

NORDIQC DATA FOR LUNG MARKERS

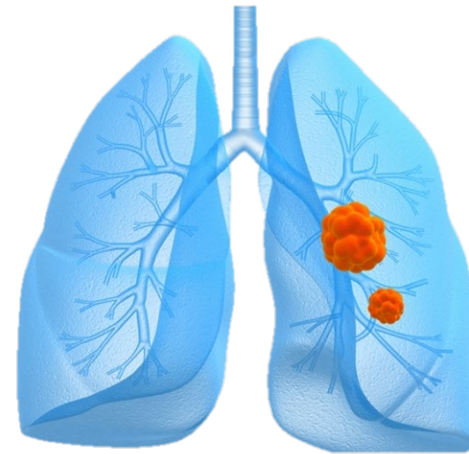
Antibody selection, protocols and controls

NordiQC Seminar, September 23rd - 25th 2026

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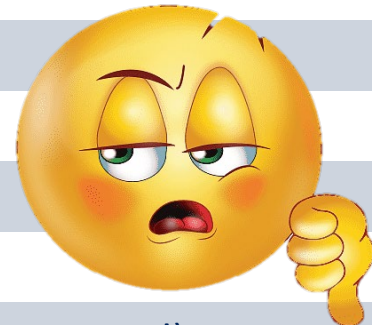
NORDIQC EQA DATA FOR IHC LUNG MARKERS

Marker	Purpose	Last run	Pass rate	No of labs
TTF1	<u>Lung</u> vs non-lung <u>Adenocarcinoma</u> vs squam.	Run 68, 2023	81%	401
Napsin A	<u>Lung</u> vs non-lung	Run 66, 2022	83%	321
Calretinin	Lung vs <u>mesothelioma</u>	Run 76, 2026	85%	408
WT1	Lung vs <u>mesothelioma</u>	Run 55, 2019	91%	291
BAP1	Reactive mesothelioma vs <u>malignant mesothelioma</u>	Run 71, 2024	63%	224
CLDN4	<u>Lung</u> vs mesothelioma	Run 74, 2025	72%	97
EpCAM	<u>Lung</u> vs mesothelioma <u>Adenocarcinoma</u> vs squam./basal cell carcinoma	Run 69, 2023	48%	372
INSM1	NSCLC vs <u>SCLC</u>	Run 71, 2024	72%	156
CGA	NSCLC vs <u>SCLC</u>	Run 70, 2024	75%	370
SYP	NSCLC vs <u>SCLC</u>	Run 73, 2025	75%	429
CD56	NSCLC vs <u>SCLC</u>	Run 64, 2022	72%	364
p40	Adenocarcinoma vs <u>squam.</u>	Run 74, 2025	94%	411
CK5	Adenocarcinoma vs <u>squam.</u>	Run 76, 2026	69%	330
ALK (lung)	Predictive for Crizotinib	Run 73, 2025	59%	307
PD-L1 TPS/CPS	Predictive for Keytruda, Imfinzi, Opdivo.....	Run C19, 2026	96%	299



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
WT1	mAbs 6F-H2 & WT49	-
BAP1	mAb C-4 & BSB-109, rmAb EPR22826-65	pAbs
CLDN4	mAb 3E2C1, rmAb EP417	pAbs
EpCAM	mAbs BS14, Ber-EP4, MOC-31 & VU-1D9	mAb Ber-EP4
INSM1	rmAbs MRQ-70 & ZR395	mAb A-8 <i>(might depend on vendor)</i>
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 – Fit For Treatment!)



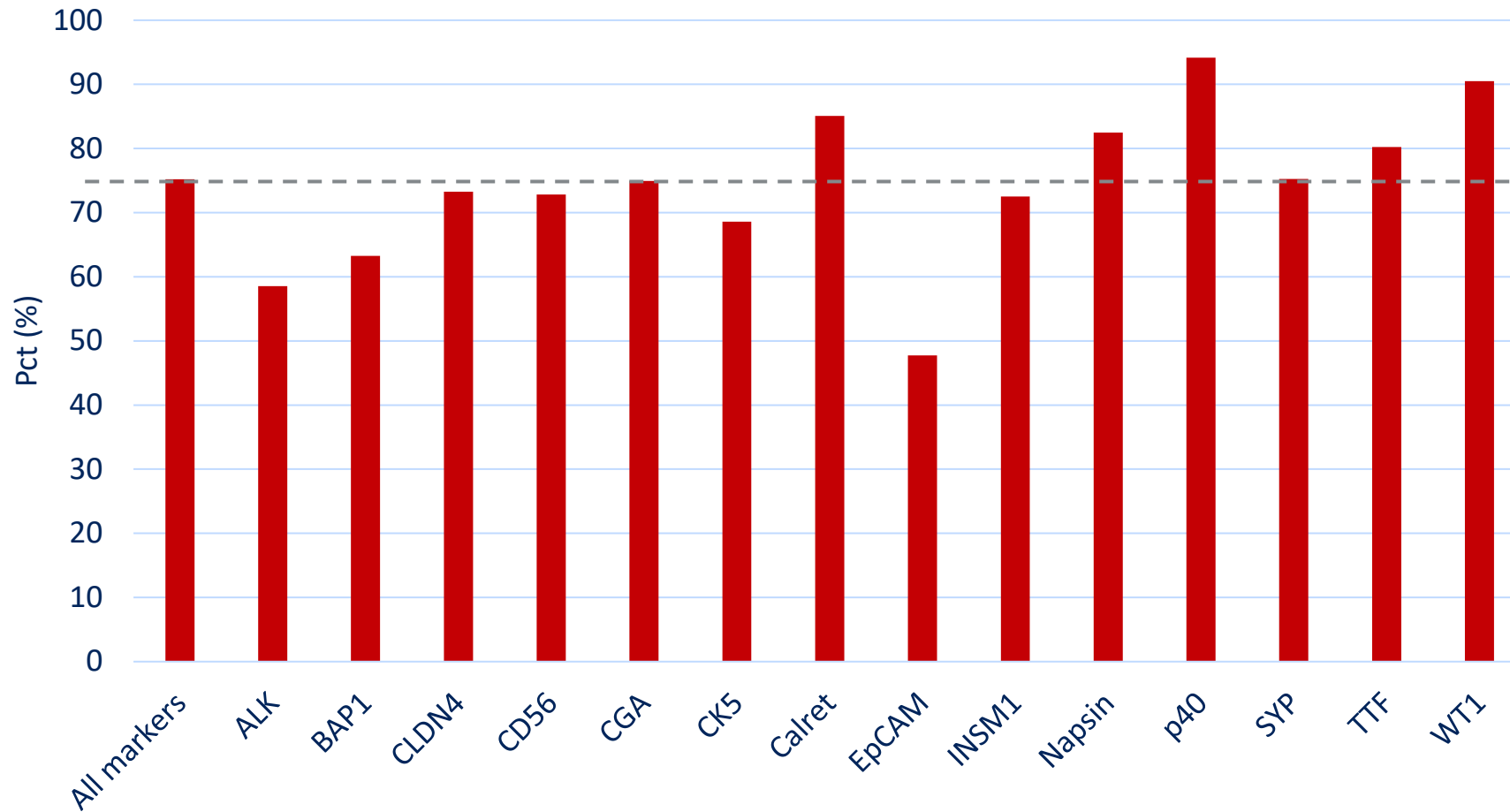
KEY-POINTS FOR BEST PROTOCOLS

- 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

Pass rates for selected lung markers



Lung markers in the General Module:

Overall pass rate: **75%**
(3.536/4.702), ranging from
48% for EpCam till 94% for p40.

KEY-POINTS FOR BEST PROTOCOLS

Lung markers in the General Module:

