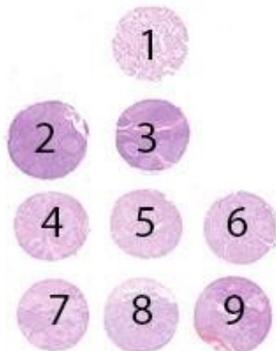


Purpose

This was the eighteenth assessment for PD-L1 in the NordiQC Companion Module. This assessment for PD-L1 TPS/CPS (KEYTRUDA®) primarily focused on the evaluation of the analytical accuracy of the IHC assays performed by the NordiQC participants to identify patients with non-small cell lung cancer (NSCLC) and triple negative breast carcinoma (TNBC) to be treated with KEYTRUDA® as immunotherapy. PD-L1 22C3 pharmDx (Dako/Agilent), was used as the reference standard method, and accuracy was evaluated in carcinomas with the dynamic and critical relevant expression levels of PD-L1 characterized by TPS and CPS. The scores obtained by NordiQC participants is indicative of the performance of the IHC tests but due to the limited number and composition of samples, additional internal validation/verification and extended quality control e.g. regularly measuring the PD-L1 results, is needed.

Material

Table 1. Content of the TMA used for the NordiQC PD-L1 TPS/CPS (KEYTRUDA®) C18 assessment.

	PD-L1 IHC TPS/CPS score*	
Tissue controls		
1. Placenta	See section for controls	
2. Tonsil	See section for controls	
3. Tonsil	See section for controls	
Carcinomas		
4. NSCLC	TPS: No; <1%	
5. NSCLC	TPS: Low; 5-49%**	
6. NSCLC	TPS: High; 95%	
7. TNBC	CPS: <10 IC [#]	
8. TNBC	CPS: ≥10; 10-60 IC ^{##}	
9. TNBC	CPS: ≥10; 25-45 IC+TC [#]	

* Tumour proportion score (TPS) and combined positive score (CPS) determined by PD-L1 IHC 22C3, pharmDx (Dako/Agilent) performed in NordiQC reference lab.

** The tumour showed heterogeneity in the different levels within and in between the TMA's used. In one of the eight TMA's used for the assessment, areas with TPS 50-60% were observed.

[#] IC, Immune cells - TC; Tumour cells.

^{##} The tumour showed heterogeneity in the different levels within and in between the TMA's used. In three of the eight TMA's used for the assessment, areas with CPS <10% were observed.

All tissues were fixed in 10% neutral buffered formalin.

The participating laboratories were asked to perform their PD-L1 IHC assay for predicting likely response to KEYTRUDA® as a treatment option, evaluate the PD-L1 expression level using the TPS and CPS scoring system, and to submit their stained slides and scores to NordiQC. This allowed assessment of the technical performance (analytical accuracy) of the PD-L1 TPS/CPS assays and provided information on the reproducibility and concordance of the PD-L1 read-out results among the laboratories.

PD-L1 TPS/CPS, Technical assessment

In order to account for heterogeneity of PD-L1 expression in the individual tumour cores included in the tissue micro array (TMA) blocks, reference slides were made throughout the blocks. The PD-L1 expression levels were thus characterized in every twenty-fifth slide and during the assessment, TPS and CPS categories for each tissue core on the submitted slides from the participants were compared to the level in the nearest reference slide.

Criteria for assessing a staining as Optimal include:

The staining is considered perfect or close to perfect in all of the included tissues.

TPS/CPS is concordant to the NordiQC reference data in all carcinomas.

Criteria for assessing a staining as Good include:

The staining is considered acceptable (correct PD-L1 TPS/CPS category) in all of the included tissues. PD-L1 expression in one or more tissues varies significantly from the expected TPS/CPS scores, but still in the correct category. The protocol may be optimized to ensure analytical accuracy. The technical quality may be improved for e.g. counter staining, morphology and signal-to-noise ratio. TPS/CPS is still concordant to the NordiQC reference data obtained in all carcinomas.

Criteria for assessing a staining as Borderline include:

The staining is considered insufficient because of a false negative or false positive staining reaction in one of the included carcinomas. The protocol should be optimized. TPS/CPS is **not** concordant to the NordiQC reference data in one of the carcinomas.

Criteria for assessing a staining as Poor include:

The staining is considered very insufficient e.g. because of a false negative or a false positive staining reaction of more than one of the included carcinomas. Optimization of the protocol is urgently needed. TPS/CPS is **not** concordant to the NordiQC reference data in two or more of the carcinomas.

An IHC result can also be assessed as **borderline/poor** related to technical artefacts, e.g. poor signal-to-noise ratio, excessive counterstaining, impaired morphology and/or excessive staining compromising the scoring.

KEY POINTS FOR PD-L1 TPS/CPS IMMUNOASSAYS

- The **CDx** IHC assays with one or more predictive claims provided an overall pass rate of 98% compared to 91% for LD assays.
- The **22C3 CDx** assay GE006, Dako/Agilent was most successful with a pass rate of 100% with VRPS, 98% optimal.
- Insufficient results were mainly caused by reduced proportion of PD-L1 positive cells compared to levels expected and defined by the NordiQC reference standard method, leading to false negative results.

Participation

Number of laboratories registered for PD-L1 KEYTRUDA IHC C18	312
Number of laboratories returning PD-L1 KEYTRUDA IHC slides	288 (92%)
Number of laboratories returning PD-L1 scoring sheet	250

