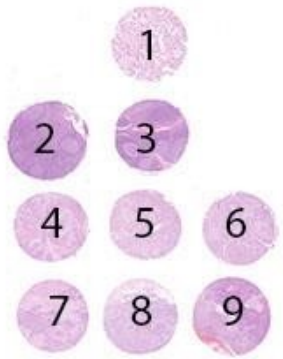
Assessment Run C17 2025
PD-L1 TPS/CPS

Purpose
This was the seventeenth 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®) C17 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: High; 70-100%
6. NSCLC	TPS: Low; 15-49%***
7. TNBC	CPS: <10 IC#
8. TNBC	CPS: ≥10; 15-60 IC#
9. TNBC	CPS: ≥10; 25-70 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 three of the eight TMA's used for the assessment, areas with TPS 1-5% were observed.
*** The tumour showed heterogeneity in the different levels within and in between the TMA's used. In two of the eight TMA's used for the assessment, areas with TPS 50-60% were observed.
# IC, Immune cells - TC; Tumour cells.

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 <u>Optimal</u> include:
The staining is considered perfect or close to perfect in all of the included tissues.
<u>TPS/CPS is concordant to the NordiQC reference data in all carcinomas.</u>

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 80% compared to 71% for LD assays.
- The **22C3 CDx** assay GE006, Dako/Agilent was most successful with a pass rate of 100% with VRPS, 71% optimal (a lower optimal rate observed compared to previous runs).
- Insufficient results were mainly caused by reduced proportion of PD-L1 positive cells compared to levels expected and defined by the NordiQC reference standard methods, leading to false negative results.

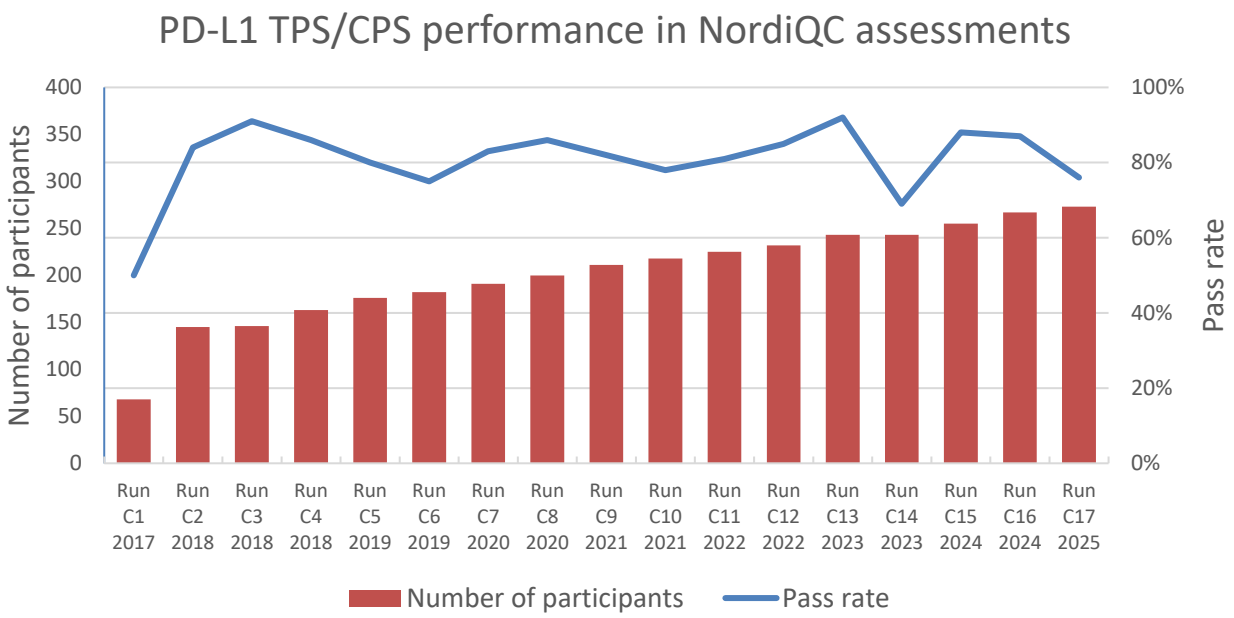
Participation

Number of laboratories registered for PD-L1 KEYTRUDA IHC C17	299
Number of laboratories returning PD-L1 KEYTRUDA IHC slides	273 (91%)
Number of laboratories returning PD-L1 scoring sheet	235

Results
273 laboratories participated in this assessment and returned slides. 76% 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. However, the rate remined steady for C15 and C16, before decreasing by 11% in this run – C17, 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, C15 and C17, reaching 273.

