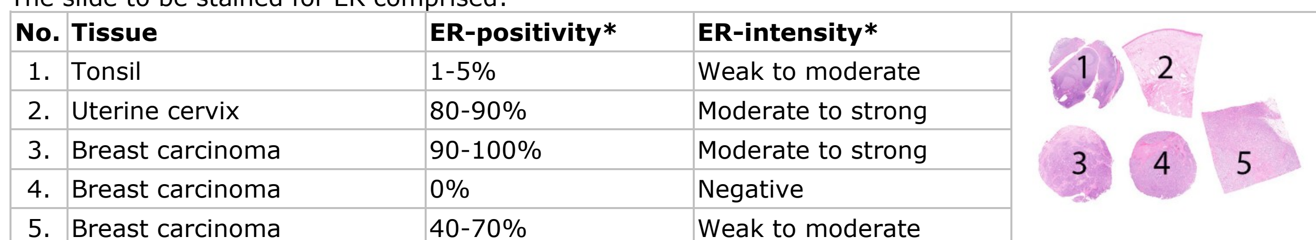
Assessment Run B41 2026

Estrogen receptor (ER)

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of IHC tests performed by the NordiQC participants for demonstration of estrogen receptor (ER) expression in breast carcinomas. IHC, based on the rmAb clones SP1 and EP1, performed in a NordiQC reference laboratory served as reference standard methods and were used to identify breast carcinomas with the dynamic, diagnostic and critical relevant expression levels of ER. The obtained score in NordiQC is indicative of the performance of the IHC tests, but due to the limited number and composition of samples, internal validation and extended quality control (e.g. regular measurement of ER results) is needed.

Material
The slide to be stained for ER comprised:

No.	Tissue	ER-positivity*	ER-intensity*
1.	Tonsil	1-5%	Weak to moderate
2.	Uterine cervix	80-90%	Moderate to strong
3.	Breast carcinoma	90-100%	Moderate to strong
4.	Breast carcinoma	0%	Negative
5.	Breast carcinoma	40-70%	Weak to moderate



* ER-status and staining pattern as characterized by NordiQC reference laboratory using the rmAb clones EP1 and SP1.

All tissues were fixed in 10% neutral buffered formalin for 24-48 hours and processed according to Allison et al.[1]

Criteria for assessing an ER staining as **optimal** included:

- A moderate to strong, distinct nuclear staining of virtually all columnar epithelial cells (if present), and most squamous epithelial and stromal cells (with the exception of endothelial cells and lymphoid cells) in the uterine cervix.
- An at least weak to moderate nuclear staining reaction in scattered follicular dendritic cells/T-cells and squamous epithelial cells in the tonsil, easily identified at low magnification.
- An at least weak to moderate distinct nuclear staining in the appropriate proportion of the neoplastic cells in the ER positive breast carcinomas, tissue cores no. 3 and 5.
- No nuclear staining in the neoplastic cells in the ER negative breast carcinoma, tissue core no. 4.
- No more than a weak cytoplasmic reaction in cells with a strong nuclear staining reaction.

- An ER IHC result was classified as **good** if ≥ 10% of the neoplastic cells in the breast carcinomas, tissue cores no. 3 and 5, showed an at least weak nuclear staining reaction but in a significantly reduced proportion compared to the reference range. Alternatively or additionally, if a reduced but still weak to moderate nuclear staining reaction in the uterine columnar and squamous epithelial cells and/or in the dispersed cells expected to be positive in the tonsil was seen.

- An IHC result was also assessed as **good**, if the signal-to-noise ratio was low, e.g., because of moderate cytoplasmic reaction, background staining, excessive or inselective counterstaining or impaired morphology. The ER IHC result was still acceptable in all tissues.