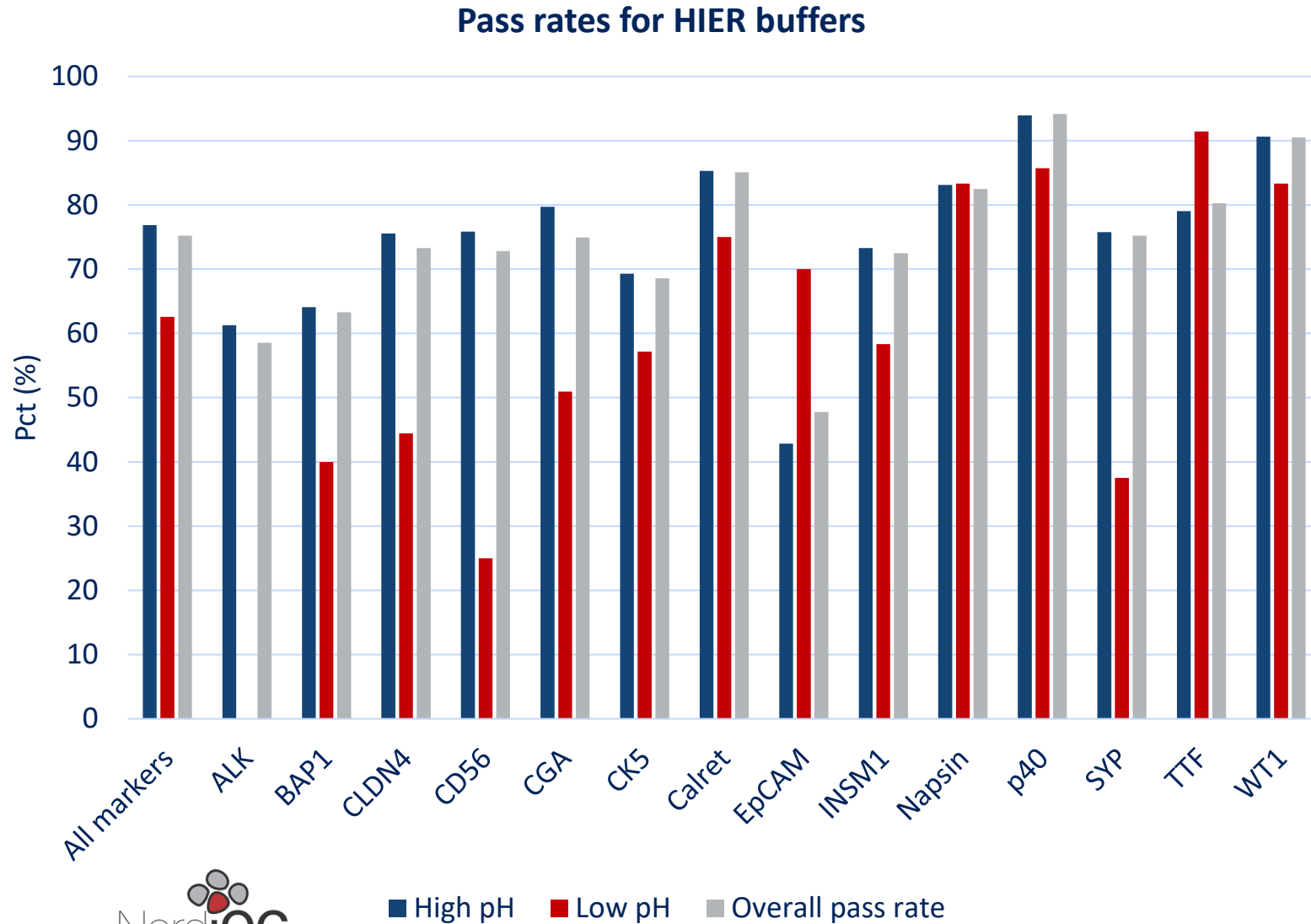
Overall pass rate: **75%** (3.536/4.702)

HIER in High pH: **77%**

Ranging from 43% for EpCAM till 94% for p40

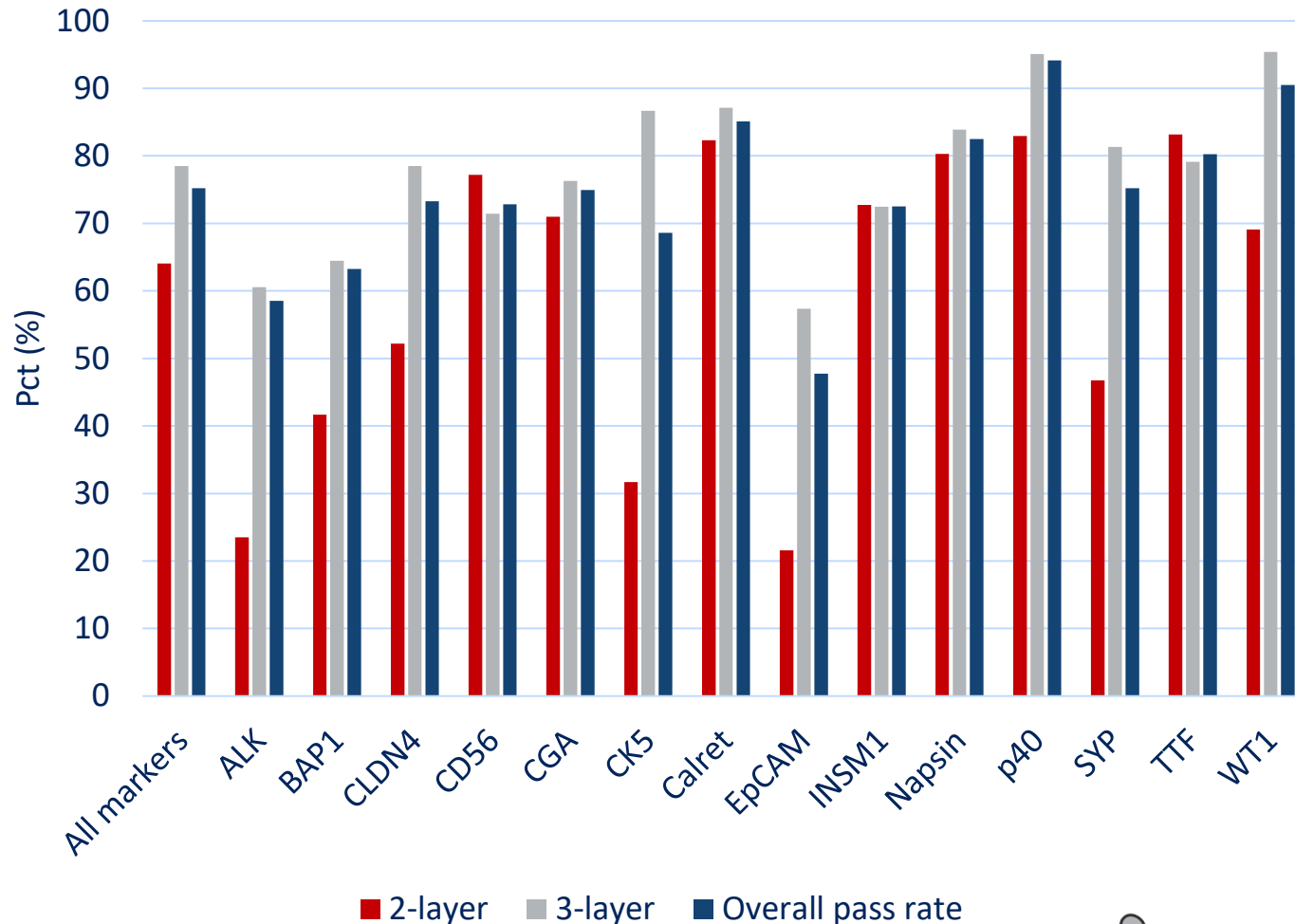
HIER in Low pH: 63%

Ranging from 0% for ALK till 91% for TTF1



KEY-POINTS FOR BEST PROTOCOLS

Pass rates for detection systems



Lung markers in the General Module:

3-layer detection system: 78%

- OptiView, UltraView DAB + amplification
- EnVision Flex +, Flex++ (4-layer)
- Bond Refine (despite host of primary Ab)