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



Conclusion

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

273 laboratories participated and a pass rate of 76% 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 71%, being superior to the other companion diagnostic assays. However, the optimal rate is considerably lower than seen in previous runs. 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 62%. This represents a significantly lower performance compared to previous NordiQC runs, although not as low as observed in Run C14.

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 methods, leading to false negative results. This finding contrasts 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 C17**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
CE-IVD / FDA approved PD-L1 assays*	168	56	79	29	4	80%	33%
Laboratory developed PD-L1 assays based on concentrated antibodies	56	14	26	10	6	71%	25%
PD-L1 assays based on Ready-To-Use antibodies without predictive claims	49	6	26	11	6	67%	13%
Total	273	76	131	50	16		
Proportion		28%	48%	18%	6%	76%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).
* Including all protocol settings - both performed as per recommneded guidelines or modified settings.

Table 2b. **Assessment marks for CE-IVD / FDA approved PD-L1 assays for PD-L1 TPS/CPS, run C17**

CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone SP263, **741-4905 (VRPS)[3]**	45	Ventana/Roche	3	29	12	1	71%	7%
rmAb clone SP263, **741-4905 (LPMS)[4]**	2	Ventana/Roche	-	2	-	-	-	-
rmAb clone SP263, **740-4907 (VRPS)[3]**	13	Ventana/Roche	-	4	7	2	31%	-
mAb clone 22C3 pharmDX, **SK006 (VRPS)[3]**	21	Dako/Agilent	6	11	4	-	81%	29%
mAb clone 22C3 pharmDX, **SK006 (LMPS)[4]**	25	Dako/Agilent	6	14	5	-	80%	24%
mAb clone 22C3 pharmDX, **GE006 (VRPS)[3]**	48	Dako/Agilent	34	14	-	-	100%	71%
mAb clone 22C3 pharmDX, **GE006 (LMPS)[4]**	13	Dako/Agilent	7	4	1	1	85%	54%
rmAb clone 28-8 pharmDX, **SK005 (VRPS)[3]**	1	Dako/Agilent	-	1	-	-	-	-
Total	168		56	79	29	4		
Proportion			33%	47%	17%	3%	80%	

1) Proportion of sufficient stains (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 C17**

Antibodies[5] for laboratory developed PD-L1 assays, concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **22C3**	46	Dako/Agilent	11	24	8	3	76%	24%
rmAb clone **28-8**	1	Cell Marque				1		
rmAb **CAL10**	1	Biocare Medical	-	1	-	-	-	-
rmAb clone **E1L3N**	3	Cell Signaling	-	1	1	1	-	-
rmAb clone **QR1**	4	Quartett	3	-	1	-	-	-
rmAb clone **MSVA-711R**	1	MSVA	-	-	-	1	-	-
Total	56		14	26	10	6		
Proportion			25%	46%	18%	11%	71%	

1) Proportion of sufficient stains (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 antibodies[6] for PD-L1 TPS/CPS, run C17**

Ready-To-Use antibodies[6]	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone SP263, **790-4905[6] (VRPS)[3]**	10	Ventana/Roche	1	8	-	1	90%	10%
rmAb clone SP263, **790-4905[6] (LMPS)[4]**	25	Ventana/Roche	2	14	7	2	64%	8%
rmAb clone 73-10 **PA0832**	5	Leica Biosystems	1	1	1	2	60%	20%
rmAb clone MX070C **MAB-0854**	1	Fuzhou Maixin	-	1	-	-	-	-
rmAb clone AC37 **PA168**	1	Abcarta	-	-	1	-	-	-
rmAb clone BP6099 **I12052E**	1	Biolynx	1	-	-	-	-	-
rmAb clone RM320 **8263-C010**	1	Sakura Finetek	-	1	-	-	-	-
rmAb clone **CAL10**	2 1	Zytomed Systems Biocare Medical	1	1	-	1	-	-
rmAb clone QR1 **P-P001-70**	1	Quartett	-	-	1	-	-	-
rmAb Clone GR110 **GT256202**	1	Gene Tech GAS	-	-	1	-	-	-
Total	49		6	26	11	6	-	
Proportion			12%	53%	23%	12%	65%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of optimal results.
3) Vendor recommended protocol settings – RTU product used in compliance to protocol settings, platform and package insert.
4) Laboratory modified protocol settings for a RTU product applied either on the vendor recommended platform(s) or other platforms.
6) Ready-To-Use antibodies without predictive claim.

Detailed Analysis

CE IVD / FDA approved assays

SP263 (741-4905, Ventana/Roche): In total, 3 of 45 (7%) 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, 32 of 45 (71%) 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).

PD-L1 IHC 22C3 pharmDx (SK006, Dako/Agilent): In total, 6 of 21 (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, 17 of 21 (81%) 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 an overall comparable performance as shown in Table 2b.