- An ER IHC result was assessed as **borderline** if ≥ 1% and < 10% of the neoplastic cells in one or both of the breast carcinomas, tissue cores no. 3 and 5, showed a nuclear staining reaction. A negative staining reaction of the cells expected to be demonstrated in tonsil/uterine cervix could also be assessed as **borderline.**

  In cases where the signal-to-noise ratio was low, e.g., because of moderate cytoplasmic reaction, excessive/inselective counterstaining or impaired morphology, to the extent where interpretation of the ER IHC result was compromised could be considered **borderline**.

- An ER IHC result was assessed as **poor** if a false negative staining (< 1%) was seen in one of the breast carcinomas, tissue cores no. 3 and 5, or false positive staining (≥ 1%) was seen in the breast carcinoma, tissue core no. 4. Poor signal-to-noise ratio or poor morphology as described above could also result in a grade of **poor** where interpretation was severely hampered.

KEY POINTS FOR ER IHC ASSAYS

- Tonsil and uterine cervix are highly recommendable tissue controls to monitor analytical sensitivity and specificity.
- RTU assays were used by 94% of all participants.
- The rmAb clone EP1 was most successful, with high pass rates both applied as RTU or LD assay.
- Following the inferior performance of the Ventana/Roche RTU system (rmAb clone SP1) seen in Run B40, caused by recalled faulty antibody batches with reduced analytical sensitivity, the pass rate for this system returned to satisfactory levels (94%) in this run.

Participation

Number of laboratories registered for ER, B41	495
Number of laboratories returning slides	400 (81%)

At the date of assessment, 400 (81%) of the participants had returned the circulated NordiQC slides.
In this assessment, run B41, general issues with the Danish postal service affected the distribution and return of slides to/from participants, resulting in a lower number of returned slides compared to previous assessments.
Slides received after the assessment were not included in this report. However, all returned slides were assessed, and participating laboratories with insufficient results received advice.

Results

400 laboratories participated in this assessment run, and of these 365 (91%) achieved a sufficient mark (optimal or good). Optimal results were obtained by 242 laboratories (61%). Table 1 summarizes antibodies (Abs) used and assessment marks given (see page 4 and 5).

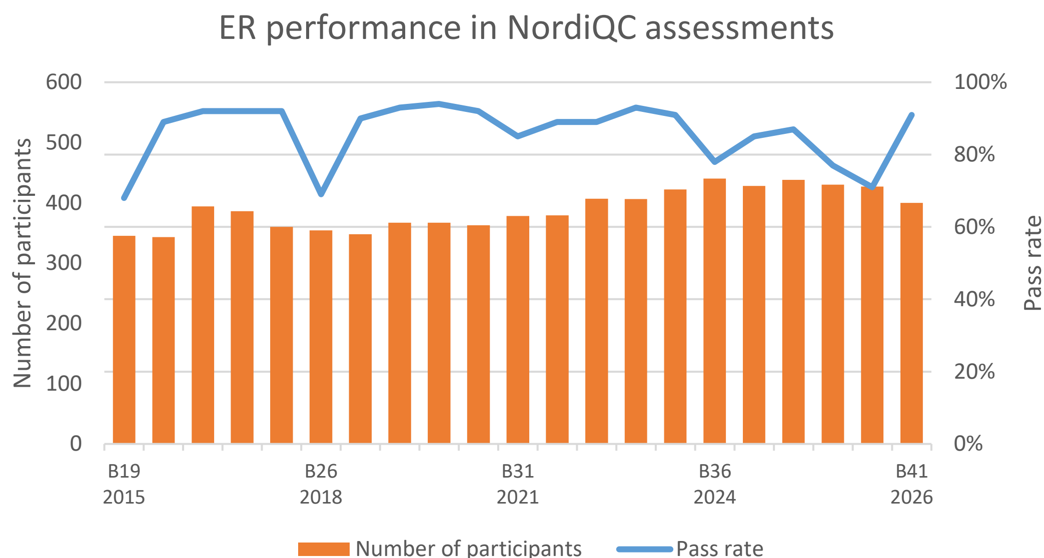
The most common issue, responsible for 97% (34 of 35) of the insufficient results in this assessment, was low analytical sensitivity. This led to either too weak staining reaction in 86% (30 of 35) of cases or a false negative reaction in 11% (4 of 35) of cases.
This generally manifested as a severely reduced number of positive cells compared to the reference slide. Low analytical sensitivity and weak demonstration of ER was occasionally complicated by excessive or "inselective" counterstaining (where nuclei were difficult to distinguish from cytoplasm), or impaired tissue morphology interfering with the interpretation.