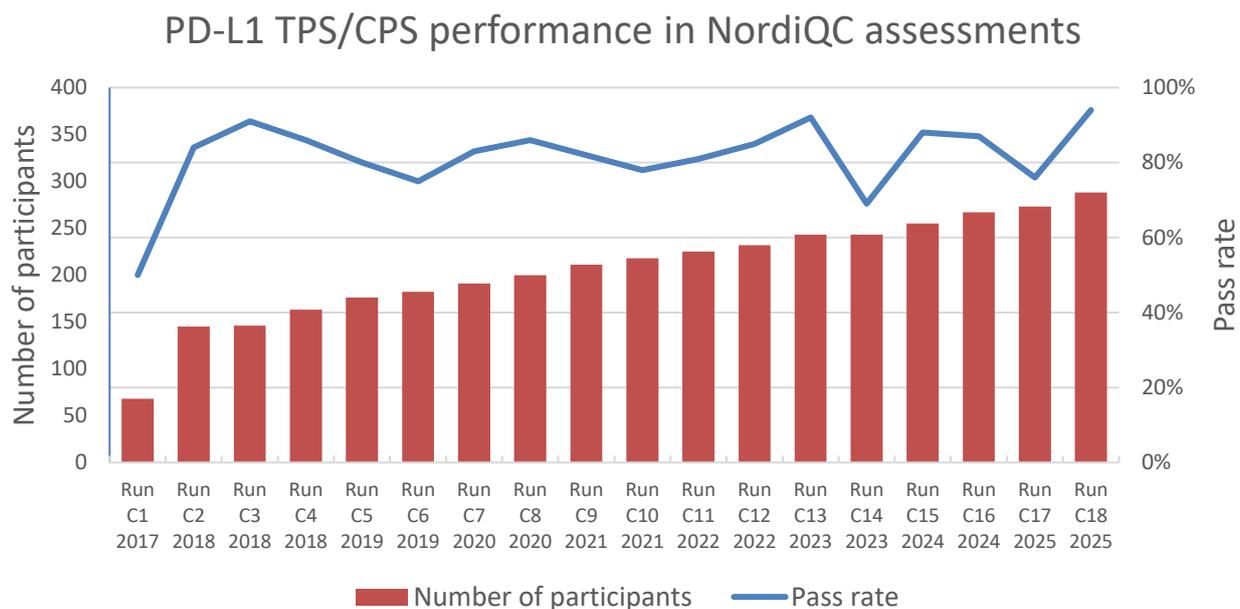
Results

288 laboratories participated in this assessment and returned slides. 94% of the participants achieved a sufficient mark. Assessment marks for IHC PD-L1 assays and PD-L1 antibodies are summarized in Table 2a-2d (see page 3-5). All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data was not included in this report.

Performance history

A relatively consistent pass rate has been observed, with an upward trend - exception of C14. The rate remained steady for C15 and C16, before decreasing again in C17, followed by an increase in this run (C18), which achieved the highest pass rate since C13, as shown in Graph 1 (see page 3). The number of new participants has consistently increased by approximately 3-5% in each run. However, it remained unchanged for C13 and C14, before rising again in the most recent runs C17 and C18, reaching 288.

Graph 1. **Proportion of sufficient results for PD-L1 TPS/CPS (KEYTRUDA®) in the NordiQC runs performed.**



Conclusion

This was the eighteenth NordiQC assessment of PD-L1 for TPS/CPS status with focus on NSCLCs and TNBCs.

288 laboratories participated and a pass rate of 94% was observed.

The PD-L1 IHC pharmDx assay, **22C3 GE006**, Dako/Agilent applied in concordance to the vendor recommended guidelines, was the most successful companion diagnostic assay providing a pass rate of 100%, with an optimal rate of 98%, being superior to the other companion diagnostic assays. The widely applied Ventana/Roche PD-L1 IHC assays 741-4905 and 740-4907 for BenchMark (Ultra/XT/GX) based on the rmAb clone SP263 provided an overall pass rate of 97% and 33% being optimal. This represents a significantly higher performance compared to C17, but still with a relatively low proportion of optimal results.

In this assessment run, the majority of insufficient results were attributed to a reduced proportion of PD-L1 positive cells compared to levels expected and defined by the NordiQC reference standard method leading to false negative results, similarly to C17. However, these findings contrast with more recent NordiQC runs, such as C15 and C16, where the majority of insufficient results were primarily due to technical issues, including extensive cytoplasmic staining reactions and poor signal-to-noise ratios, observed in one or more of the NSCLC and TNBC samples. However, the current pattern is more in line with earlier NordiQC runs for PD-L1 TPS/CPS, such as C14 and some prior assessments.

Table 2a. **Overall results for PD-L1 TPS/CPS, run C18**

	n	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
CE-IVD / FDA approved PD-L1 assays*	192	115	70	5	2	96%	60%
Laboratory developed PD-L1 assays based on concentrated antibodies	60	28	27	5	0	92%	47%
PD-L1 assays based on Ready-To-Use antibodies without predictive claims	36	15	16	3	2	86%	42%
Total	288	158	113	13	4		
Proportion		55%	39%	5%	1%	94%	

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

2) Proportion of optimal results (≥5 assessed protocols).

* Including all protocol settings - both performed as per recommended guidelines or modified settings.

Table 2b. **Assessment marks for CE-IVD / FDA approved PD-L1 assays for PD-L1 TPS/CPS, run C18**

CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
rmAb clone SP263, 741-4905 (VRPS) ³	68	Ventana/Roche	23	43	1	1	97%	34%
rmAb clone SP263, 741-4905 (LMPS) ⁴	4	Ventana/Roche	1	3	-	-	-	-
rmAb clone SP263, 740-4907 (VRPS) ³	8	Ventana/Roche	2	6	-	-	100%	25%
rmAb clone SP142, 741-4860 (VRPS) ³	1	Ventana/Roche	-	-	1	-	-	-
mAb clone 22C3 pharmDX, SK006 (VRPS) ³	28	Dako/Agilent	20	7	1	-	96%	71%
mAb clone 22C3 pharmDX, SK006 (LMPS) ⁴	13	Dako/Agilent	8	3	1	1	85%	62%
mAb clone 22C3 pharmDX, GE006 (VRPS) ³	47	Dako/Agilent	46	1	-	-	100%	98%
mAb clone 22C3 pharmDX, GE006 (LMPS) ⁴	19	Dako/Agilent	13	5	1	-	95%	68%
rmAb clone 28-8 pharmDX, SK005 (VRPS) ³	2	Dako/Agilent	-	2	-	-	-	-
rmAb clone 28-8 pharmDX, SK005 (LMPS) ⁴	2	Dako/Agilent	2	-	-	-	-	-
Total	192		115	70	5	2		
Proportion			60%	36%	3%	1%	96%	

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

2) Proportion of optimal results (≥5 assessed protocols).

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.

Table 2c. **Assessment marks for concentrated antibodies for PD-L1 TPS/CPS, run C18**

Antibodies⁵ for laboratory developed PD-L1 assays, concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 22C3	47	Dako/Agilent	22	21	4	-	92%	47%
rmAb clone QR1	4	Quartett	2	2	-	-	-	-
rmAb clone E1L3N	3	Cell Signaling	-	2	1	-	-	-
rmAb clone H302	3	Gene Tex	2	1	-	-	-	-
rmAb clone 28-8	1	Abcam	-	1	-	-	-	-
rmAb CAL10	1	Biocare Medical	1	-	-	-	-	-
rmAb clone BSR90	1	Nordic Biosite	1	-	-	-	-	-
Total	60		28	27	5	0		
Proportion			47%	45%	8%	0%	92%	

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

2) Proportion of optimal results (≥5 assessed protocols).