PD-L1 IHC 22C3 pharmDx (GE006, Dako/Agilent): In total, 34 of 48 (71%) 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, 48 of 48 (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**	32/45 (71%)	3/45 (7%)	-	-
Ventana BenchMark Ultra rmAb SP263, **740-4907**	4/13 (31%)	0/13 (0%)	-	-
Dako Autostainer Link 48+ mAb 22C3 pharmDX, **SK006**	17/21 (81%)	6/21 (29%)	11/13 (85%)	4/13 (31%)
Dako Omnis mAb 22C3 pharmDX, **GE006**	48/48 (100%)	34/48 (71%)	8/8 (100%)	7/8 (88%)
Dako Autostainer Link 48+ rmAb 28-8 pharmDX, **SK005**	1/1	0/1	-	-

*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, 11 of 46 (24%) protocols were assessed as optimal of which 24 were stained on the BenchMark Ultra platform (Ventana/Roche), 6 on the BenchMark Ultra Plus platform (Ventana/Roche), 1 on BenchMark XT platform (Ventana/Roche), 8 on the Omnis platform (Dako/Agilent), 2 on Autostainer Link 48 (Dako/Agilent), 4 on Bond III platform (Leica Biosystems), and 1 manually.

On BenchMark Ultra, the protocols providing optimal results were based on a titre of 1:20-40 for mAb clone 22C3, incubation time of 60-120 min., HIER in CC1 for 48-56 min. and OptiView as the detection system. Using these protocol settings, 3 of 24 (13%) laboratories produced optimal staining results, and 19 of 24 (79%) laboratories produced sufficient staining results.
On Omnis, the protocols providing optimal results for mAb clone 22C3 were based on a titre of 1:20-50 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 8 (63%) laboratories produced optimal results and 8 of 8 (100%) laboratories produced a sufficient staining result.

rmAb clone **QR1**: 3 of 4 protocols was assessed as optimal.
The optimal protocols for this clone were based on a titre of 1:100 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. and Bond™ Refine as the detection system and performed on Bond III (2/3) & MAX (1/3) platforms (Leica Biosystems). Using these protocol settings, 3 of 3 (100%) 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**	4/31** (13%)	-	-	2/2**	-	5/8** (63%)	0/3**	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

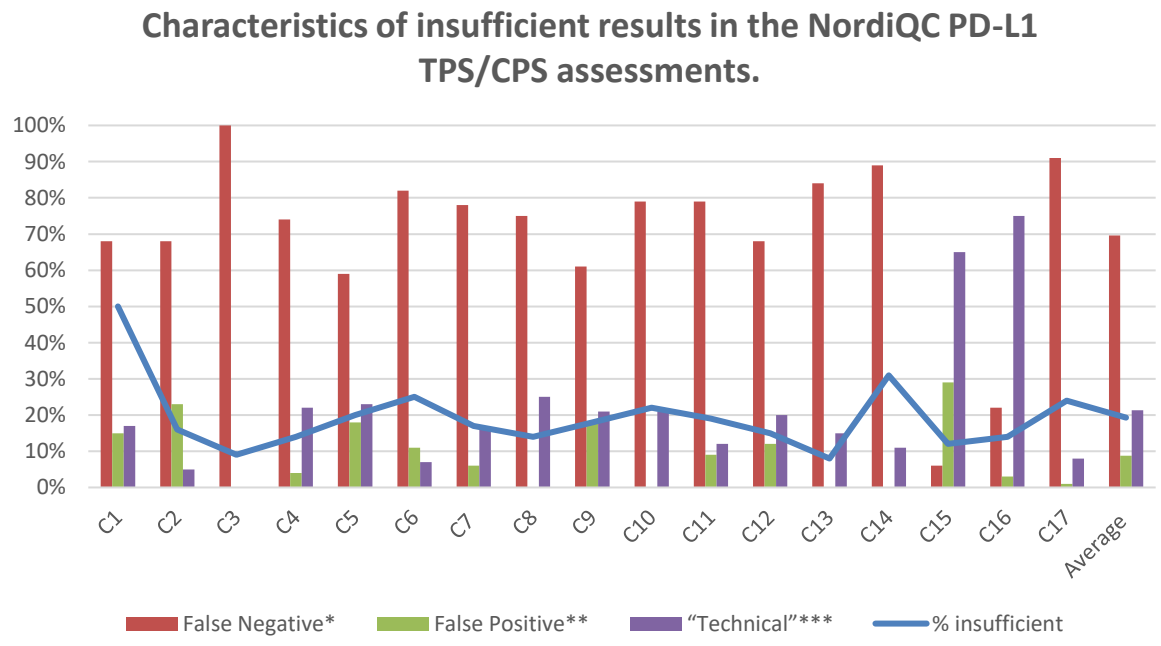
The tissue micro array (TMA) blocks constructed for this PD-L1 run consisted of three NSCLCs, three TNBCs, two tonsils and one placenta. The NSCLCs were selected to comprise PD-L1 expression levels for each TPS category: TPS negative (<1% PD-L1 positive tumour cells), TPS low (≥1-49%) and TPS high (≥50%). 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 seven 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 cores no. 1 and 6, areas with TPS 1-5% (TPS low) and TPS 50-60% (TPS high) were observed respectively. 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 seventeenth 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 70% of the insufficient results. 21% of the insufficient results were caused by 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, and 9% caused by a false negative staining result. 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 this run – C17 of the NordiQC PD-L1 TPS/CPS (KEYTRUDA®) assessments.