2-layer detection system: 64%

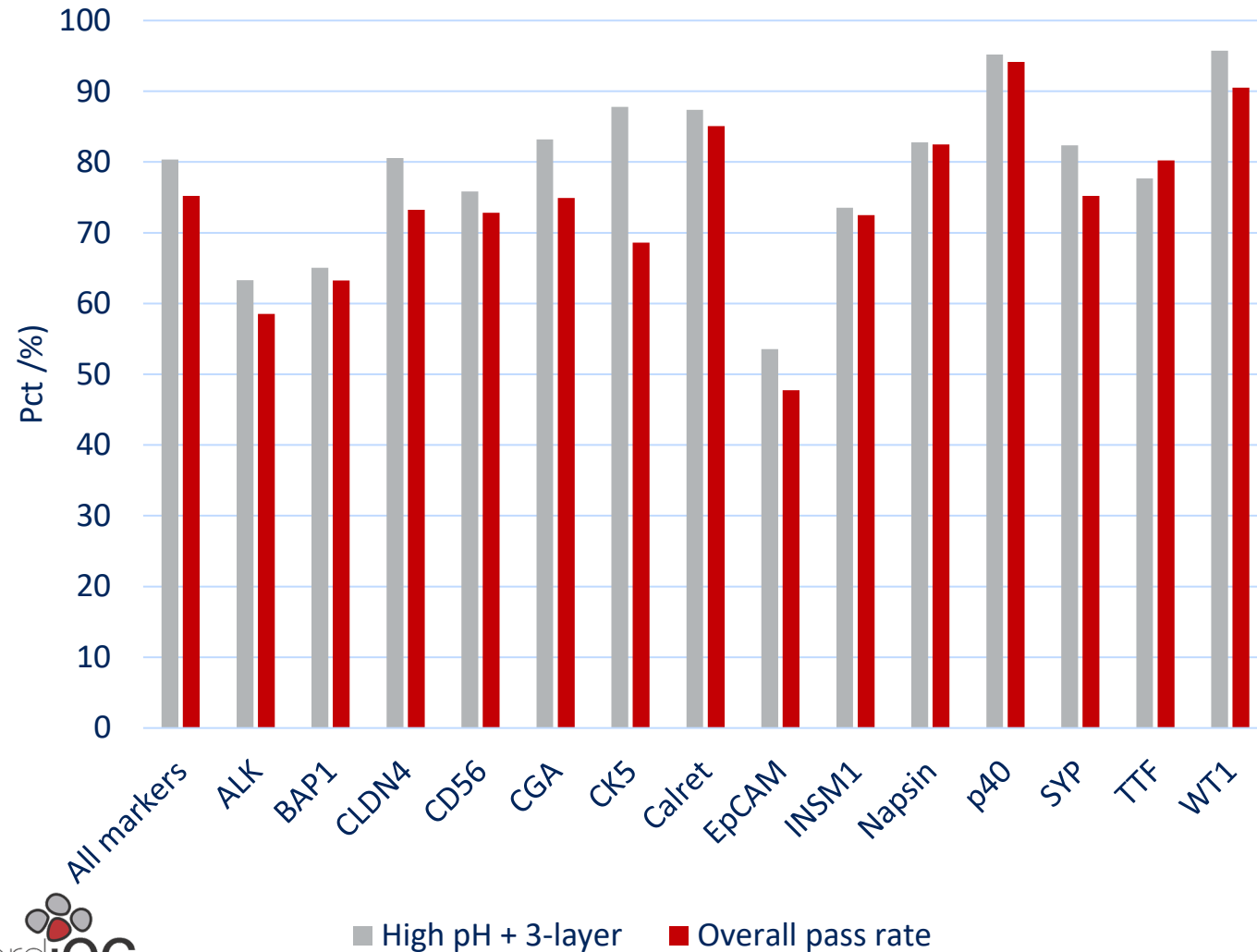
KEY-POINTS FOR BEST PROTOCOLS

Lung markers in the General Module:

- Overall pass rate: 75%
- “Best practice protocol”*: HIER in a high pH buffer AND a 3-layer detection system: 80%
- Ranging from 54% for EpCAM till 96% for WT1

*Clone selection is NOT included.

Pass rates for “Best practice protocol” - settings



KEY-POINTS FOR BEST PROTOCOLS

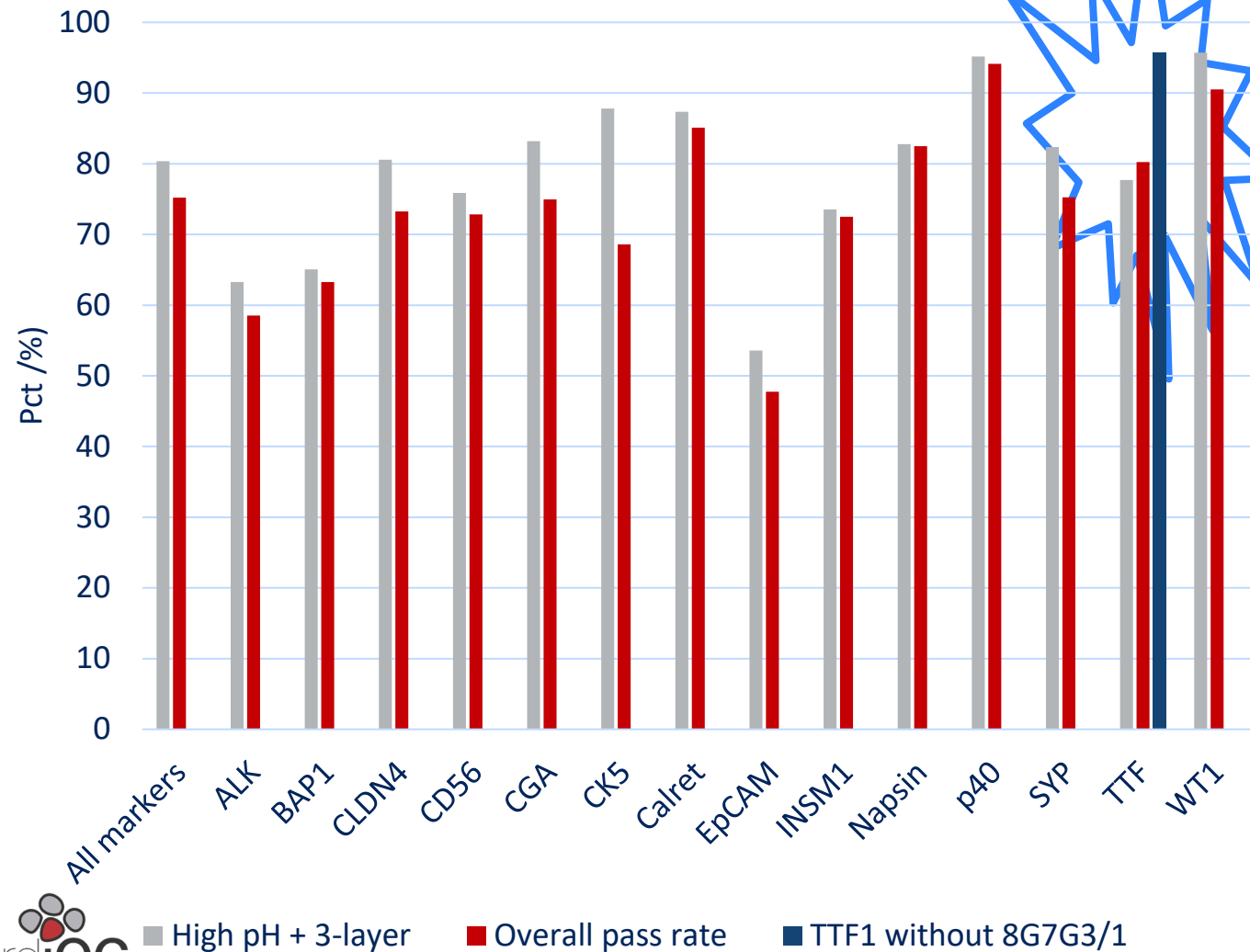
Lung markers in the General Module:

- Overall pass rate: 74%
- “Best practice protocol”*: HIER in a high pH buffer AND a 3-layer detection system: 80%
- Ranging from 54% 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 80% if including the clone.

Pass rates for "Best practice protocol" - settings





NOW TIME TO LOOK
AT SOME SPECIFIC
MARKERS

CLDN4 – ‘NEW’ MARKER IN NORDIQC

Table 1b. Concentrated antibodies and assessment marks for CLDN4, run 74

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 3E2C1	15	Biocare Medical	3	7	4	1	67%	20%
	24	Invitrogen	3	15	3	3	75%	13%
mAb clone A-12	2	Gennova	0	0	0	2	-	-
rmAb clone EP417	8	BioSB	1	4	2	1	63%	13%
	8	Cell Marque	6	2	0	0	100%	75%
rmAb clone EPRR17575	4	Abcam	1	1	2	0	-	-
rmAb clone QR137	1	Quartett	1	0	0	0	-	-
pAb, ab15104	4	Abcam	1	1	2	0	-	-
pAb, PA580481	1	ThermoFisher	0	0	0	1	-	-
pAb, 16195-1-AP	1	Proteintech	0	0	0	1	-	-
Total	68		16	30	13	9		
Proportion			24%	44%	19%	13%	68%	

¹ Proportion of sufficient slides (optimal + good) / (Suff. assessed antibodies)

Table 1c. Ready-To-Use antibodies and assessment marks for CLDN4, run 74

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 3E2C1 MAB-1106	1	Fuzhou Maixin	0	1	0	0	-	-
mAb clone 3E2C1 API 3121 AA	8	Biocare Medical	5	2	0	1	88%	63%
mAb clone A-12 AMB08-5M	1	BioGenex	0	0	0	1	-	-
mAb clone AA11 MFM-0188	1	Bioin Biotechnology	0	1	0	0	-	-
mAb clone GM104 GT2339	1	Gene Tech	1	0	0	0	-	-
rmAb clone EP417 BSB 3792-3/7/15	7	Bio SB	0	5	1	1	71%	0%
rmAb clone EP417 468-17/18	9	Cell Marque	5	3	1	0	89%	56%
Ab clone 172D5F1 PA616	1	Abcarta	1	0	0	0	-	-
Total	29		12	12	2	3		
Proportion			41%	41%	7%	11%	82%	

¹ Proportion of sufficient slides (optimal + good) / (Suff. assessed antibodies)

Table 2. Proportion of optimal results for CLDN4 for the most commonly used antibody concentrates on the four main IHC systems*

Concentrated antibodies	Dako/Agilent Autostainer ¹		Dako/Agilent Omnis		Ventana/Roche BenchMark ²		Leica Biosystems Bond ³	
	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 3E2C1	0/1**	-	5/10 (50%)	-	1/18 (6%)	0/1	0/3	-
rmAb clone EP417	2/2	-	0/2	1/1	4/10 (40%)	-	-	-

3E2C1 on Omnis:
HIER in TRS High & EnVision Flex+ gave a 100% pass rate (10/10)

EP417 on BenchMark:
HIER in CC1 & OptiView gave a 90% pass rate (9/10)

Bond: HIER in BERS2: 100% pass rate (5/5)
Omnis: HIER in TRS High, EnVision Flex+: 1/1 optimal

BenchMark: HIER in CC1 & OptiView: 100% pass rate (3/3)
Omnis: HIER in TRS High, EnVision Flex+: 1/1 optimal