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

Table 2d. **Assessment marks for Ready-To-Use antibodies⁶ for PD-L1 TPS/CPS, run C18**

Ready-To-Use antibodies ⁶	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
rmAb clone SP263, 790-4905 (VRPS)⁴	15	Ventana/Roche	7	8	-	-	100%	47%
rmAb clone SP263, 790-4905 (VRPS)³	7	Ventana/Roche	4	2	1	-	86%	57%
rmAb clone SP142, 740-4859 (VRPS)⁴	1	Ventana/Roche	-	-	-	1	-	-
rmAb clone SP142, 790-4860 (VRPS)⁴	1	Ventana/Roche	-	-	-	1	-	-
rmAb clone 73-10 PA0832	4	Leica Biosystems	2	2	-	-	-	-
rmAb clone MX070C MAB-0854	1	Fuzhou Maixin	1	-	-	-	-	-
rmAb clone 28-8 438R-18-ASR	1	Cell Marque	-	-	1	-	-	-
rmAb clone BP6099 I12052E	1	Biolynx	-	1	-	-	-	-
rmAb clone RM320 8263-C010	1	Sakura Finetek	1	-	-	-	-	-
rmAb clone CAL10 API3171	1	Biocare Medical	-	1	-	-	-	-
rmAb clone QR1 P-P001-70	1	Quartett	-	1	-	-	-	-
rmAb Clone GR110 GT256202	1	Gene Tech GAS	-	-	1	-	-	-
rmAb Clone DY49268 492291	1	DAKEWE/BioSci	-	1	-	-	-	-
Total	36		15	16	3	2	-	
Proportion			42%	44%	8%	6%	86%	

1) Proportion of sufficient results (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.

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

Detailed Analysis

CE IVD / FDA approved assays

SP263 (741-4905, Ventana/Roche): In total, 23 of 68 (34%) protocols were assessed as optimal. This product has a locked protocol on all BenchMark platforms and cannot be changed. The protocol is based on Heat Induced Epitope Retrieval (HIER) in Cell Conditioning 1 (CC1) at 100°C for 64 min., 16 min. incubation of primary Ab and OptiView as detection system. Using these protocols settings and applied on the BenchMark platform, 66 of 68 (97%) laboratories produced a sufficient staining result (optimal or good).

SP263, 741-4905 was also applied on other non-intended platforms as Leica Biosystems Bond III and Prime with an overall performance as shown in Table 2b (LMPS).

SP263 (740-4907, Ventana/Roche): In total, 2 of 8 (25%) protocols were assessed as optimal. This product has a locked protocol on the BenchMark platform and cannot be changed. The protocol is based on HIER in CC1 at 100°C for 64 min., 16 min. incubation of primary Ab and OptiView as detection system. Using these protocols settings, 8 of 8 (100%) laboratories produced a sufficient staining result.

PD-L1 IHC 22C3 pharmDx (SK006, Dako/Agilent): In total, 20 of 28 (29%) protocols were assessed as optimal, when applied accordingly to the vendor recommended protocol settings based on HIER using EnVision™ FLEX Target Retrieval Solution (TRS) low pH 6.1 at 95-99°C for 20 min. in PT Link, 30 min. incubation of the primary antibody, EnVision™ FLEX+ as the detection system and performed on Autostainer Link 48. Using these protocol settings, 27 of 28 (96%) laboratories produced a sufficient staining result.

SK006 was also used with modified protocol settings e.g., electing for other platforms such as Ventana BenchMark, Leica Bond III or Dako/Agilent Omnis with a slightly inferior pass rate (85%) and performance as shown in Table 2b (data points relatively few and must be interpreted with caution).

PD-L1 IHC 22C3 pharmDx (GE006, Dako/Agilent): In total, 46 of 47 (98%) protocols were assessed as optimal, when applied accordingly to the vendor recommended protocol settings HIER using EnVision™ FLEX TRS low pH 6.1 (GV805) at 95-99°C for 40 min., 40 min. incubation of the primary antibody, EnVision™ FLEX+ as the detection system and performed on Omnis. Using these protocol settings, 47 of 47 (100%) laboratories produced a sufficient staining result.

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used CE IVD / FDA approved assays. The performance was evaluated both as “true” plug-and-play systems performed strictly accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended automated IHC platform are included (in Table 1 LMPS also includes off label use on deviant IHC stainers).

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	66/68 (97%)	23/68 (34%)	4/4	1/4
Ventana BenchMark Ultra rmAb SP263, 740-4907	8/8 (100%)	2/8 (25%)	-	-
Dako Autostainer Link 48+ mAb 22C3 pharmDX, SK006	27/28 (96%)	20/28 (71%)	11/13 (85%)	8/13 (62%)
Dako Omnis mAb 22C3 pharmDX, GE006	47/47 (100%)	46/47 (98%)	18/19 (95%)	13/19 (68%)
Dako Autostainer Link 48+ rmAb 28-8 pharmDX, SK005	2/2	0/2	2/2	2/2

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

**Modifications in one or more of above-mentioned parameters. Only protocols performed on the specified vendor IHC stainer are included.

Concentrated antibodies for laboratory developed (LD) assays

mAb clone **22C3**: In total, 22 of 47 (47%) protocols were assessed as optimal of which 11 were stained on the BenchMark Ultra platform (Ventana/Roche), 2 on the BenchMark Ultra Plus platform (Ventana/Roche), 5 on the Omnis platform (Dako/Agilent), and 4 on Autostainer Link 48 (Dako/Agilent).

On BenchMark Ultra, the protocols providing optimal results were based on a titre of 1:20-50 for mAb clone 22C3, incubation time of 60-80 min., HIER in CC1 for 32-80 min. and OptiView as the detection system. Using these protocol settings, 11 of 24 (46%) laboratories produced optimal staining results, and 22 of 24 (92%) laboratories produced sufficient staining results.

On Omnis, the protocols providing optimal results for mAb clone 22C3 were based on a titre of 1:20-30 of the primary antibody, incubation time of 30-40 min., HIER in TRS low pH 6.1 at 97°C for 30-50 min. and EnVision™ FLEX+ as detection system. Using these protocol settings, 5 of 7 (71%) laboratories produced optimal results and 7 of 7 (100%) laboratories produced a sufficient staining result.

mAb clone **QR1**: 2 of 4 protocols was assessed as optimal. The optimal protocols for this clone were based on a titre of 1:50-100 of the primary antibody, incubation time of 15-60 min., HIER in Bond Epitope Retrieval Solution (BERS2, Leica Biosystems) pH 9 at 100°C for 20-30 min., Bond™ Refine was applied as the detection system and performed on Bond III (3/4) & MAX (1/4) platforms (Leica Biosystems). Using these protocol settings, 2 of 4 (50%) laboratories produced optimal results.