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



* 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. 6, characterized as TPS low by the NordiQC reference standard method, was the most challenging to obtain an optimal result.

48% (n=131) of the slides submitted were marked as "Good". In 94% of these (123 of 131), 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. 0% (0 of 131) had an increased TPS/CPS level observed compared to the level expected. In the remaining 6% (8 of 131) 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 21% (58 of 273) of the participants and in total provided an overall pass rate of 62% (36 of 58), with 5% (3 of 58) 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. Both in this assessment run and the runs from C10, 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.

The Dako/Agilent **22C3** pharmDx assay GE006 for Dako Omnis was used by 18% (48 of 273) of the participants providing a pass rate of 100% (71% 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 8% (21 of 273) of the participants and provided a pass rate of 81% (29% optimal) when applied by protocol settings in compliance with vendor recommendations (see Table 3). The 22C3 SK006 assay was also applied off-label (n=25), 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 similar performance when applied as per recommendations or by modified off-label settings.

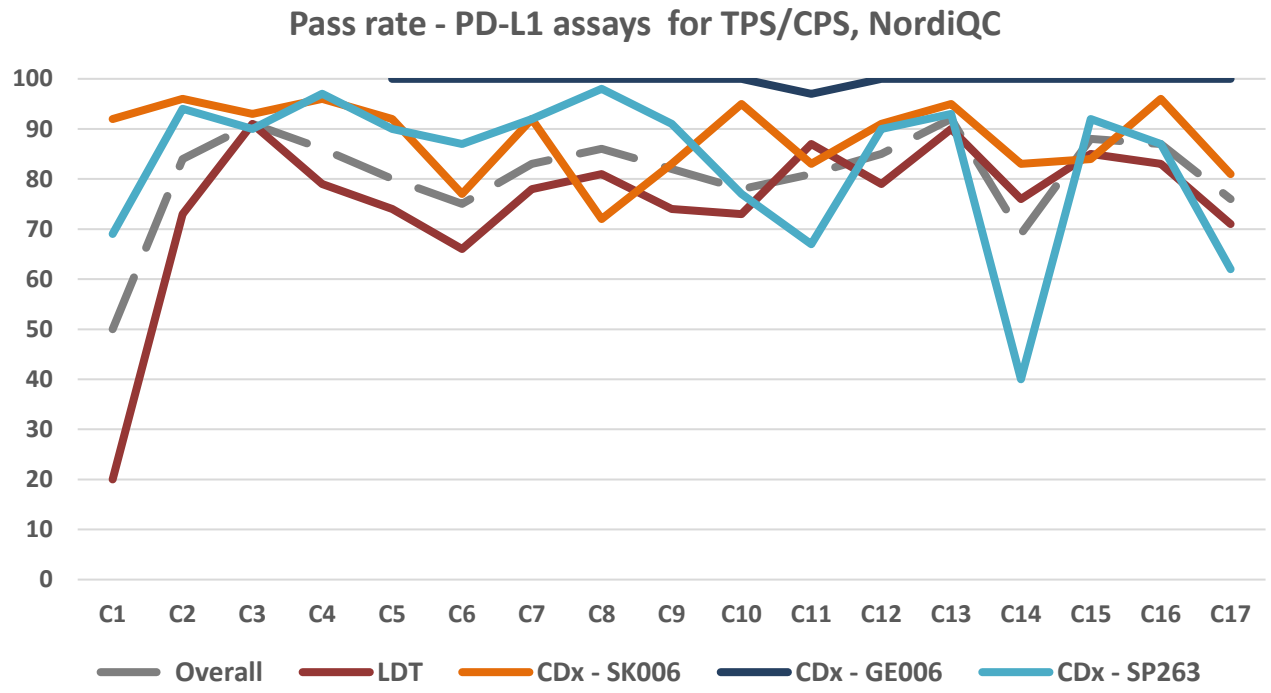
The Dako/Agilent pharmDx SK005 rmAb **28-8** for Autostainer Link 48 was used by 1 laboratory, using the recommended protocol settings being assessed as good.