The most frequent causes of insufficient staining reactions were:
- Use of detection systems with low analytical sensitivity
- Insufficient HIER time or HIER in acidic buffer (weak staining)

Performance history

In this run, the pass rate reversed the decline observed in runs B39-B40, returning to a pass rate of 91%; comparable to the stable and satisfactory level of 85-94% in runs between 2015-2024, with the exception of runs B19, B26 and B36 (see Graph 1). The reduced pass rates for runs B39 and B40 were associated with reduced sensitivity of the Roche/Ventana SP1 RTU system (see run reports B39 and B40 for a full discussion).

Graph 1. **Participant numbers and pass rates for ER from 2015 - 2026**



Fluctuations in pass rates in between assessment runs can be caused by many factors, related both to the protocols applied by the participants, circulation of more challenging material, variations in assessment criteria or similar factors. To ensure the consistency of the material circulated, NordiQC evaluates the selected tissue materials with two reference standard methods and in addition monitors the ER expression levels throughout all TMAs used in the assessment. NordiQC also strives to include testing material that shows comparable and diagnostically-relevant levels of antigen expression in between each run. However, as in previous runs, similar pass rates continue to be observed for both existing and newly registered participants. The reduced pass rate attributed to inferior performance of the Ventana/Roche RTU system based on rmAb clone SP1 (790-4324/790-4325) on the BenchMark platform group seen in runs B36, B39 and B40 was not present in this run. In run B40, which contained lot numbers of the recalled product by Roche for poor analytical sensitivity, the pass rate for this RTU system was 67%, in contrast with a more typical pass rate of 94% in this run.

Conclusion
In this assessment, the rabbit monoclonal antibodies (rmAb) clones **SP1** and **EP1** and the mouse monoclonal Ab (mAb) clone **6F11** could all be used to provide an optimal result for demonstration of ER. The majority of participants (94%, 377 of 400) used RTU systems, with the majority of these (238 of 377, 63%) using the Ventana/Roche SP1 RTU product on the BenchMark platforms. Both RTU and concentrated primary antibody formats could be used successfully: the pass rate for participants using RTU antibodies was 92% (345 of 377), slightly superior to the pass rate of 87% (20 of 23) for concentrated formats (Table 1a). In this run, continuing the trend across runs B36-B41, the most robust overall performance was seen using the Dako/Agilent RTU system based on rmAb clone EP1 for Omnis, with a pass rate of 95% (76% optimal). This was successfully used either as a true "plug and play" (vendor-recommended) assay, with a pass rate of 93% (77% optimal), or with modifications (97% pass, 75% optimal) (Tables 1a, 1c and 3). The most widely used RTU IHC system from Ventana/Roche based on rmAb clone SP1 showed slightly lower pass and optimal rates when used with vendor recommended settings (89% and 48% respectively), although the pass and optimal rates were improved when applied with laboratory modified protocol settings (96% and 64% respectively). In particular, laboratory modified protocols employing OptiView detection system showed improved pass and optimal rates of 98% and 82% respectively (see Comments below).

Controls
In concordance with previous NordiQC runs, uterine cervix and tonsil were found to be valuable positive and negative tissue controls for ER staining: In the uterine cervix, optimal results were characterized by a moderate to strong, distinct nuclear staining reaction in virtually all epithelial cells throughout the squamous epithelium and in the glands. In the stromal compartment, moderate to strong nuclear staining reaction was seen in most cells except endothelial and lymphatic cells.