Table 4. **Optimal results for PD-L1 for the most commonly used antibody as concentrate on the four main IHC systems***

Concentrated antibodies	Ventana/Roche BenchMark ¹		Dako/Agilent Autostainer ²		Dako/Agilent Omnis		Leica Biosystems Bond III/ MAX	
	CC1 pH 8.5	CC2 pH 6.0	TRS pH 9.0	TRS pH 6.1	TRS High pH	TRS Low pH	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone 22C3	13/29** (45%)	-	-	4/4**	0/1	5/6** (83%)	0/4**	0/1**

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

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

1) BenchMark, XT, Ultra, Ultra Plus

2) Autostainer Link 48

Block construction and assessment reference standards

The tissue micro array (TMA) blocks constructed for this PD-L1 run consisted of three NSCLCs, three TNBCs, two tonsils and one placenta. The NSCLCs were selected to comprise PD-L1 expression levels for each TPS category: TPS negative (<1% PD-L1 positive tumour cells), TPS low (≥1-49%) and TPS high (≥50%). The TNBCs were selected to comprise one carcinoma with CPS<10 and two carcinomas with CPS≥10 - one with PD-L1 expression primarily in immune cells and one with PD-L1 expression in both tumour cells and immune cells. Reference slides throughout the individual TMA blocks (interval at each twenty-fifth slide) were stained using the companion diagnostic assay 22C3 pharmDX GE006 (Dako/Agilent).

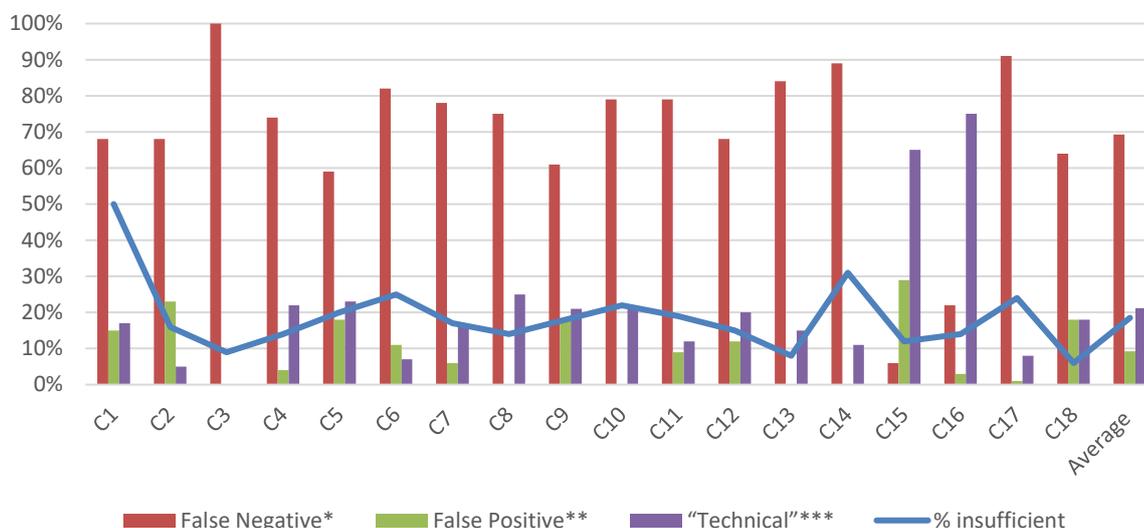
In total, eight identical TMA blocks were constructed and all eight of these were used for this assessment. Reviewing the reference slides from the blocks, a heterogenic expression of PD-L1 was seen in two of the tumour cores. Of particular importance for the NSCLC, tissue core no. 5, most areas showed a TPS of 10-20% (TPS low), but in a few areas a TPS 50-60% (TPS high) was observed. For tissue core no. 8, most areas showed a CPS of 10-60 (CPS high) but focally CPS <10 (CPS low) were observed. During the assessment, TPS and CPS categories for each tissue core on the submitted slides were compared to the level in the nearest reference slides. Heterogeneity in PD-L1 expression is well known in NSCLCs and the assessment in this sense emulated clinical settings.

Comments

In this eighteenth NordiQC assessment for PD-L1 TPS/CPS (KEYTRUDA®), the prevalent feature of an insufficient staining result was reduced proportion of PD-L1 positive cells compared to levels expected and defined by the NordiQC reference standard methods, leading to false negative results, being observed in 64% of the insufficient results. 18% of the insufficient results were caused by false positive staining results and 18% due to technical issues such as poor-signal-to-noise ratio, excessive cytoplasmic staining reaction or a coarse and indistinct granular staining reaction compromising the scoring of the PD-L1 status in one or more of the carcinomas. As shown in Graph. 2, a false negative staining result has been the most common reason for insufficient staining results up until C15, and then again in run C17 and this run - C18 of the NordiQC PD-L1 TPS/CPS (KEYTRUDA®) assessments.

Graph 2. **Prevalence and characteristics of insufficient results**

Characteristics of insufficient results in the NordiQC PD-L1 TPS/CPS assessments.



* TPS changes from high to low or low to negative. And/or CPS changes from ≥ 10 to < 10 .

** TPS changes from negative to low or low to high. And/or CPS changes from < 10 to ≥ 10 .

*** Interpretation compromised e.g. by poor-signal-to noise ratio, poor morphology, excessive cytoplasmic staining reaction etc.

In order to evaluate IHC accuracy, NordiQC strives to include neoplasms with PD-L1 levels close to the critical and clinically relevant thresholds for positivity focusing on both intensity, proportion and subtypes of cells to be scored mimicking real-life diagnostics.

The NSCLC, tissue core no. 5, characterized as TPS low by the NordiQC reference standard method, was the most challenging to obtain an optimal result.

39% (n=113) of the slides submitted were marked as "Good". In 60% of these (68 of 113), this was due to a significantly reduced TPS/CPS level, but with no change of the TPS/CPS-category in any of the carcinomas and thus still an accurate PD-L1 status for treatment decision. 5% (6 of 113) had an increased TPS/CPS level observed compared to the level expected. In the remaining 35% (39 of 113) of the results assessed as "Good" these were characterized by poor signal-to-noise ratio, impaired morphology, too weak or excessive counterstaining and/or a coarse granular staining reaction compromising the evaluation of the membranous staining reaction. The latter only seen for protocols based on OptiView with amplification kit (Ventana/Roche).

The Ventana/Roche PD-L1 IHC assays 741-4905 and 740-4907 for BenchMark (Ultra/XT/GX) with predictive claims, based on the **SP263** clone, were used by 26% (76 of 288) of the participants and in total provided an overall pass rate of 97% (74 of 76), with 33% (25 of 76) being assessed as optimal when applied by protocol settings in compliance with vendor recommendations (see Table 3). The assays are locked for central protocol settings and based on HIER in CC1 for 64 min., incubation in primary Ab for 16 min. and use of OptiView as the detection system. The proportion of optimal results seen are still inferior to the level seen for the 22C3 IHC pharmDx assays (Dako/Agilent), but the gap has significantly improved in this run. In previous assessment runs - C10 onwards, a relatively high number of SP263 results have been characterized by a reduced analytical sensitivity providing a lower TPS level compared to the level seen for the 22C3 pharmDx assays. At present, no explanation for this discrepancy has been identified. However, the observation of a reduced analytical sensitivity of the Ventana/Roche SP263 PD-L1 IHC assays compared to the 22C3 pharmDx assay has recently also been addressed in a publication by Javier Perez et al*. This study revealed that the SP263 assay showed a low positive percent agreement at 82.7% to the 22C3 IHC assay.