Table 1a. Overall results for Calret, run 76

	n	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
Concentrated antibodies	60	32	14	9	5	77%	53%
Ready-To-Use antibodies	348	232	69	34	13	86%	67%
Total	408	264	83	43	18		
Proportion		65%	20%	11%	4%	85%	

Table 1c. Ready-to-Use antibodies and assessment marks for Calret, run 76

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone CAL6 PA0346 ³	21	Leica Biosystems	18	3	-	-	100%	86%
mAb clone CAL6 PA0346 ⁴	9	Leica Biosystems	5	3	1	-	89%	56%
mAb clone DAK-Calret 1 IR627 ³	12	Dako/Agilent	1	5	5	1	50%	8%
mAb clone DAK-Calret 1 IR627 ⁴	59	Dako/Agilent	8	17	22	12	42%	14%
mAb clone C5G4 CCM-0222	1	Celnovte	1	-	-	-	-	-
mAb clone IHC523 IHC523-7	1	GenomeMe	-	1	-	-	-	-
mAb clone BY219 BFM-0581	1	Bioin Biotechnology	1	-	-	-	-	-
Ab clone DY49410 4910642	1	Dakewe	1	-	-	-	-	-
mAb clone MX027 MAB-0716	1	Fuzhou Maixin	1	-	-	-	-	-
rmAb clone SP13 232R	2	Cell Marque	2	-	-	-	-	-
rmAb clone SP13 AY747	1	BioGenex	-	1	-	-	-	-
rmAb clone BSR235 MAD-000784QD	3	Master Diagnostica	1	1	1	-	-	-
rmAb clone RM324 8522-C010	3	Sakura Finetek	3	-	-	-	-	-
rmAb clone RM324 BO6011	1	Guangzhou Biotron	1	-	-	-	-	-
rmAb clone SP65 790-4467 ³	78	Ventana/Roche	71	7	-	-	100%	91%
rmAb clone SP65 790-4467 ⁴	150	Ventana/Roche	116	29	5	-	97%	77%
rmAb clone 554J188 PA262	1	Abcarta	1	-	-	-	-	-
pAb IP092	1	Biocare Medical	-	1	-	-	-	-
pAb HAP134	1	PathnSitu	-	1	-	-	-	-
pAb GT200902	1	Gene Tech	1	-	-	-	-	-
Total	348		232	69	34	13		
Proportion			66%	20%	10%	4%	86%	

CALRETININ – PITFALLS

Table 2. Proportion of optimal results for Calret for the most commonly used antibody concentrates on the four main IHC systems by optimal settings as listed above.

Concentrated antibodies	Dako/Agilent Autostainer ¹		Dako/Agilent Omnis		Ventana/Roche BenchMark ²		Leica Biosystems Bond ³	
	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 CAL6	-	-	9/10 (90%)	-	1/1	-	8/12 (67%)	1/2
mAb clone DAK-Calret 1	2/2	-	0/2	-	-	-	2/2	-
pAb 18-0211	-	-	2/2	-	2/3	-	-	-

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 Calret 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% (21/21)	86% (18/21)	100% (8/8)	63% (5/8)
Dako AS mAb DAK-Calret 1 IR627	50% (6/12)	8% (1/12)	90% (9/10)	50% (5/10)
Ventana BenchMark mAb SP65 UltraView 790-4467	100% (56/56)	88% (49/56)	96% (64/67)	78% (52/67)
Ventana BenchMark mAb SP65 OptiView 790-4467	100% (22/22)	100% (22/22)	98% (81/83)	77% (64/83)

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

EnVision Flex:
60% pass rate (7% optimal)

EnVision Flex+
85% pass rate (71% 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 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, but limited data..

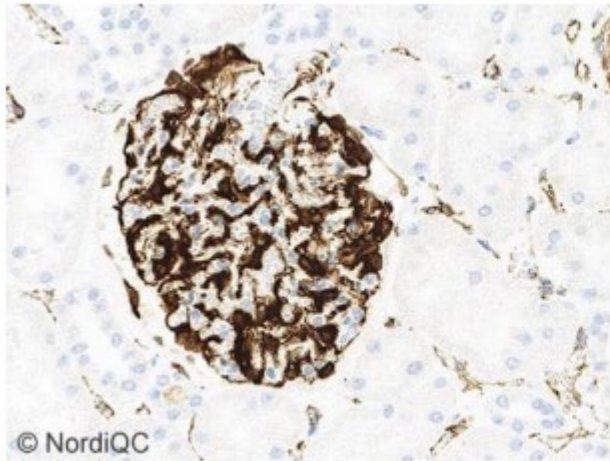
Table 3. Proportion of optimal results for WT1 for the most commonly used antibodies as concentrates on the four main IHC systems*

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.

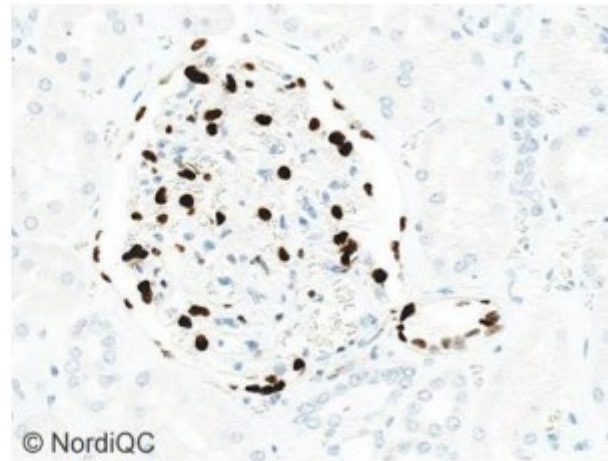
** Number of optimal results/number of laboratories using this buffer

WT1 – PITFALLS



© NordiQC
Fig. 7a (x300)

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



© NordiQC
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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Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone BS14	25	Nordic Biosite	19	6	0	0	100%	76%
mAb clone Ber-Ep4	40	Dako/Agilent	5	11	30	3	33%	10%
	8	Cell Marque						
	1	Thermo Scientific						
mAb clone MOC-31	16	Dako/Agilent	1	14	4	1	75%	5%
	1	Biocare Medical						
	2	Cell Marque						
	1	Leica Biosystems						
mAb clone VU-1D9	9	Thermo Scientific	4	8	2	1	80%	27%
	1	Merck Millipore						
	4	Diagnostic Biosystems						
	1	Monosan						
mAb clone C-10	2	Santa Cruz	0	0	2	0	-	-
mAb clone ZM131	1	Zeta Corporation	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone Ber-Ep4 760-4383³	13	Ventana/Roche	0	0	13	0	0%	0%
mAb clone Ber-Ep4 760-4383⁴	86	Ventana/Roche	1	20	60	5	24%	1%
mAb clone Ber-Ep4 248M-98	15	Cell Marque	0	1	13	1	7%	0%
mAb clone Ber-Ep4 IR/IS637³	1	Dako/Agilent	0	0	1	0	-	-
mAb clone Ber-Ep4 IR/IS637⁴	15	Dako/Agilent	3	2	10	0	33%	20%
mAb clone Ber-Ep4 GA637³	44	Dako/Agilent	12	30	2	0	95%	27%
mAb clone Ber-Ep4 GA637⁴	16	Dako/Agilent	3	8	5	0	69%	19%
mAb clone Ber-Ep4 PM107	3	Biocare Medical	0	0	1	2	-	-
mAb clone Ber-Ep4 MAD-001709QD	3	Master Diagnostica	0	0	2	1	-	-
mAb clone Ber-Ep4 PDM131	2	Diagnostic Biosystems	0	0	0	2	-	-
mAb clone Ber-Ep4 P-E002	1	Quartett	0	0	0	1	-	-
mAb clone Ber-Ep4 BMS048	1	Zytomed Systems	0	1	0	0	-	-
mAb clone Ber-Ep4 GM080402	2	Gene Tech	0	1	1	0	-	-
mAb clone MOC-31 790-4561¹	3	Ventana/Roche	0	1	2	0	-	-
mAb clone MOC-31 790-4561⁴	10	Ventana/Roche	2	6	2	0	80%	20%
mAb clone MOC-31 248M-18	4	Cell Marque	0	1	3	0	-	-
mAb clone MOC-31 CEM-0051	1	Celnovte	0	0	1	0	-	-
mAb clone VU-1D9 PDM077	2	Diagnostic Biosystems	0	1	0	1	-	-
mAb clone VU-1D9 8230-C010	3	Sakura FineTek	0	1	2	0	-	-
mAb clone BS14 8377-C010	1	Sakura FineTek	1	0	0	0	-	-
mAb clone SPM491 ab228023	1	abcam	0	0	0	1	-	-
mAb clone MX066 MAB-0850	1	Fuzhou Maxin	0	1	0	0	-	-
mAb clone BP6056 BX50051	1	Biolynx	0	0	1	0	-	-
mAb clone IHC567 IHC567-7/25	1	GenomeMe	0	0	1	0	-	-
rmAb clone EP155 AN820	1	BioGenex	0	0	0	1	-	-
rmAb clone BY118 BFM-0436	1	Bioin Biotechnology	0	0	1	0	-	-
rmAb clone DA056 RMA1A068	1	Shenzhen Dartmon Biotechnology	0	0	0	1	-	-
Total	345		51	113	159	22		
Proportion			15%	33%	46%	6%	48%	

EP-CAM – PITFALLS

Table 2. 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 / Ultra Plus			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 2/3	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone BS14	-	-	1/2**	-	6/7 (86%)	10/13 (77%)	-	1/2	-
mAb clone Ber-EP4	0/1	1/1	-	5/6 (83%)	0/2	0/4	0/1	0/1	0/2
mAb clone MOC-31	0/1	-	-	1/3	0/4	-	-	-	0/1
mAb clone VU-1D9	-	-		0/1	4/5 (80%)		-	0/1	-

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

Table 3. 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% (0/13)	0% (0/13)	25% (21/85)	1% (1/85)
Autostainer +/-Link mAb Ber-EP4 IS/IR637	(0/1)	(0/1)	40% (4/10)	30% (3/10)
Omnis mAb Ber-EP4 GA637	95% (42/44)	27% (12/44)	73% (11/15)	27% (3/11)
BenchMark XT/Ultra mAb MOC-31 790-4561	(1/3)	(0/3)	80% (8/10)	20% (2/10)

RTU for Dako Omnis a obtained high pass rate, but few optimal. Use of a 3-layer detection system for IR637 increases sufficient results.