Use of tonsil as a control tissue is particularly recommended as a tool to monitor analytical sensitivity for the demonstration of ER, and this tissue is superior to uterine cervix in this context. It was observed, that dispersed cells (most likely follicular dendritic cells[2]) in germinal centers and squamous epithelial cells were prominently demonstrated in virtually all protocols providing an optimal result. If the follicular

dendritic cells were negative or weakly demonstrated, a reduced proportion of ER positive cells were seen in the other tissues and, most critically, an unsatisfactory weak or even false negative staining pattern was seen in breast carcinomas, tissue cores no. 3 and 5. In addition, tonsil can be used as supplementary negative tissue control, as B-cells in mantle zones and within germinal centers must be negative.

To validate the specificity of the IHC protocol further, an ER negative breast carcinoma must be included as primary negative tissue control, in which only remnants of normal epithelial and stromal cells should be ER positive, serving as internal positive tissue control. Positive staining reaction of the stromal cells in breast tissue indicates that the IHC protocol provides a high analytical sensitivity for ER, whereas the analytical sensitivity cannot reliably be evaluated in normal epithelial cells in breast as they typically express moderate to high levels of ER.

1. Kimberly H. Allison, M. Elizabeth H. Hammond, Mitchell Dowsett, Shannon E. McKernin, Lisa A. Carey, Patrick L. Fitzgibbons, Daniel F. Hayes, Sunil R. Lakhani, Mariana Chavez-MacGregor, Jane Perlmutter, Charles M. Perou, Meredith M. Regan, David L. Rimm, W. Fraser Symmans, Emina E. Torlakovic, Leticia Varella, Giuseppe Viale, Tracey F. Weisberg, Lisa M. McShane, and Antonio C. Wolff.
Estrogen and Progesterone Receptor Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update. Arch Pathol Lab Med. 2020 May;144(5):545-563

2. Sapino A, Cassoni P, Ferrero E, Bongiovanni M, Righi L, Fortunati N, Crafa P, Chiarle R, Bussolati G.
Estrogen receptor alpha is a novel marker expressed by follicular dendritic cells in lymph nodes and tumor-associated lymphoid infiltrates. Am J Pathol. 2003 Oct;163(4):1313-20. PubMed PMID: 14507640

Table 1a. **Overall results for ER, run B41**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	23	16	4	3	0	87%	70%
Ready-To-Use antibodies	377	226	119	26	6	92%	60%
Total	400	242	123	29	6		
Proportion		61%	31%	7%	1%	91%	

1) Proportion of sufficient results (optimal or good).
2) Proportion of optimal results.

Table 1b. **Concentrated antibodies and assessment marks for ER, run B41**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **6F11**	10	Leica Biosystems	7	1	2	0	80%	70%
rmAb clone **EP1**	1 4	Dako/Agilent CellMarque	2	2	1	0	80%	40%
rmAb clone **SP1**	2 3	Thermo Scientific CellMarque	4	1	0	0	100%	80%
rmAb clone **QR013**	3	Quartett	3	-	-	-	-	-
Total	23		16	4	3	0		
Proportion			70%	17%	13%	0%	87%	

