	95%	74%
mAb clone 22C3 pharmDX, **SK006 (LMPS)**[4]	20	Dako/Agilent	13	5	2	-	90%	65%
mAb clone 22C3 pharmDX, **GE006 (VRPS)**[3]	29	Dako/Agilent	23	6	-	-	100%	79%
mAb clone 22C3 pharmDX, **GE006 (LMPS)**[4]	18	Dako/Agilent	12	4	2	-	89%	67%
rmAb clone 28-8 pharmDX, **SK005 (VRPS)**[3]	3	Dako/Agilent	2	1	-	-	-	-
Antibodies[5] for laboratory developed PD-L1 assays, concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **22C3**	44	Dako/Agilent	18	19	7	-	84%	41%
rmAb **CAL10**	4 1	Zytomed Systems Biocare Medical	2	2	-	1	80%	40%
rmAb clone **E1L3N**	4	Cell Signaling	1	3	-	-	-	-
rmAb clone **QR1**	2	Quartett	2	-	-	-	-	-
rmAb clone **28-8**	1	Dako/Agilent	-	1	-	-	-	-
rmAb clone **ZR3**	1	Zeta Corporation	-	1	-	-	-	-
rmAB clone **SP142**	1	Abcam	1	-	-	-	-	-
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	-	11	2	-	85%	-
rmAb clone SP263, **790-4905[6] (LMPS)**[4]	16	Ventana/Roche	1	15	-	-	100%	6%
rmAb clone 73-10 **PA0832**	6	Leica Biosystems	5	1	-	-	100%	83%
rmAb MX070C **MAB-0854**	2	Fuzhou Maixin	1	1	-	-	-	-
mAb clone C9C9 **CPM-0278**	1	Celnovte	-	1	-	-	-	-
rmAb clone AC37 **AD80167**	1	Abcarta	1	-	-	-	-	-
rmAb clone RM320 **8263-C010**	1	Sakura Finetek	1	-	-	-	-	-
rmAb clone BP6099 **I12052E**	1	Biolynx	1	-	-	-	-	-
Total	243		106	118	17	2		
Proportion			44%	48%	7%	1%	92%	





PD-L1 – ICAPS - TONSIL



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

Different assays → different staining patterns.

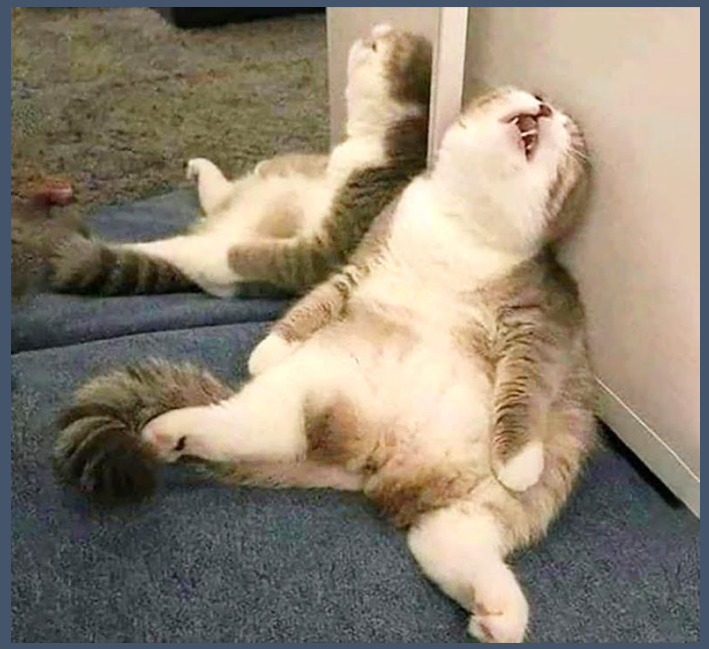
All 9 assays achieved an optimal score for PD-L1 TPS/CPS.

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	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

THANK YOU FOR YOUR ATTENTION!

NordiQC



BONUS – ROS1

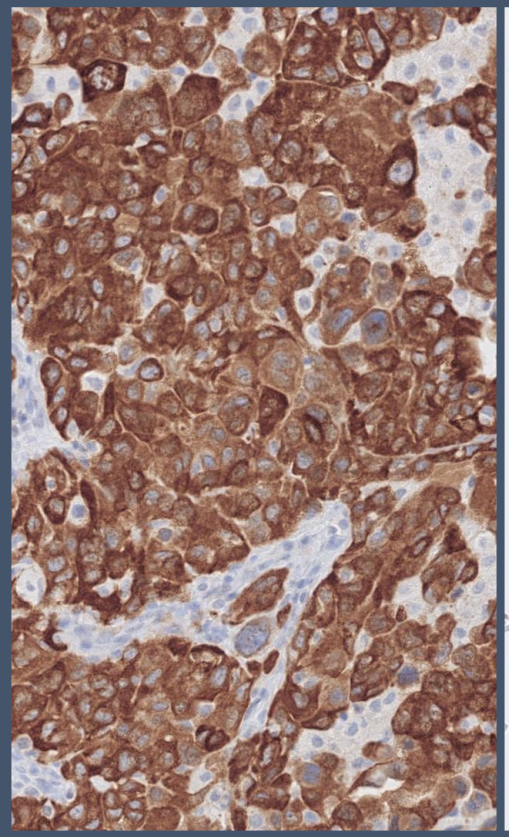
No NordiQC data available for ROS1.

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

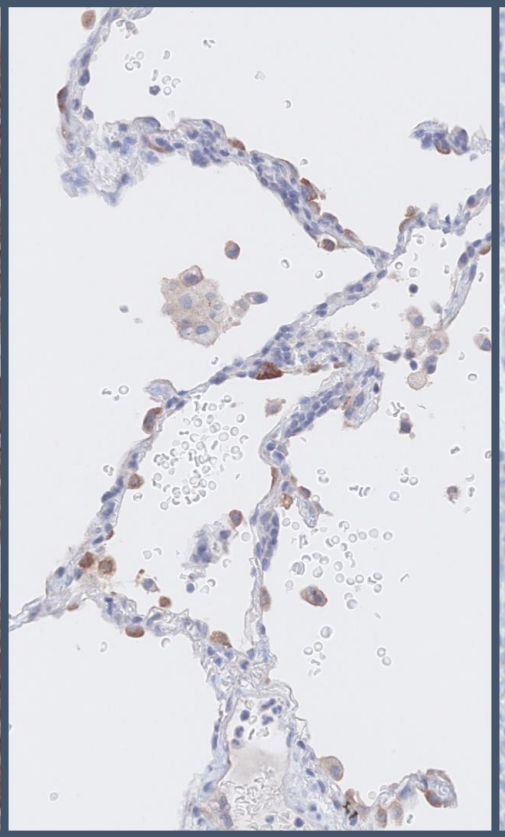
Positive controls:
Tumor with known ROS1-translocation
Type II-pneumocytes in normal lung

Negative control:
Appendix

Tumor with ROS1-translocation (lung adenocarc.)



Normal lung



Appendix