Overall, 128 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 80% (102/128), 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 49% (135 of 273) of the participants. For this group a pass rate 71% was observed which is lower compared to the level of 82% seen in the last assessment run – C16. Focusing on the performance of PD-L1 LD assays from C2-C17, 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), 88% for 22C3 SK006 pharmDx (Dako/Agilent) and 84% 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=46), achieving a pass rate of 76% and an optimal rate of 24%. This performance is comparable in pass rate to run C16 (77%) but shows a lower optimal rate (24% vs. 46%). It is also lower compared to run C15, which had a higher pass rate (81%) and optimal rate (63%).

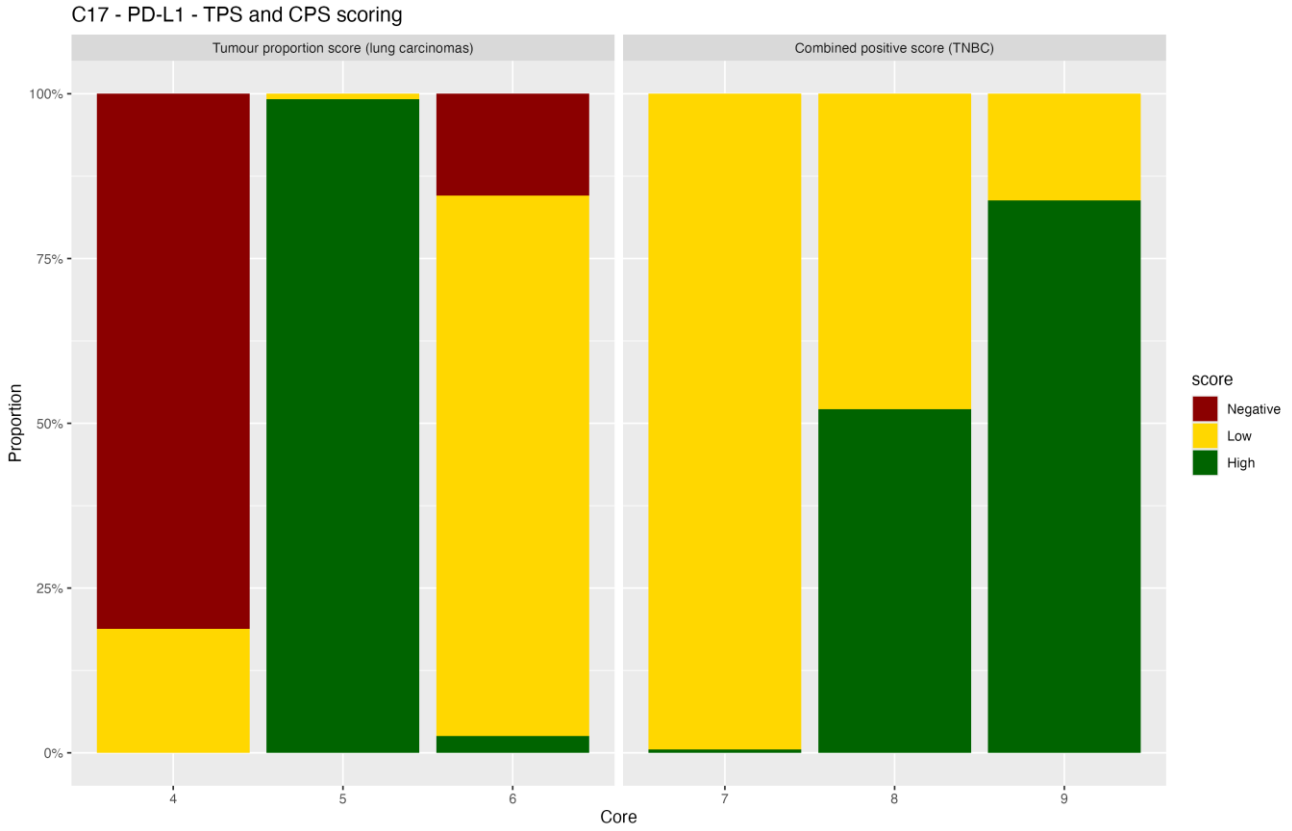
As described above for optimal protocol settings for mAb clone 22C3 as concentrated format, successful and interlaboratory reproducible settings have been identified for BenchMark (Ventana/Roche) and Omnis (Dako/Agilent) and these seem to be widely consolidated within the laboratories providing a pass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

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. Cumulated data for runs C8 - C17 evaluating the performance of mAb clone 22C3 on the Bond platforms have shown a pass rate of 33% (13 of 39), 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.