Table 1c. Ready-to-Use antibodies and assessment marks for CK5, run 76								
Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suffl ¹	OR ²
mAb clone D5/16 B4* 790-4554³	22	Ventana/Roche	3	3	16	-	27%	14%
mAb clone D5/16 B4* 790-4554⁴	40	Ventana/Roche	6	16	17	1	55%	15%
mAb D5/16 B4* GA780⁵	19	Dako/Agilent	-	3	16	-	16%	0%
mAb D5/16 B4* GA780⁴	25	Dako/Agilent	2	9	14	-	44%	8%
mAb clone D5/16 B4* IR780⁴	15	Dako/Agilent	2	5	7	1	47%	13%
mAb clone D5/16 B4* 8295-C010	2	Sakura Finetek	1	1	-	-	-	-
mAb clone D5/16 B4* 356M-10	1	Cell Marque	-	-	-	1	-	-
mAb clone 150A8C1 PA018	1	Abcarta	1	-	-	-	-	-
mAb clone 15D1 B64011	1	Guangzhou Biotron	-	1	-	-	-	-
mAb clone C6H1 CCM-0975	1	Celnovte	-	-	1	-	-	-
mAb clone IHC556* IHC556	1	GenomeMe	1	-	-	-	-	-
mAb clone XM26 PA0468³	14	Leica Biosystems	13	1	-	-	100%	93%
mAb clone XM26 PA0468⁴	13	Leica Biosystems	11	1	1	-	92%	85%
mAb clone XM26 PM234	2	Biocare Medical	2	-	-	-	-	-
mAb clone XM26/LL002** BMS023	2	Zytomed	-	1	1	-	-	-
mAb clone ZM186 ZM186	1	Zeta Corporation	-	-	1	-	-	-
rmAb clone EP1601Y 305R-17/18	2	Cell Marque	-	2	-	-	-	-
rmAb clone EP1601Y/LL002** 905H-08	1	Cell Marque	-	-	1	-	-	-
rmAb clone EP42 BSB6599	1	BioSB	-	-	1	-	-	-
rmAb clone EP42 NPAI430	1	Biocare Medical	-	1	-	-	-	-
rmAb clone EP24/EP67* MAD-0006510D	1	Master Diagnostica	-	-	1	-	-	-
rmAb clone SP27 760-4935³	32	Ventana/Roche	31	-	1	-	97%	97%
rmAb clone SP27 760-4935⁴	24	Ventana/Roche	22	2	-	-	100%	92%
rmAb clone SP27 MAD-000491QD	1	Master Diagnostica	1	-	-	-	-	-
rmAb clone BY160 BFM-0482	1	Bioin Biotechnology	1	-	-	-	-	-
rmAb clone BP6021 BX50016	1	Biolynx Biotechnology	-	1	-	-	-	-
rmAb clone DY49108 4922072	1	Dakewe	-	1	-	-	-	-
rmAb clone GR301 GT215202	1	Gene Tech	1	-	-	-	-	-
rmAb clone MXR029 RMA-1064	1	Fuzhou Maixin	1	-	-	-	-	-
Total	228		99	48	78	3		
Proportion			44%	21%	34%	1%	65%	

CK5, RTUs – PITFALLS

Table 1a. Overall results for CK5, run 76							
	n	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
Concentrated antibodies	102	56	23	21	2	77%	55%
Ready-To-Use antibodies	228	99	48	78	3	65%	44%
Total	330	155	71	99	5		
Proportion		47%	22%	30%	1%	69%	

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, UltraView 790-4554	6% (1/16)	0% (0/16)	20% (3/15)	7% (1/15)
Ventana Benchmark mAb clone D5/16 B4, OptiView 790-4554	83% (5/6)	50% (3/6)	79% (19/24)	21% (5/24)
Dako Omnis mAb clone D5/16 B4, GA780	16% (3/19)	0% (0/19)	50% (11/22)	9% (2/22)
Dako Autostainer mAb clone D5/16 B4, IR780	-	-	43% (3/7)	14% (1/7)
Leica Bond mAb clone XM26, PA0468	100% (14/14)	93% (13/14)	100% (11/11)	91% (10/11)
Ventana Benchmark rmAb clone SP27, UltraView 760-4935	100% (12/12)	100% (12/12)	100% (8/8)	100% (8/8)
Ventana Benchmark rmAb clone SP27, OptiView 760-4935	95% (19/20)	95% (19/20)	100% (14/14)	86% (12/14)



Fig. 3a
Optimal CK5 staining of the prostate hyperplasia using same protocol as in Figs. 1a-2a. A strong and distinct cytoplasmic staining reaction is seen in most basal cells in the hyperplastic prostate glands. No background staining.

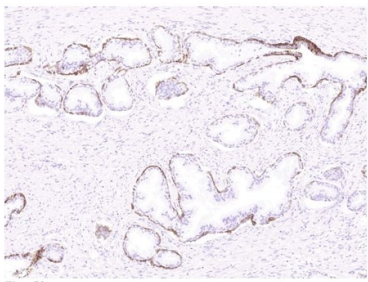


Fig. 3b
Insufficient CK5 staining of the prostate hyperplasia using same protocol as in Figs. 1b-2b – same field as in Fig. 3a. A reduced number of positive basal cells in the hyperplastic prostate glands show a weak staining reaction, compromising the diagnostic utility of the test in prostate samples.

Lower pass rate of RTUs compared to concentrates.

Less successful performance of the widely used mAb D5/16 B4 on both Ventana and Dako platforms

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*‡

mAb XM26 outperforms mAb D5/16, and is also available as a conc.

Left: XM26 // Right: D5/16 B4

Recommended protocol for CK5

Obtained in run 76

03 Feb 2026

Immunostainer

Type: Dako Omnis

Primary antibody

Clone: XM26
Producer: Leica/novocastra
Product no. / lot no.: NCL-L-CK5 / 6117631
Diluent: Antibody Diluent
Diluent producer / no.: Dako / K8006
Dilution factor: 1:100
Incubation time / temperature: 30 min. / 36°C

Epitope retrieval, HIER

Device: On Board / On Machine
Buffer: Dako Omnis Target Retrieval Solution, High pH
Heating time at max. temp.: 24 min.
Maximum heating temp.: 97°C

Visualization system

Producer: Dako Omnis
Product / no: EnVision Flex / GV800/GV823/GV900
Linker: Mouse LINKER
Incubation time linker: 10 min.
Incubation time polymer: 20 min.
Incubation temperature: 36°C

Chromogen

Producer: Dako Omnis
Product / no: DAB+ Substrate Chromogen System / GV825
Incubation time / temperature: 5 min. / 36°C
Enhancement: None

Disclaimer:

NordiQC makes every attempt to provide accurate and up-to-date information, yet NordiQC does not make any claim or warranty regarding the accuracy of the provided information nor does it represent that the contents of the web site and protocols reflect the most recent developments in immunohistochemistry at any point in time.

CK5, CONCs – PITFALLS

Table 1a. Overall results for CK5, run 76

	n	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
Concentrated antibodies	102	56	23	21	2	77%	55%
Ready-To-Use antibodies	228	99	48	78	3	65%	44%
Total	330	155	71	99	5		
Proportion		47%	22%	30%	1%	69%	

Table 1b. Concentrated antibodies and assessment marks for CK5, run 76

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff ¹	OR ²
mAb clone D5/16 B4*	1	BioSB	1	-	-	-	48%	19%
	3	Cell Marque	1	1	1	-		
	23	Dako/Agilent	3	7	11	2		
mAb clone XM26	3	Abcam	1	1	1	-	93%	72%
	1	Biocare Medical	1	-	-	-		
	3	Diagnostic Biosystems	2	1	-	-		
	61	Leica Biosystems	45	12	4	-		
mAb clone 3E2F1	1	Thermo Fisher Scientific	-	-	1	-	-	-
rmAb clone SP27	1	Abcam	1	-	-	-	-	-
rmAb clone EP1601Y	1	Cell Marque	1	-	-	-	-	-
rmAb clone EP24/EP67*	1	PathnSitu	-	-	1	-	-	-
rmAb clone EP24	1	Epitomics	-	1	-	-	-	-
rmAb clone EP42	1	Epitomics	-	-	1	-	-	-
rmAb clone ZR280	1	Zeta Corporation	-	-	1	-	-	-
Total	102		56	23	21	2		
Proportion			55%	22%	21%	2%	77%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).