* Perez J, et al. Clinical Interchangeability of PD-L1 Immunohistochemistry Assays in First-Line Non-Small Cell Lung Cancer Management With Cemiplimab. *JCO Precis Oncol.* 2025 Sep;9:e2500177. doi: 10.1200/PO-25-00177. Epub 2025 Sep 24. PMID: 40991883;

The Dako/Agilent **22C3** pharmDx assay GE006 for Dako Omnis was used by 16% (47 of 288) of the participants providing a pass rate of 100% (98% optimal) when applied by protocol settings in compliance with vendor recommendations (see Table 3).

Consistent with findings from previous runs, it was observed that the PD-L1 22C3 GE006 assay for Omnis was more successful compared to the **22C3** pharmDx SK006 for Autostainer Link 48. The superior performance of GE006 might in part be related to a more consistent reproducibility of the 22C3 pharmDx assay on the fully automated Dako Omnis platform compared to the assay when applied on the semi-automated Autostainer Link 48. In this context it has to be emphasized that the 22C3 GE006 assay for Dako Omnis is by Dako/Agilent only validated for PD-L1 status and predictive claim in NSCLC with TPS as scoring system and at present not validated by Dako/Agilent for any indication with CPS as scoring system including TNBC.

The Dako/Agilent **22C3** pharmDx assay SK006 for Autostainer Link 48 was used by 10% (28 of 288) of the participants and provided a pass rate of 96% (71% optimal) when applied by protocol settings in compliance with vendor recommendations (see Table 3). The 22C3 SK006 assay was also applied off-label (n=13), both on Autostainer 48 Link using modified protocol settings or on non-Autostainer Link 48 platforms as e.g. BenchMark Ultra (Ventana/Roche), BOND III (Leica Biosystems) and Omnis (Dako/Agilent), and as shown in Table 2b in this run with a pass rate of 85% and as such slightly inferior as when applied as per recommendations.

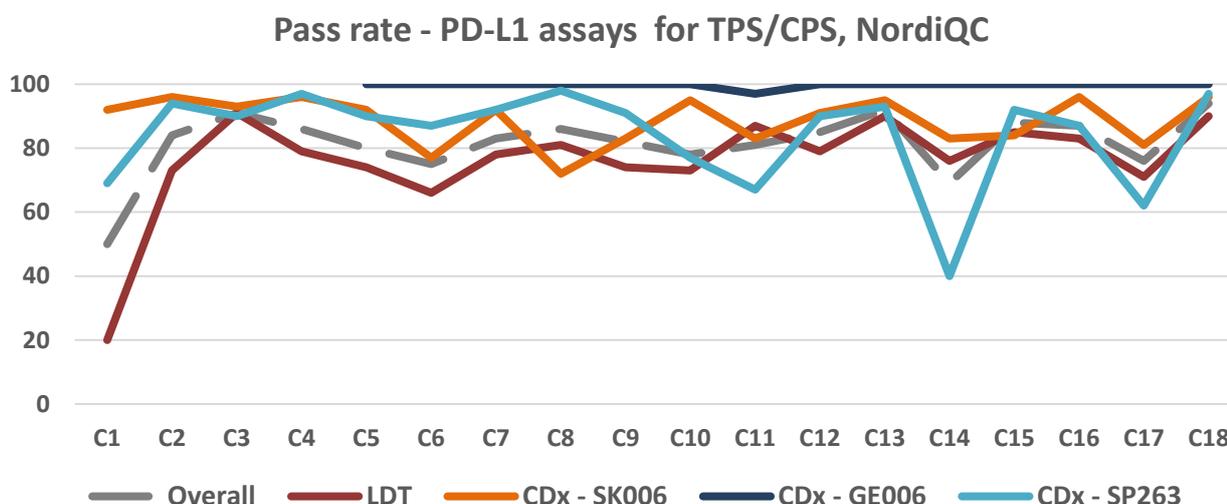
The Dako/Agilent pharmDx SK005 rmAb **28-8** for Autostainer Link 48 was used by 2 laboratories, using the recommended protocol settings being assessed as good. It was applied off-label by 2 laboratories using modified protocol settings 1 on the Autostainer Link 48 and the other on the Omnis with both being assessed as Optimal.

Overall, 158 participants used one of the PD-L1 IHC CDx assays with one or more predictive claims for immune-oncology (22C3 SK006/GE006, SP263 741-4905/740-4907 and 28-8, SK005) by VRPS and a pass rate of 98% (155/158), was achieved.

Laboratory developed (LD) assays either based on a concentrated antibody, a RTU antibody without any predictive claim or a companion diagnostic assay not used strictly accordingly to vendor recommendations were applied by 47% (134 of 288) of the participants. For this group a pass rate 90% was observed which is considerably higher compared to the level of 71% seen in the last assessment runs (C17 and C16) (82%). Focusing on the performance of PD-L1 LD assays from C2-C18, excluding the initial run C1 and start-up phase to identify "best practice LD assays", the mean pass rate for LD assays has been 79% (range 66%-91%) compared to e.g., 100% for the 22C3 GE006 pharmDx (Dako/Agilent), 89% for 22C3 SK006 pharmDx (Dako/Agilent) and 85% for the SP263 assay (Ventana/Roche).

The performance of CDx and LD IHC assays for PD-L1 is summarized and shown in Graph 3 below.

Graph 3. **Proportion of pass rates for PD-L1 TPS/CPS IHC assays in the NordiQC runs performed.**



The mAb clone **22C3** was the most widely used concentrated antibody within a LD assay (n=47), achieving a pass rate of 92% and an optimal rate of 47%. This performance is the highest seen to date, and significantly higher than the C17 pass rate of 76%, and optimal rate of 24%.

As described above for optimal protocol settings for mAb clone 22C3 as concentrated format, successful and interlaboratory reproducible settings have been identified for BenchMark (Ventana/Roche) and Omnis (Dako/Agilent) and these seem to be widely consolidated within the laboratories providing a pass rate largely comparable to most companion diagnostic assays in this assessment as show in Graph 3.