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

Table 1c. **Ready-To-Use antibodies and assessment marks for ER, run B41**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **6F11** **PA0009/PA0151 (VRPS[3])**	2	Leica Biosystems	0	0	1	1	-	-
mAb clone **6F11** **PA0009/PA0151 (LMPS[4])**	12	Leica Biosystems	3	6	2	1	75%	25%
rmAb clone **SP1** **790-4324/4325 (VRPS[3])**	75	Ventana/Roche	36	31	8	0	89%	48%
rmAb clone **SP1** **790-4324/4325 (LMPS[4])**	168	Ventana/Roche	107	55	5	1	96%	64%
rmAb **EP1** **IR084 (VRPS[3])**	2	Dako/Agilent	1	1	0	0	-	-
rmAb **EP1** **IR084 (LMPS[4])**	18	Dako/Agilent	11	1	4	2	67%	61%
rmAb **EP1** **GA084 (VRPS[3])**	43	Dako/Agilent	33	7	3	0	93%	77%
rmAb **EP1** **GA084 (LMPS[4])**	32	Dako/Agilent	24	7	1	0	97%	75%
rmAb **EP1** **8361-C010**	3	Sakura Finetek	1	2	0	0	-	-
rmAb **EP1** **BFM-0596**	1	Bioin Biotechnology	0	0	1	0	-	-
rmAb clone **SP1** **249R-17/18**	4	Cell Marque	3	1	0	0	-	-
rmAb clone **SP1** **MAD-0003060-DS-2/ MAD-000306QD**	3	Master Diagnostica Vitro SA	0	2	1	0	-	-
rmAb clone **SP1** **RMPD001**	1	Diagnostic BioSystems	0	1	0	0	-	-
rmAb clone **SP1** **GT205602**	1	Gene Tech	1	0	0	0	-	-
rmAb clone **SP1** **BRB053** **NOA-RB053**	4	Zytomed Systems	2	2	0	0	-	-
rmAb clone **SP1** **ALR 301 G7**	1	BioCare Medical	0	1	0	0	-	-
rmAb clone **SP1** **M3011**	1	Spring Biosystems	0	0	0	1	-	-
rmAb clones **SP1+6F11** **PM308**	1	BioCare Medical	1	0	0	0	-	-
Ab clone **DY49837** **4911432**	1	Dakewe/BioSci	1	0	0	0	-	-
rmAb clone **QR013** **P-E001-30**	1	Quartett	0	1	0	0	-	-
rmAb clone **MXR030** **RMA-1065**	1	Fuzhou Maixin	1	0	0	0	-	-
Ab clone **AD55** **PA623**	1	Abcarta	1	0	0	0	-	-
Ab clone **BP6139** **I12192E**	1	Biolynx Biotechnology	0	1	0	0	-	-
Total	377		226	119	26	6		
Proportion			60%	32%	7%	1%	92%	

1) Proportion of sufficient results (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) refer to a specific RTU product applied on the vendor recommended platform(s) according to the manufacturer's recommended protocol settings.
4) Laboratory Modified Protocol Settings (LMPS) refer to a specific RTU product applied either on the vendor recommended platform(s) or other platforms.

Detailed analysis of ER: Run B41
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **6F11**: 7 of 10 laboratories obtained optimal results: based on Heat Induced Epitope Retrieval (HIER) using Bond/Bond PRIME Epitope Retrieval Solution 2 (BERS2) pH 9.0 (Leica Biosystems) or CC1/Ultra CC1 (Ventana/Roche) as retrieval buffer. The mAb was diluted in the range of 1:25-1:100 and combined with a 2- or 3-layer detection system (Leica Bond Polymer Refine, Ventana UltraView with amplification or OptiView with amplification). Using these protocol settings, 7 of 8 (88%) laboratories produced a sufficient staining result (optimal or good).

rmAb clone **EP1**: 2 of 5 laboratories obtained an optimal result, using a protocol based on HIER using BERS2 pH 9.0 (Leica Biosystems) as retrieval buffer and a retrieval time of 30 minutes. The rmAb was diluted 1:10-1:50 and combined with a 2-layer detection system. Using these protocol settings, 3 of 3 laboratories produced a sufficient staining result (optimal or good).

rmAb clone **SP1**: 4 of 5 laboratories obtained an optimal result, all using high pH HIER (Ventana/Roche CC1/Ultra CC1 or Leica Biosystems BERS2 pH 9.0). The rmAb was diluted 1:50-1:100 and combined with a 3-layer detection system (Ventana OptiView or Leica Bond PRIME Polymer). The OptiView detection system was combined with amplification by one laboratory. Using these protocol settings, 5 of 5 (100%) laboratories produced a sufficient staining result (optimal or good).

Table 2 summarizes the overall proportion of optimal staining results when using the three most frequently used concentrated Abs on the most commonly used IHC staining platforms.