2) Proportion of Optimal Results (≥5 assessed protocols).

*) Cytokeratin 5 and 6.

Table 2. Proportion of optimal results for CK5 for the most commonly used antibody concentrates on the four main IHC systems by optimal settings as listed above.

Concentrated antibodies	Dako/Agilent Autostainer ¹		Dako/Agilent Omnis		Ventana/Roche BenchMark ²		Leica Biosystems Bond ³	
	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 D5/16 B4	-	-	0/1	-	3/9 (33%)	-	2/5 (40%)	0/3
mAb clone XM26	2/3	-	20/22 (91%)	-	17/28 (61%)	-	9/13 (69%)	1/1

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

mAb XM26 obtained optimal results on the main systems. Recommended protocols for the main systems can be found on the NordiQC website – remember to do your internal validation!

Table 1b. Concentrated antibodies and assessment marks for ALK (lung), run 73

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 5A4	1	Abcam	0	0	1	0	-	-
	1	Biocare Medical	0	0	1	0	-	-
	1	Diagnostic BioSystems	0	0	1	0	-	-
	17	Leica Biosystems	1	8	8	0	39%	4%
	1	Master Diagnostica	0	0	0	1	-	-
	1	Monosan	0	0	1	0	-	-
	1	Zytomed Systems	0	0	1	0	-	-
mAb clone OTI1A4*	7	Origene	3	4	0	0	100%	43%
mAb clone IHC509	1	GenomeMe	0	0	1	0	-	-
rmAb clone D5E3	18	Cell Signaling	0	10	8	0	56%	0%
rmAb clone ALK1	5	Dako/Agilent	0	1	0	4	20%	0%
rmAb clone QR017	1	Quartett	0	1	0	0	-	-
rmAb clone ZR305	1	Zeta Corporation	0	0	0	1	-	-
Total	56		4	24	22	6		
Proportion			7%	43%	39%	11%	50%	

Table 1c. Ready-To-Use antibodies and assessment marks for ALK (lung), run 73

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 5A4 PA0831 (VRPS)³	9	Leica Biosystems	4	4	1	0	89%	44%
mAb clone 5A4 PA0831 (LMPS)⁴	15	Leica Biosystems	2	10	1	2	80%	13%
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-001720QD	2	Master Diagnostica	0	0	1	1	-	-
mAb clone ALK1 GA641 (VRPS)³	4	Dako/Agilent	0	0	0	4	-	-
mAb clone ALK1 GA641 (LMPS)⁴	6	Dako/Agilent	0	0	1	5	0%	0%
mAb clone ALK1 IR641	3	Dako/Agilent	0	0	0	3	-	-
mAb clone ALK1 790/800-2918 (VRPS)³	1	Ventana/Roche	0	0	0	1	-	-
mAb clone ALK1 790/800-2918 (LMPS)⁴	10	Ventana/Roche	0	1	1	8	10%	0%
mAb clone ALK1 204M-17/18	1	Cell Marque	0	0	0	1	-	-

ALK-LUNG – PITFALLS

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

In run 73, a pass rate of 7% was obtained (2 of 30).

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

ALK-LUNG – PITFALLS

Run 65, 2022

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.

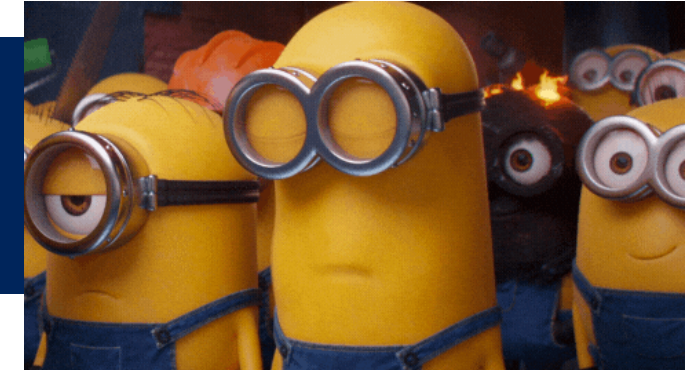
Run 73, 2025

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 790-4794/970-4796/741-7157	57% (63/110)	13% (14/110)	45% (14/31)	16% (5/31)
Dako Omnis mAb OTI1A4 GA785	100% (32/32)	84% (27/32)	89% (8/9)	67% (6/9)
Leica BOND mAb 5A4 PA0831	89% (8/9)	44% (4/9)	83% (10/12)	17% (2/12)

* 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 products for the automated systems, *usual* works as plug-and-play...

However, in run 73, the Ventana RTU gave an aberrant cytoplasmic staining reaction in epithelial cells of appendix, seen in 79% (111 of 141).

VENTANA®



SUBJECT Important Information Regarding Optiview DAB Detection Kit (760-700 / 06396500001)

Dear Valued Customer,

We are writing to inform you about an observation of **specific off-target mucin staining** with certain lots of our OptiView DAB Detection Kit (Product Code: 760-700 / 06396500001). The affected lot numbers are as follows: M17402, M18297, M21054, M21187, M23649, M25973, M25974, M28116, M29508, N01440, and N02309.

Our investigation indicates that this **off-target staining is associated with a raw material used in the OptiView HQ Universal Linker component**. Despite this observation, we want to assure you that these affected lots are deemed acceptable for use.

May 2025

INSM1 – (RELATIVELY) NEW MARKER IN NORDIQC

Table 1b. Concentrated antibodies and assessment marks for INSM1, run 71

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone A-8	1	Diagnostic Biosystems	1	-	-	-	-	-
	5	Gennova	1	2	1	1	60%	20%
	1	Master Diagnostica	-	-	1	-	-	-
	1	Monosan	1	-	-	-	-	-
	2	Nordic Biosite	-	1	1	-	-	-
	50	Santa Cruz	10	15	13	12	50%	20%
	5	Zeta Corporation	4	1	-	-	100%	80%
mAb clone BSB-123	3	Bio SB	-	3	-	-	-	-
	1	LS Bio	-	1	-	-	-	-
rmAb clone MSVA-456R	1	MS Validated Antibodies	-	1	-	-	-	-
rmAb clone BP6240	1	Biolyx Biotechnology	1	-	-	-	-	-
rmAb clone EPR23199-37-6-1	1	Abcam	-	-	1	-	-	-
rmAb clone MRQ-70	27	Cell Marque	19	6	2	-	93%	70%
rmAb clone QR116	1	Quartett	-	-	-	1	-	-
rmAb clone RBT-INSM1	2	Bio SB	-	1	1	-	-	-
rmAb clone ZR395	2	Zeta Corporation	2	-	-	-	-	-
Total	104		39	31	20	14		
Proportion			38%	30%	19%	13%	68%	

Table 2. Proportion of optimal results for INSM1 for the most commonly used antibody concentrates on the four main IHC systems*

Concentrated antibodies	Dako/Agilent Autostainer ¹		Dako/Agilent Omnis		Ventana/Roche BenchMark ²		Leica Biosystems Bond ³	
	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 A-8	2/5** (40%)	1/1	6/12 (50%)	0/2	2/30 (7%)	1/2	5/10 (50%)	0/1
rmAb clone MRQ-70	1/2	-	5/9 (56%)	-	12/14 (86%)	-	1/2	-

The performance and stability of mAb clone A-8 seems to depend on the vendor. Many different lot numbers from the Santa Cruz Ab were submitted, with varying results:

- Optimal
- Good – poor signal-to-noise ratio, weak
- Borderline/poor – false positive and/or false negative

INSM1 – (RELATIVELY) NEW MARKER IN NORDIQC

Table 1c. Ready-To-Use antibodies and assessment marks for INSM1, run 71

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb A-8 AMB44-5M	2	BioGenex	1	-	-	1	-	-
mAb clone A-8 MAD-000777QD	10	Master Diagnostica/ Vitro SA	2	6	2	-	80%	20%
mAb clone A-8 PDM586	3	Diagnostic Biosystems	1	1	-	1	-	-
mAb clone A-8 MAB-1017	1	Fuzhou Maixin	-	1	-	-	-	-
mAb clone BSB-123 BSB 3553/4/5	11	Bio SB	-	7	2	2		
mAb clone DA267 DMRD0168	1	Dartmon Biotechnology	1	-	-	-	-	-
mAb clone BY059 BFM-0177	1	Bioin Biotechnology	-	1	-	-	-	-
rmAb clone IHC741 IHC741	1	GenomeMe	-	1	-	-	-	-
rmAb clone BLR272L API3299	1	Biocare Medical	-	1	-	-	-	-
rmAb clone 315I4E7 PA598	1	Abcarta	-	1	-	-	-	-
rmAb clone GR013 GT246802	1	Gene Tech	1	-	-	-	-	-
rmAb clone INSM1/6286R ANC07-5M	1	BioGenex	-	-	-	1	-	-
rmAb clone MRQ-70 475-97/98	16	Cell Marque	9	6	-	1	94%	56%
rmAb clone RBT-INSM1 BSB-3780-3/7/15	1	Bio SB	-	1	-	-	-	-
rmAb clone ZR395 Z2751RP	1	Zeta Corporation	1	-	-	-	-	-
Total	52		16	26	4	6		
Proportion			31%	50%	8%	11%	81%	