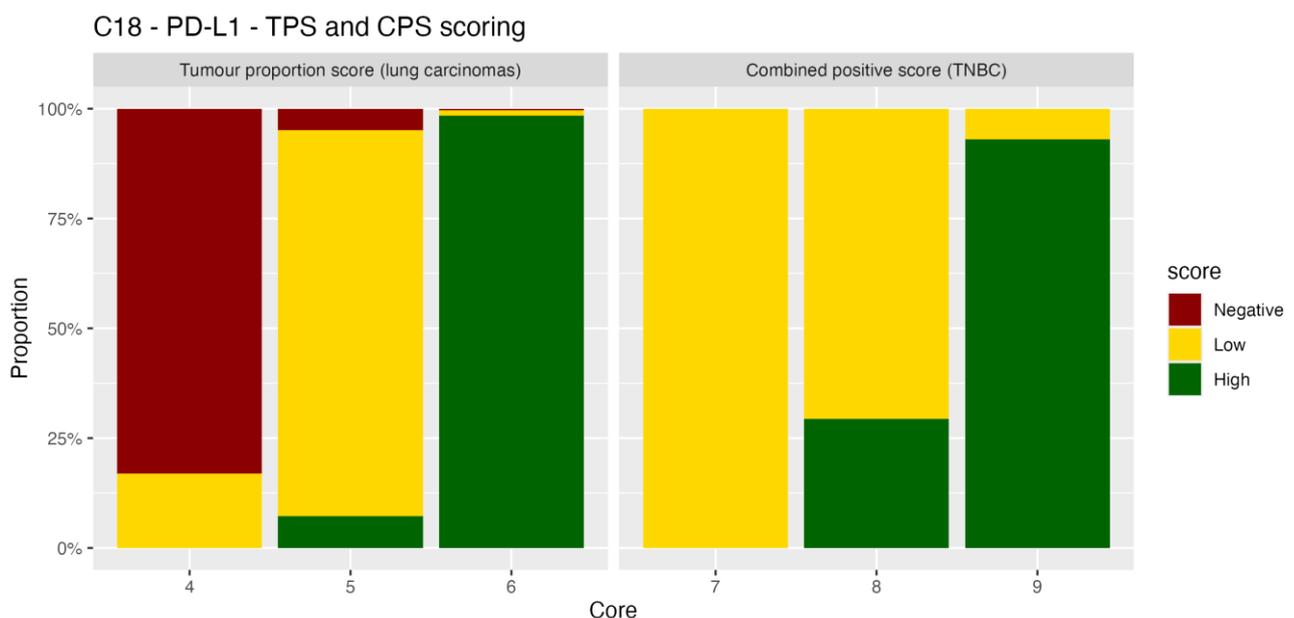
As noted in previous reports, the performance of the mAb clone 22C3 on the Bond III / Bond MAX (Leica Biosystems) platforms has generally been inferior. However, in run C13, a 100% sufficient pass rate was observed, with one participant achieving an optimal result. This improvement was not sustained in subsequent runs C14 and C15, where no participants achieved an optimal result. Interestingly, in run C16, one participant again achieved an optimal result, but this was not maintained in run C17, where no participants achieved either an optimal or sufficient result. However, in C18 three of the five participants achieved a 'Good' result and two achieving an insufficient result. Cumulated data for runs C8 - C18 evaluating the performance of mAb clone 22C3 on the Bond platforms have shown a pass rate of 36% (16 of 44), with only 2 optimal results achieved to date. Given the limited number of observations, these findings should be interpreted with caution. However, in this context it should be mentioned that the rmAb clone QR1 (Quartett) seemed to be a more reproducible antibody for the Leica Bond IHC platforms, as 3 out of 3 protocols gave an optimal result when applied by the protocol settings listed on page 6 in C17. In this run a slightly inferior performance was obtained as only 1 of 3 submitted protocols gave an optimal result while the remaining achieving a result assessed as good. However, in total, six of six protocols based on QR1 have provided a sufficient result in these two consecutive runs, being promising.

The Leica Biosystems PD-L1 IHC RTU assay based on rmAb clone **73-10** (PA0832) with intended use on Bond III, was used by 4 participants and all 4 results were assessed as sufficient, 2 being optimal. From Run C13 to this run C18 an overall pass rate of 88% (23/26) has been achieved and 39% being optimal.

The commonly used Ventana/Roche IHC RTU assay 790-4905, **SP263** without predictive claim showed a slightly superior performance in run C18 (as was also seen in C17) compared to the corresponding locked assay 741-4905 giving a pass rate of 100% (47% optimal) when applied by vendor recommended protocol settings. When used by laboratory modified protocol settings a lower pass rate of 86% but higher optimal scores (57%) was observed. No single modified protocol settings as prolonging HIER or Ab incubation time explaining the improved performance could be identified.

PD-L1 interpretation and scoring consensus:

Participants were asked to score each of the cores using either tumour proportion score (TPS) for the NSCLCs or combined positive score (CPS) for the TNBCs.



Graph 4. NordiQC PD-L1 run C18: Tumour Proportion scores (TPS) in NSCLCs (core no. 4-6) and Combined Positive Score (CPS) in TNBCs (core no. 7-9).

As shown in Graph 4, relatively high consensus rates were observed for tissue cores 5–7 and 9. In contrast, cores 4 and 8 showed lower consensus. The NSCLC, tissue core 4 was by the NordiQC reference method characterized as TPS negative, but contained many immune cells, especially large macrophages, that might have been scored as tumour cells. Within the TNBC tissue core 8, immune cells were positive while tumour cells were negative. Moreover, the CPS in many blocks were near the CPS 10 threshold that distinguishes high from low expression, likely contributing to the disagreement observed in core 8.

Controls

Throughout all assessments for PD-L1 TPS/CPS tonsil and placenta have been used as positive and negative tissue controls and tonsil has been found to be superior to placenta, as tonsil typically display a dynamic and clinically relevant range of PD-L1 expression levels from low to high, whereas placenta typically only contain cells (trophoblasts) with high level PD-L1 expression.

In tonsil, protocols with optimal results for PD-L1 TPS/CPS status typically provide the following reaction pattern:

A moderate to strong predominantly membranous staining reaction in the crypt epithelial cells, a weak to moderate, typically punctuated membranous staining reaction of the majority of germinal centre macrophages and scattered intra- and interfollicular lymphocytes and macrophages showing a coarse punctuated granular cytoplasmic staining reaction. No staining reaction in the vast majority of lymphocytes and normal stratified squamous epithelial cells.

It has been observed that different assays and/or clones for PD-L1 TPS/CPS status give different staining patterns in tonsil, which must be taken into account when evaluating the reaction pattern and to verify if the result is as expected. The rmAb clone SP263 (741-4905, 790-4905, 740-4907), Ventana/Roche) typically provide a higher proportion of positive inter- and intra-follicular immune cells compared to the Dako/Agilent 22C3 PD-L1 assays (SK006 and GE006).

For other clones, e.g. rmAb clone CAL10 and E1L3N typically a stronger staining reaction in more germinal centre macrophages were seen compared to mAb clone 22C3, when the clones still provided otherwise optimal and accurate results in the carcinomas. This emphasizes that the expected test performance characteristics in tonsil must be correlated to the PD-L1 IHC test/clone used both for the inter- and intra-PD-L1 IHC reproducibility evaluation.