Table 2. **Proportion of optimal results for ER 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 High pH 9.0	TRS Low pH 6.1	TRS High pH 9.0	TRS Low pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **6F11**	-	-	-	-	4/4**	-	3/4	-
rmAb clone **EP1**	-	-	-	-	-	-	2/3	-
rmAb clone **SP1**	-	-	-	-	3/4	-	1/1	-

1) Autostainer Classical, Link 48.
2) BenchMark GX, XT, Ultra, Ultra Plus
3) Bond III, Prime

Ready-To-Use antibodies and corresponding systems
mAb clone **6F11,** product. no. **PA0009/PA0151**, Leica Biosystems Bond III/Bond Max/Bond PRIME:
3 of 14 laboratories (21%) obtained an optimal result, using the following protocol: HIER in BERS2 (high pH) for 20-40 min., 15 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) or Bond-PRIME Polymer DAB Detection (DS9284) as detection system. Using these protocol settings, 7 of 7 (100%) laboratories were able to achieve sufficient results, 43% optimal. Four laboratories used a protocol using HIER with Bond Epitope Retrieval Solution 1 (BERS1, low pH), with one obtaining a sufficient result.
5 laboratories used product no PA0009/PA0151 on the Bond Prime with a pass rate of 80%.

rmAb clone **EP1**, product no. **IR084**, Dako/Agilent, Dako Autostainer+/Autostainer Link:
8 of 16 laboratories (50%) obtained an optimal result. Protocols with optimal results were based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 20 min. at 97-98°C), 20-40 min. incubation of the primary Ab (mode 20 min.) and EnVision FLEX (K8000/SM802, K8024/SM802), EnVision FLEX+ (K8002/SM802) or EnVision+ Rabbit (K4003) as detection system, most commonly with Rabbit Linker (K8009, K8019)(7 of 8). Of the laboratories using these protocol settings, 9 of 13 (69%) produced a sufficient staining result, 62% optimal.
4 laboratories used product no IR084 on other platforms. These were not included in the description above.

rmAb clone **EP1**, product no. **GA084**, Dako/Agilent, Dako Omnis:
57 of 75 laboratories (76%) obtained an optimal result. Protocols with optimal results were typically based on HIER using TRS High pH (efficient heating time 20-30 min. at 97°C, mode = 30 min.), 10-28 min. incubation of the primary Ab (mode = 10 min.) and EnVision FLEX (GV800/GV823) with or without rabbit linker (GV809) as detection system. One laboratory used TRS low pH and obtained an optimal result. Of the laboratories using these protocol settings, 69 of 73 (95%) produced a sufficient staining result, 56 of 73 (77%) optimal.

rmAb clone **SP1**, product no. **790-4324/4325**, Ventana/Roche, BenchMark XT, ULTRA, ULTRA Plus:
138 of 235 laboratories (59%) obtained an optimal result. Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 30-95 min. (mode = 64 min.), 12-64 min. incubation of the primary Ab (modes = 16 and 32 min.) and UltraView (760-500), or OptiView (760-700) with or without the corresponding amplification kit as detection system. Using these protocol settings, 221 of 234 (94%) laboratories produced a sufficient staining result, 137 of 234 (59%) optimal.
7 laboratories used product no 790-4324/4325 on other platforms. These were not included in the description above.

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocol assays performed on the specific IHC platform(s) indicated on the datasheet are included.

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

RTU systems	**Vendor recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS48 rmAb EP1 **IR084**	2/2	1/2	8/14 (57%)	7/14 (50%)
Dako Omnis rmAb EP1 **GA084**	40/43 (93%)	33/43 (77%)	31/32 (97%)	24/32 (75%)
Leica Bond III/Max*** mAb 6F11 **PA0009/PA0151**	0/2	0/2	5/7 (71%)	1/7 (14%)
VMS Ultra/XT/Ultra Plus rmAb SP1 **790-4324/4325**	67/75 (89%)	36/75 (48%)	155/160 (97%)	102/160 (64%)

* 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, detection kit and use of amplification. Only protocols performed on the specified vendor IHC stainer are included.
*** Bond PRIME excluded, as there is currently no official protocol recommendation for this platform

Comments
Since run B30, the assessment criteria continue to be centered on the tissue controls, tonsil and uterine cervix, in concordance to the ASCO/CAP 2020 recommendation on ER IHC testing. The results of previous NordiQC ER assessments, along with the ASCO/CAP guidelines (2020) strongly suggest that the use of tonsil as positive control material is essential to ensure an appropriate lower limit of sensitivity for demonstration of ER.