The Leica Biosystems PD-L1 IHC RTU assay based on rmAb clone **73-10** (PA0832) with intended use on Bond III, was used by 6 participants in run C13, 3 participants in C14, 4 participants in C15, 5 participants in C16 (with 2 participants achieving an optimal result), and 5 participants in C17 (with 1 participant archiving an optimal result). However, the overall pass rate of 100% obtained when used by vendor recommended protocol settings has not been maintained in this run, as 3 out of 5 participants achieved a borderline and poor score in this run.

The commonly used Ventana/Roche IHC RTU assay 790-4905, **SP263** without predictive claim showed a superior performance in C17 compared to the corresponding locked assay 741-4905 giving a pass rate of 90%, 10% optimal when applied by vendor recommended protocol settings. When used by laboratory modified protocol settings a significantly lower pass rate of 64% and 8% optimal scores was observed.

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 C17: 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 4–7 and 9. In contrast, core 8 showed lower consensus. In this core, 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 weak, 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.

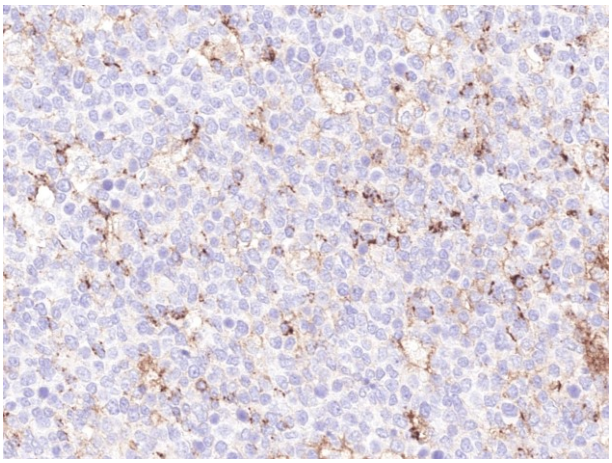


Fig. 1a
Optimal staining result of tonsil using the PD-L1 IHC 22C3 GE006 pharmDx kit, Dako/Agilent, following the vendor recommended protocol settings.
A weak to moderate, but distinct punctuated membranous staining reaction of germinal centre macrophages and dispersed lymphocytes is seen.
No staining reaction is seen in the vast majority of lymphocytes.
Also compare with Figs. 2a – 4a, same protocol.

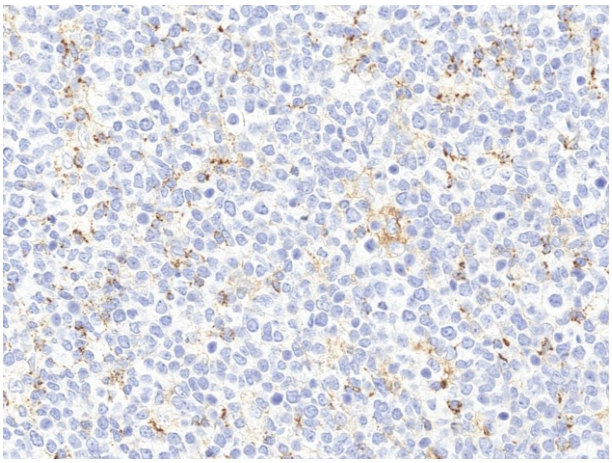


Fig. 1b
Staining result of tonsil, using the rmAb clone SP263 assay 741-4905 from Ventana/Roche, with locked protocol settings.
Mainly dispersed T-cells are demonstrated showing a weak granular punctuated membranous staining reaction.
Virtually no staining reaction is seen the germinal centre macrophages.
Also compare with Figs. 2b - 3b, same protocol.

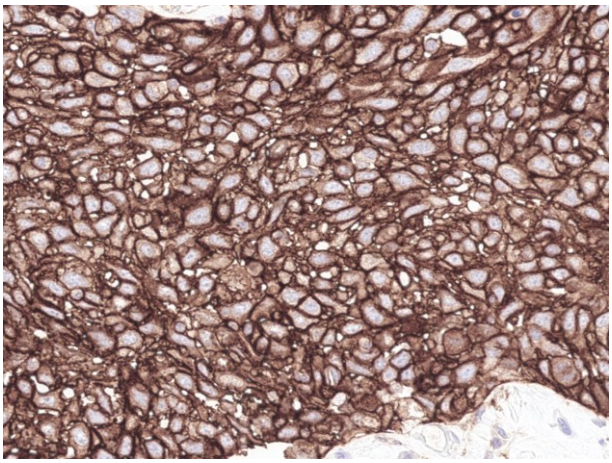


Fig. 2a
Optimal staining result of the NSCLC, tissue core no. 5, using the same protocol as in Fig. 1a.
A strong, distinct membranous staining reaction is seen in virtually all tumour cells.
The tumour was categorized as TPS High (≥50%) and thus eligible for first line immune therapy with KEYTRUDA® (different regional cut-offs occur).