No RTUs from “main” vendors at the time of the assessment (new assessment coming up in the next run)

Applied on BenchMark Ultra and Dako Omnis

Applied on BenchMark Ultra, Dako Omnis and Leica Bond

PD-L1 TPS/CPS

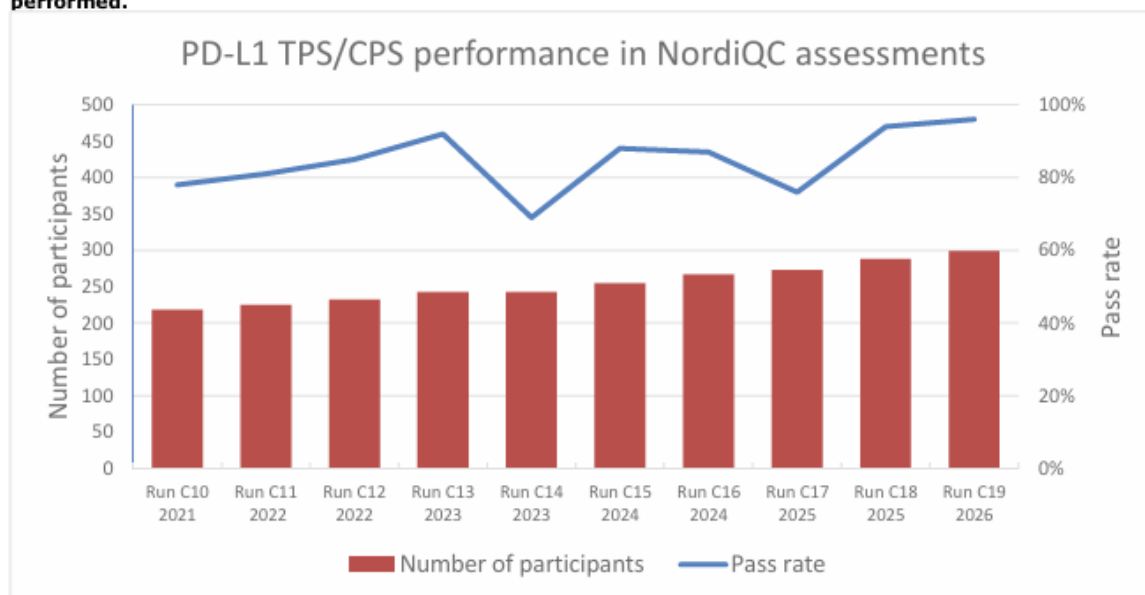
Table 2a. Overall results for PD-L1 TPS/CPS, run C19

	n	Optimal	Good	Borderline	Poor	Sufficient results ¹	Optimal results
CE-IVD / FDA approved PD-L1 assays*	188	131	52	3	2	97%	70%
Laboratory developed PD-L1 assays based on concentrated antibodies	67	40	26	1	0	99%	60%
PD-L1 assays based on Ready-To-Use antibodies without predictive claims	44	21	17	4	2	86%	48%
Total	299	192	95	8	4		
Proportion		64%	32%	3%	1%	96%	

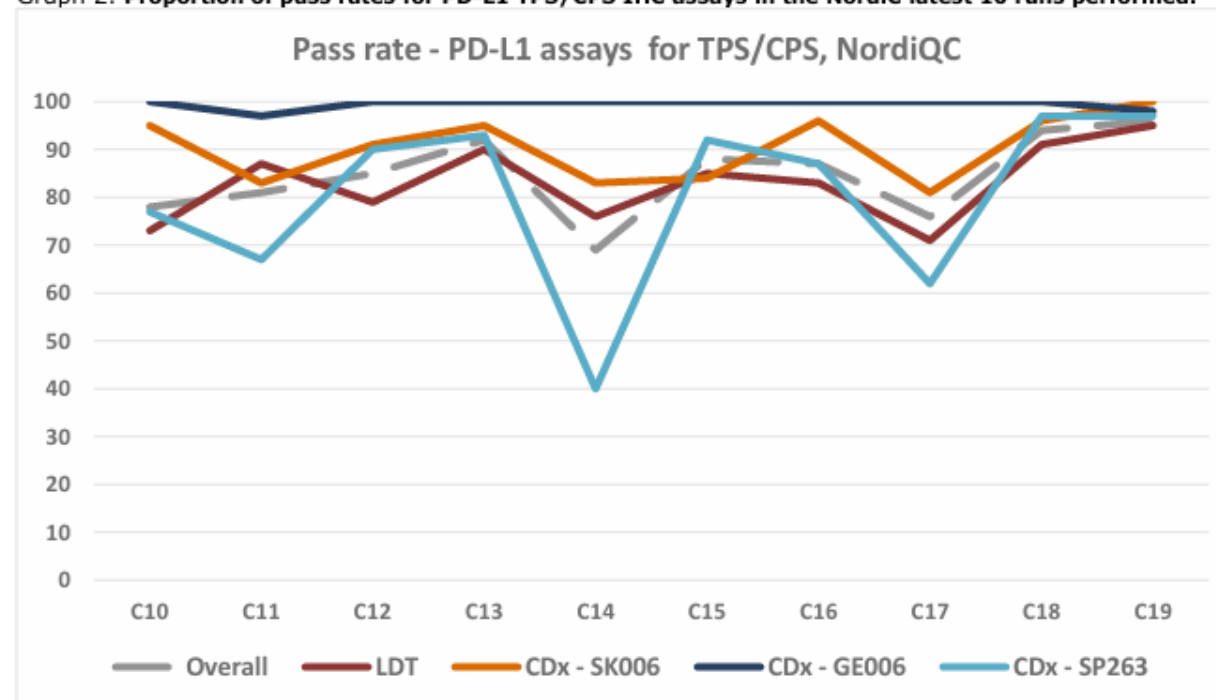
Table 3. Comparison of pass rates for vendor recommended and laboratory modified protocols

CDx assay*	Vendor recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Ventana BenchMark XT, GX, Ultra rmAb SP263, 741-4905	58/60 (97%)	27/60 (45%)	-	-
Ventana BenchMark Ultra rmAb SP263, 740-4907	14/14 (100%)	7/14 (50%)	-	-
Dako Autostainer Link 48+ mAb 22C3 pharmDx, SK006	28/28 (100%)	20/28 (71%)	5/5 (100%)	4/5 (80%)
Dako Omnis mAb 22C3 pharmDx, GE006	64/65 (98%)	60/65 (92%)	9/9 (100%)	9/9 (100%)
Dako Autostainer Link 48+ rmAb 28-8 pharmDx, SK005	0/1	0/1	-	-