As in previous NordiQC runs for ER, the most common feature of an insufficient staining result in this assessment was inadequate analytical sensitivity, resulting in a weak or false negative staining reaction, with reduced detection of the ER epitope. This was seen in 97% of the insufficient results (34 of 35), with 6% of these weak staining reactions further complicated by excessive counterstaining. Clinically relevant false positive staining reaction, where >1% of cells in the negative breast tumour showed positive signal was not observed in this run.

Demonstration of ER was successfully achieved by the majority of laboratories in the high-level ER-expressing breast carcinoma (tissue core no. 3), in which 90-100% of the neoplastic cells showed a moderate to strong intensity with the NordiQC reference standard assays. However, as in previous runs, adequate detection of ER in the heterogeneous mid-level ER-expressing breast carcinoma (tissue core no. 5), in which an at least weak nuclear staining reaction of 40-70% of the neoplastic cells was expected (ER positivity level depending on TMA), proved more challenging.

In this run, the proportion of sufficient results assessed as good was 31% (123 of 400 results). This returns to a level in line with runs B32-B35, which showed an average of 32% of all results in this category. The results assessed as good were largely characterized by reduced analytical sensitivity manifesting as significantly fewer cells staining positive for ER than expected.

Ready-To-Use (RTU) Abs were used by 94% (377 of 400) of the participants. 92% (345 of 377) of the laboratories using RTU formats obtained a sufficient staining result, 60% optimal (226 of 377).

The Ventana/Roche RTU system, 790-4324/4325 for BenchMark based on the rmAb clone SP1 was in this assessment the most widely applied assay being used by 59% (235 of 400) of all participants and gave an overall pass rate of 94% (222 of 235), 59% optimal. Laboratory modified protocols (LMPS)

were used by the majority (68%, 160 of 235) of participants using this system. Optimal results could be obtained both by the vendor recommended protocol settings (VRPS) (16 min. incubation of the primary Ab, HIER in CC1 for 64 min. and UltraView as detection kit) and by laboratory modified protocol settings (LMPS) adjusting incubation time of the primary Ab, HIER time, detection systems and use of amplification as shown in Table 3. In this assessment, VRPS, used by only 32% (75 of 235) of the laboratories, gave both pass and optimal rates lower than those given by LMPS as shown in Tables 1c and 3, with a pass rate of 97% for LMPS.
Increasing the incubation time in primary antibody to 32 min. was the most commonly used single modification to the VRPS. This modification gave an improved pass rate (100%) compared to the VRPS (89%) for laboratories using this protocol (25 of 25), with a slightly increased optimal rate of 52% versus 48% for VRPS. Increasing the primary antibody incubation time to 40 min. (n=3) also gave a 100% pass rate, although the optimal rate fell to 33%.

Similarly to run B40, use of OptiView as a substitute for UltraView detection as the sole modification to the VRPS was the second most commonly used (and highly successful) single modification; in this run giving a pass rate of 100% (14 of 14), 79% optimal. Protocol modifications using OptiView detection (with or without alteration of primary incubation and HIER time) were in general highly successful, resulting in a pass rate of 98% (48 of 49 users), and a significantly improved optimal score rate of 82% (40 of 49) compared to 48% obtained using VRPS (see Table 3). Addition of UltraView Amplification as the sole modification to the VRPS was the most successful single modification, with an optimal rate of 100% (5 of 5). Together, as in runs B38-B40, these observations suggest that use of the recommended UltraView detection system is associated with relatively reduced analytical sensitivity compared to the OptiView system, although the reduced analytical sensitivity of UltraView may be successfully increased by the addition of amplification.