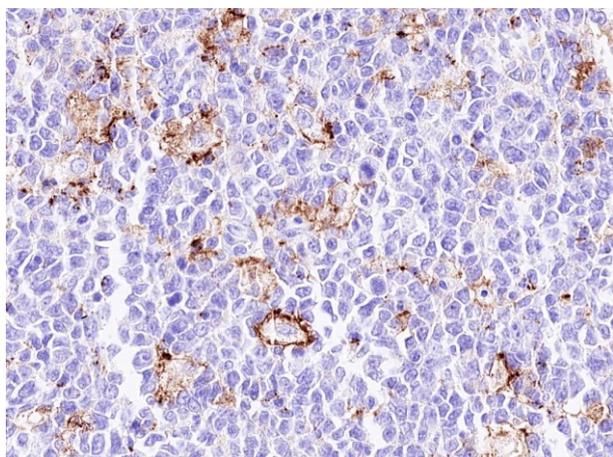


Fig. 1a
Optimal staining result of tonsil using the PD-L1 IHC 22C3 GE006 pharmDx kit, Dako/Agilent, following the vendor recommended protocol settings.

A weak to moderate, but distinct punctuated membranous staining reaction of germinal centre macrophages and dispersed lymphocytes is seen.

No staining reaction is seen in the vast majority of lymphocytes.

Also compare with Figs. 2a – 5a, same protocol.

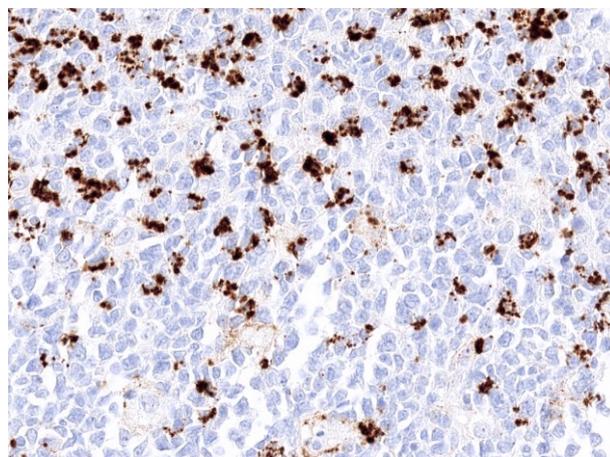


Fig. 1b
Staining result of tonsil, using the rmAb clone SP142 assay 790-4860 from Ventana/Roche.

An increased proportion of T-cells are demonstrated showing a strong granular punctuated staining reaction. The germinal centre macrophages are almost negative and only show a focal punctuated membranous staining reaction.

Also compare with Figs. 2b - 4b, same protocol.

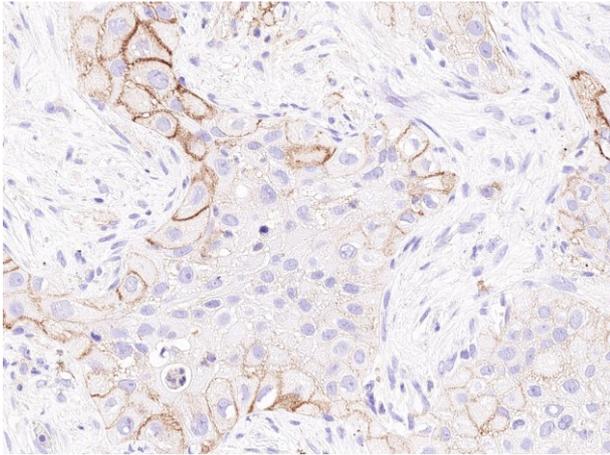


Fig. 2a
Optimal staining result of the NSCLC, tissue core no. 5, using the same protocol as in Fig. 1a.
A weak to moderate, distinct membranous staining reaction is seen in about 40% of all tumour cells.
Overall, the tumour was categorized as TPS Low ($\geq 1-49\%$) and thus eligible for second line immune therapy with KEYTRUDA® (different regional cut-offs occur).

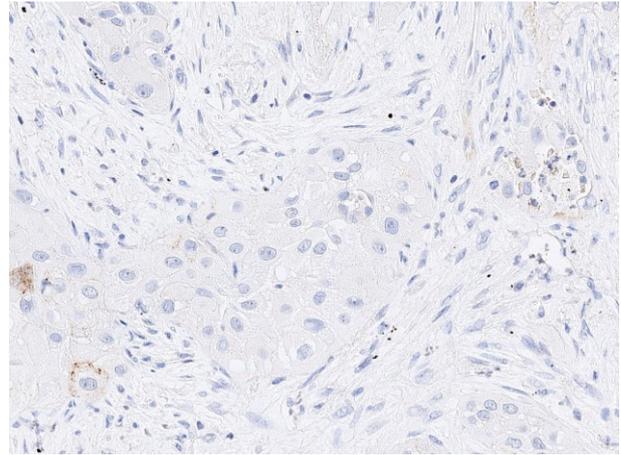


Fig. 2b
Insufficient staining result of the NSCLC, tissue core no. 5, using the same protocol as in Fig. 1b.
Overall, less than 1% of the tumour cells showed a membranous staining reaction and the PD-L1 category being changed from the expected TPS Low to TPS Negative and the tumour not being eligible for immune therapy.
Compare to the expected result as shown in Fig. 2a.

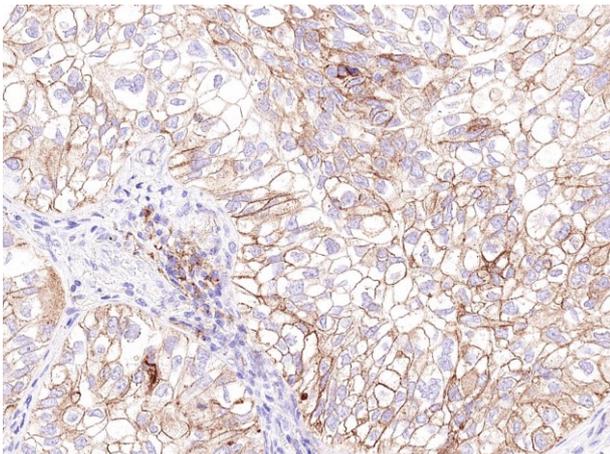


Fig. 3a
Optimal staining result of the NSCLC, tissue core no. 6, using the same protocol as in Figs. 1a and 2a.
A weak to strong, distinct membranous staining reaction is seen in virtually all tumour cells. The tumour was categorized as TPS High ($\geq 50\%$) and thus eligible for first line immune therapy with KEYTRUDA® (different regional cut-offs occur).