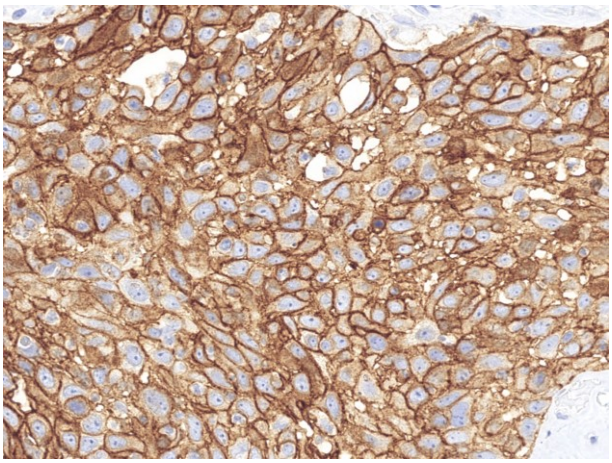


Fig. 2b
Staining result of the NSCLC, tissue core no. 5, using the same protocol as in Fig. 1b.
A moderate to strong, distinct membranous staining reaction is seen in virtually all tumour cells. The tumour still categorized as TPS High (≥50%) and thus eligible for first line immune therapy with KEYTRUDA® (different regional cut-offs occur). However, also compare with Fig. 3b, same protocol.

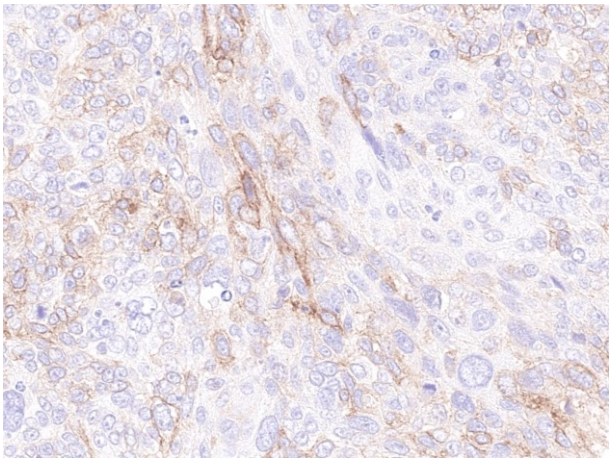


Fig. 3a
Optimal staining result of the NSCLC, tissue core no. 6, using the same protocol as in Figs. 1a and 2a.
In this area of the tumour a weak to moderate membranous staining reaction is seen in 20-25% of the neoplastic cells.
Overall, the tumour was categorized as TPS Low (≥1-49%) and thus eligible for second line immune therapy with KEYTRUDA® (different regional cut-offs occur).

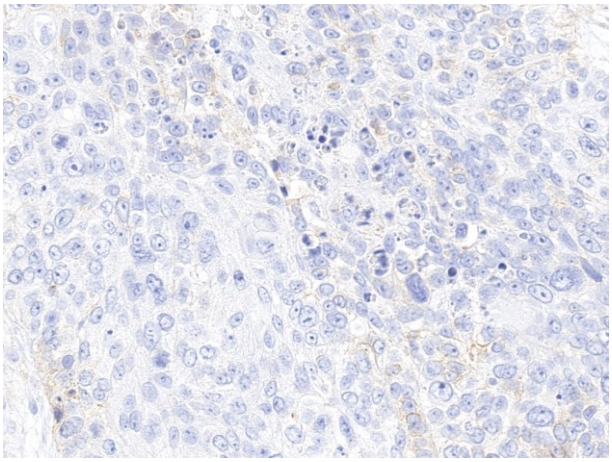


Fig. 3b
Insufficient staining result of the NSCLC, tissue core no. 6, using the same protocol as in Figs. 1b and 2b.
Less than 1% of the tumour cells show a membranous staining reaction and the PD-L1 category being changed from the expected TPS Low to TPS Negative and the tumour not being eligible for immune therapy.
Both the participating laboratory and the NordiQC assessor panel scored the result as TPS Negative.
Compare to the expected result as shown in Fig. 3a.