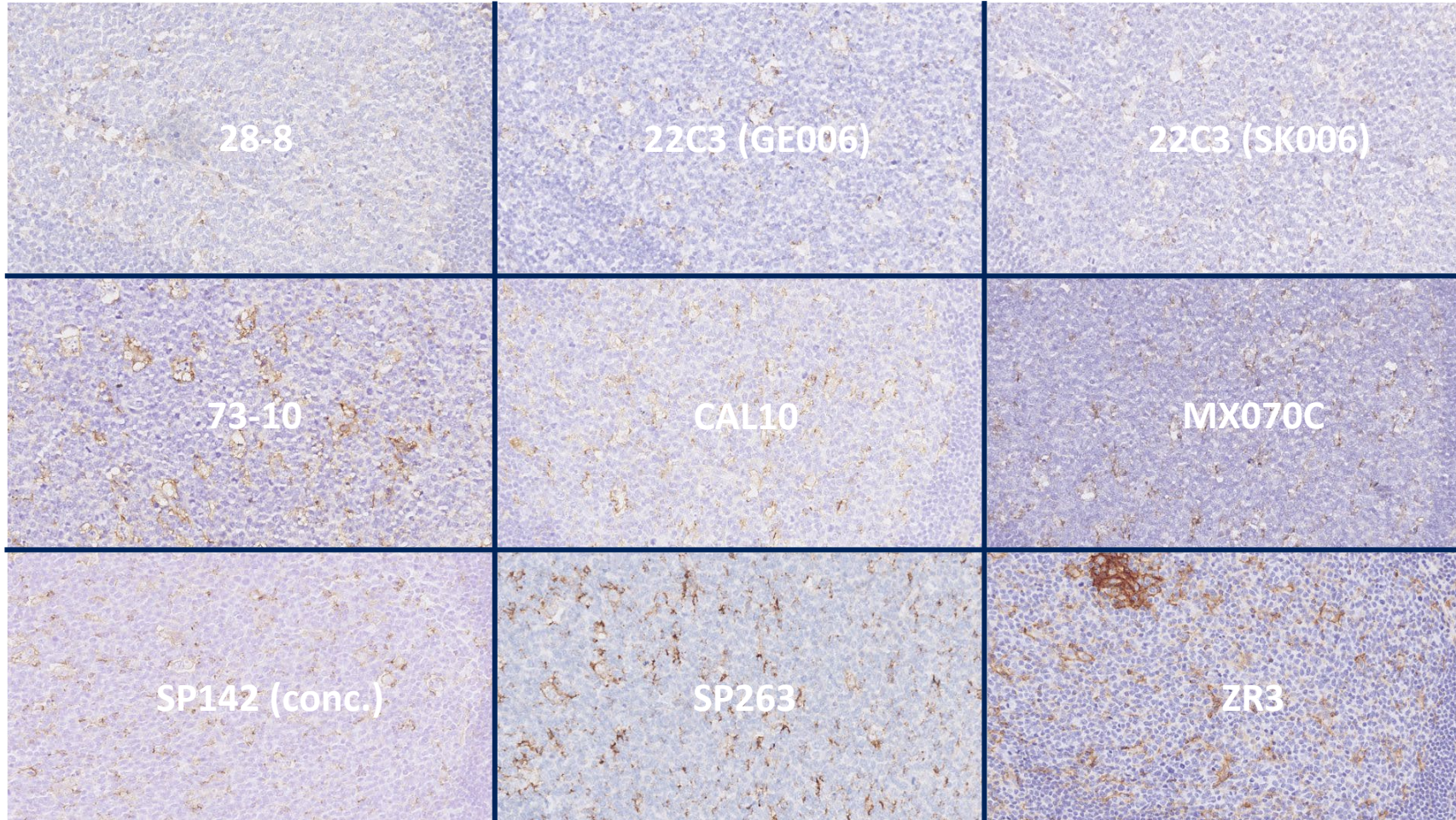
Graph 4. Proportion of sufficient results for PD-L1 TPS/CPS (KEYTRUDA®) in the latest 10 NordiQC runs performed.



Graph 2. Proportion of pass rates for PD-L1 TPS/CPS IHC assays in the NordiQC latest 10 runs performed.



PD-L1 – ICAPS - TONSIL



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



Different assays → different staining patterns.

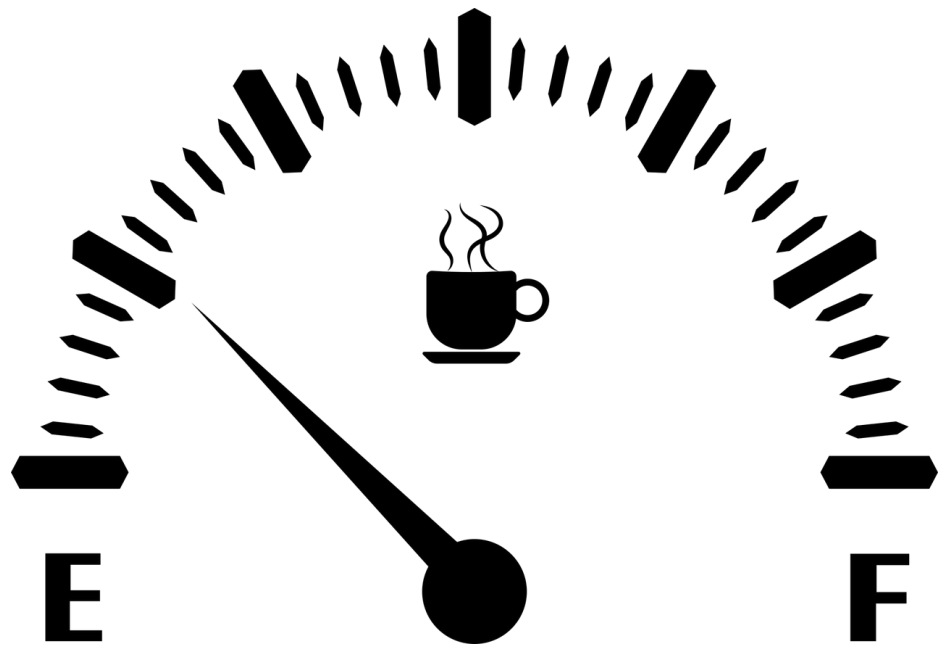
All 9 assays achieved an optimal score for PD-L1 TPS/CPS.

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.
Napsin A	Kidney: Epithelial cells of proximal tubules.	Appendix/Colon: Epithelial cells and macrophages.
Calretinin	Adrenal gland: Cortical epithelial cells.	Appendix/Colon: Epithelial cells.
WT1	Kidney: Podocytes and parietal epithelial cells of Bowman's capsule.	Kidney: Epithelial cells of the tubules.
BAP1	Tonsil: Mantle zone lymphocytes and germinal centre lymphocytes.	Malignant Mesothelioma: Neoplastic cells
CLDN4	Placenta: Cytotrophoblasts.	Appendix: Lymphocytes and smooth muscle cells.
INSM1	Pancreas: Endocrine islets of Langerhans.	Appendix: Columnar epithelial cells and smooth muscle cells.
CGA	Appendix/Colon: Axons and ganglion cells in the nerve plexus.	Appendix/Colon: Epithelial cells and smooth muscle cells.
SYP	Appendix/Colon: Neuroendocrine and scattered goblet cells in epithelial mucosa.	Appendix/Colon: Smooth muscle cells
CD56	Tonsil: NK-cells and scattered T-cells.	Appendix/Colon: Epithelial cells.
p40	Placenta: Dispersed cytotrophoblastic cells.	Tonsil: Lymphocytes.
CK5	Pancreas: Scattered epithelial cells of intercalated ducts.	Liver. All cell types.
ALK (lung)	Appendix/Colon: Dispersed axons of nerve cells.	Tonsil: All cell types.
PD-L1 TPS/CPS	Tonsil: Germinal center macrophages and T-cells.	Tonsil: Stratified normal squamous epithelial cells and vast majority of lymphocytes.



THANK YOU FOR YOUR ATTENTION!



BONUS – ROS1

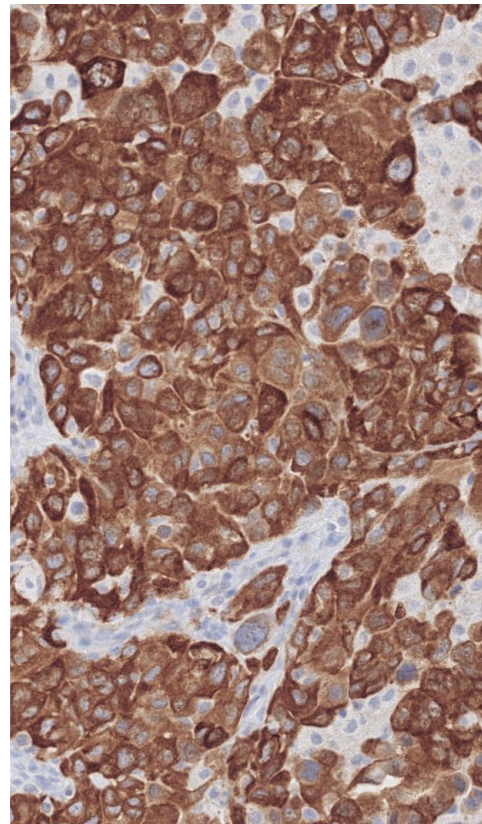
No NordiQC data available for ROS1.

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

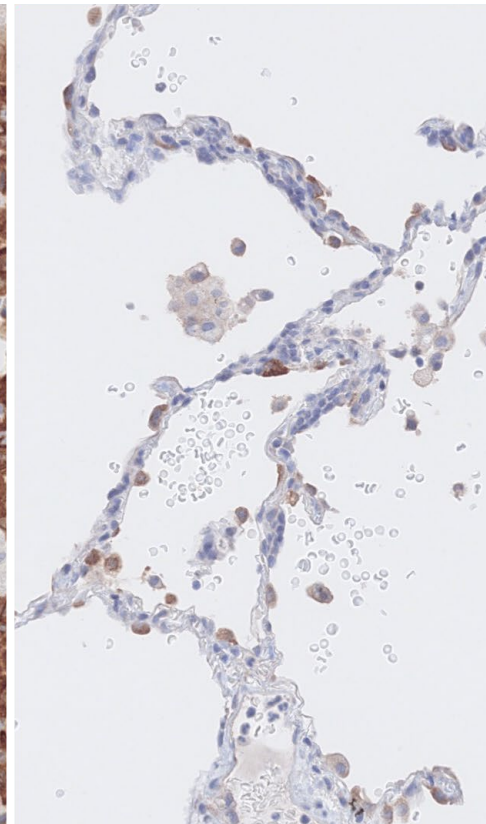
Positive controls:
Tumor with known ROS1-translocation
Type II-pneumocytes in normal lung

Negative control:
Appendix

Tumor with ROS1-
translocation
(lung adenocarc.)



Normal lung



Appendix