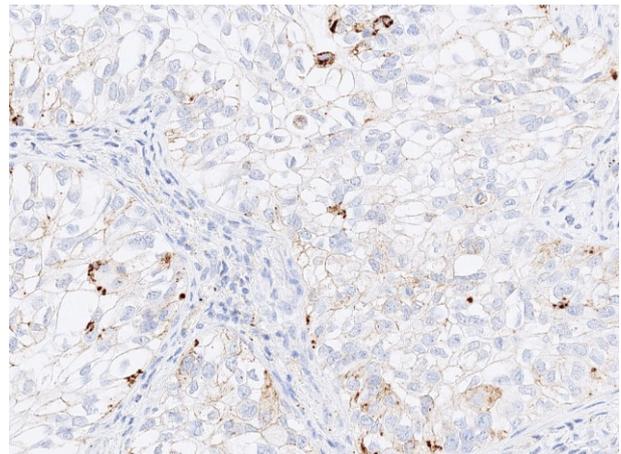


Fig. 3b
Insufficient staining result of the NSCLC, tissue core no. 6, using the same protocol as in Figs. 1b and 2b.
Less than 50% of the tumour cells show a membranous staining reaction and the PD-L1 category being changed from the expected TPS High to TPS Low and the tumour not being eligible for first line immune therapy with KEYTRUDA® (different regional cut-offs occur).
Both the participating laboratory and the NordiQC assessor panel scored the result as TPS Low.
Compare to the expected result as shown in Fig. 3a.

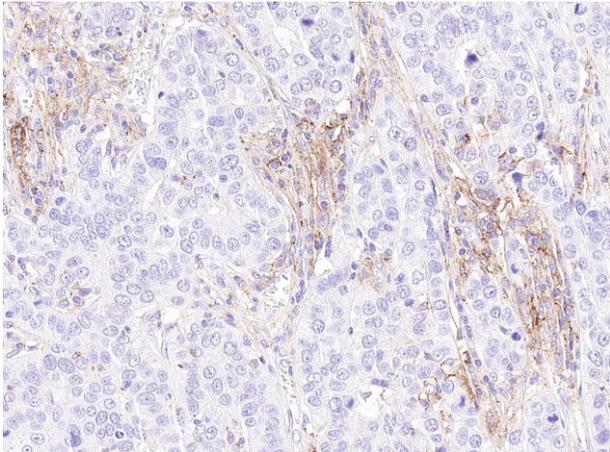


Fig. 4a
Optimal staining result of the TNBC, tissue core no. 8, using the same protocol as in Figs. 1a - 3a.
No staining reaction is observed.
The tumour was categorized as CPS High (≥ 10) and thus eligible for immune therapy with KEYTRUDA®.

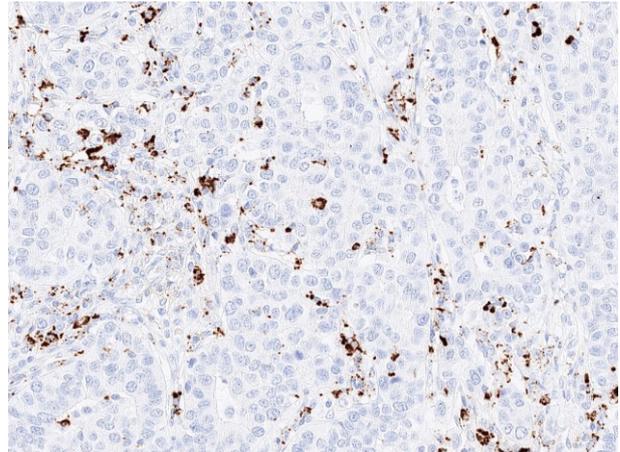


Fig. 4b
Staining result of the TNBC, tissue core no. 8, using the same protocol as in Figs. 1b-3b.
The staining pattern not fully identical to the result obtained by the Dako/Agilent 22C3 pharmDx assay, as a more granular staining reaction is seen and an increased number of immune cells being demonstrated. However, the tumour is still categorized as CPS High (≥ 10) and thus eligible for immune therapy with KEYTRUDA®.

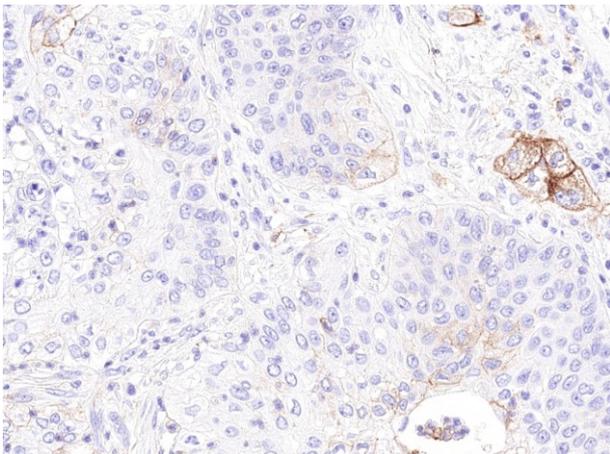


Fig. 5a
Optimal staining result of the NSCLC, tissue core no. 5, using the same protocol as in Fig. 1a.
Same tumour as in Fig. 2a, but from another TMA. Overall, the tumour typically showed a TPS ranging from 5-49%.
In this area a weak but distinct membranous staining reaction is seen in about 5% of all tumour cells.
The result as such being categorized as TPS Low ($\geq 1-49\%$) and thus eligible for second line immune therapy with KEYTRUDA® (different regional cut-offs occur).

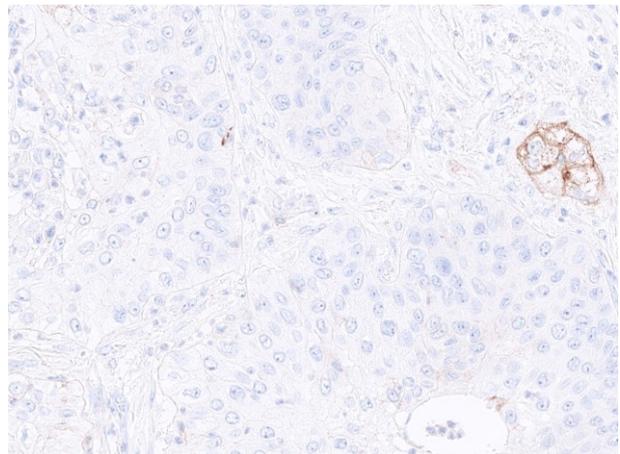


Fig. 5b
Staining result of the NSCLC, tissue core no. 5, using the Ventana/Roche SP263 IHC assay, 741-4905.
Same area and virtual neighbouring section to the optimal result shown in Fig. 5a.
A very weak but interpretable membranous staining reaction is seen in $\geq 1\%$ of the tumour cells and still being categorized as TPS Low.
The overall result was evaluated as sufficient with the assessment mark "Good" with a comment reduced proportion of tumour cells demonstrated.

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