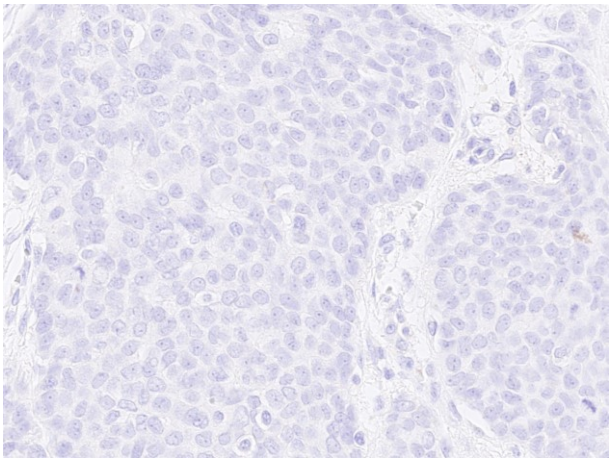


Fig. 4a
Optimal staining result of the TNBC, tissue core no. 7, using the same protocol as in Figs. 1a - 3a.
No staining reaction is observed.
The tumour was categorized as CPS Low (<10) and thus not eligible for immune therapy with KEYTRUDA®.

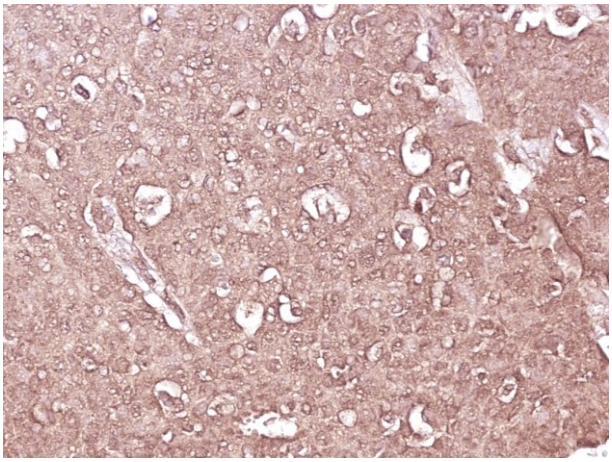


Fig. 4b
Insufficient staining result of the TNBC, tissue core no. 7, using the PD-L1 IHC 22C3 SK006 pharmDx kit, Dako/Agilent, following the vendor recommended protocol settings.
An excessive cytoplasmic and background staining reaction is hampering the read-out for PD-L1 status.
The aberrant result most likely related to a technical issue of the slide being dried durng IHC staining proces.
Compare to the expected result as shown in Fig. 4a and also the aberrant patterns and artefacts seen in Figs. 5a and 5b.

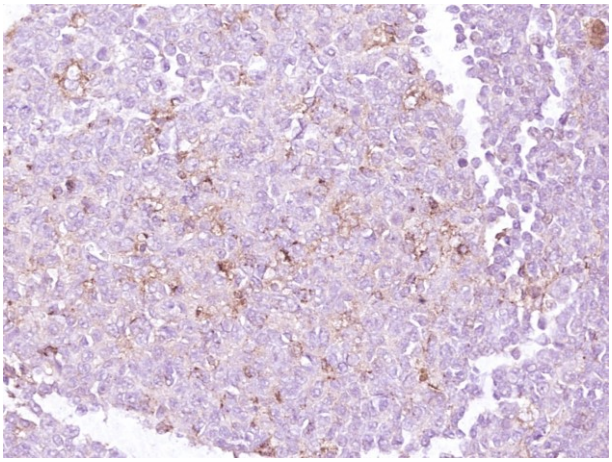


Fig. 5a
Staining result of tonsil using same protocol as in Fig. 4b. Virtually no staining reaction is seen the germinal centre macrophages compared to the level expected (see Fig. 1a).

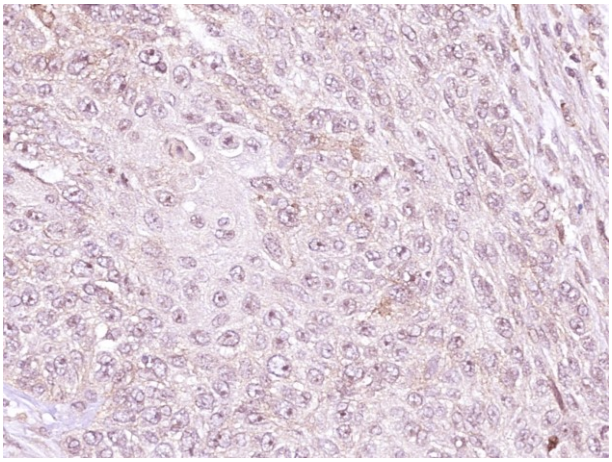


Fig. 5b
Insufficient staining result of the NSCLC, tissue core no. 6, using same protocol as in Fig. 4b providing both a too weak specific staining reaction and a diffuse background staining hampering the read-out for PD-L1 status.
A diffuse granular, membranous and cytoplasmic staining reaction is observed and PD-L1 status cannot be settled with confidence – compare with Fig. 3a for optimal result.

JH/RR/LE/HLK/SN 03.07.2025