The reduced sensitivity and lower pass rate recorded for the Ventana/Roche RTU system for ER across assessments B36-B40 (latterly attributed to 11 faulty lots of primary Ab recalled by the manufacturer in September 2025) was not observed in this run. The overall pass rate for this system of 94% in the present run is comparable to the previous stable average of 95% (range 90-97%) recorded across runs B30-B35. However, NordiQC still encourages the participants to update the registered protocols on the NordiQC website to ensure that the actual data including lot no. for primary Ab is updated for the protocol applied for the result submitted for assessment.

The Dako/Agilent RTU system GA084 for Omnis, based on rmAb clone EP1 was used by 19% of the participants (75 of 400) and gave an overall pass rate of 95%, 76% optimal. The proportion of sufficient and optimal results were very similar for laboratories using the VRPS or LMPS.
The Dako/Agilent RTU system GA084 for Dako Omnis has shown a stable performance throughout the latest 8 assessment runs for ER. In runs B34-B41, the average pass rate has been 94% (range 87-100%) when applied by VRPS.

Eight laboratories used the VRPS with the addition of rabbit linker, resulting in a slightly reduced pass rate of 88%, and a similar optimal rate to the VRPS of 75%. Increasing the primary Ab incubation time to 15 min. in conjunction with use of rabbit linker was used by 5 laboratories with a 100% pass rate, 80% optimal. Overall, modified protocols including rabbit linker obtained a pass rate of 94% (16 of 17), 88% optimal. Nine laboratories increased the primary antibody incubation time to 12-27 minutes as the sole modification from VRPS, obtaining a pass rate of 100%, 67% optimal.

The Dako/Agilent RTU system IR084 for Autostainer, also based on the rmAb EP1 was used by 4% (16 of 400) of the participants and provided an overall pass rate of 63%, 50% optimal. As shown in Table 3, 88% (14 of 16) of the laboratories modified the protocol settings, obtaining a pass rate of 57% (50% optimal). Both laboratories using the VRPS obtained a satisfactory result.
The most successful modification (used by 9 laboratories) included addition of a rabbit linker, with identical pass and optimal rates of 78%. EnVision FLEX was the most successful detection system (pass rate 83%, 67% optimal), with EnVision FLEX+ giving a pass rate of 50%, 38% optimal. However, the low number of laboratories using this format precludes a definitive analysis of protocol efficacy.

The Leica RTU system PA0009/PA0151 for BOND III and Max based on mAb 6F11, was in total used by 2% (9 of 400) of the participants and gave an overall pass rate of 56%, 11% optimal. As in the previous assessment, in this run, VRPS based on HIER in BERS1 (low pH) for 20 min., 15 min. incubation of the primary Ab and Bond Refine DAB as detection system was used by two participants, with neither achieving sufficient results. Laboratories using a protocol modification increasing analytical sensitivity by using HIER in BERS2 (high pH) for 20-30 min. without extending the incubation time in primary antibody obtained a pass rate of 100% (4 of 4), although none obtained an optimal result. One laboratory further extended use of BERS2 to 40 minutes without increasing the primary Ab incubation time and achieved an optimal result. As noted in previous runs, increasing analytical sensitivity by using BERS2 and/or extended

incubation times must be balanced against the risk of inducing false positivity in the negative tumour, or a poor signal to noise ratio.
In this analysis only protocols performed on Bond III and Max were included, as the RTU remains unvalidated by Leica Biosystems for Bond Prime.

In general, it must be emphasized that modifications of vendor recommended protocol settings for the RTU systems including migration of the RTU Abs to another platform than the intended, require a meticulous validation process for the end-users. As seen in this and previous assessments, modifications can be very successful but may also generate sub-optimal or aberrant results and therefore must be carefully monitored.

Concentrated antibody formats with laboratory-developed (LD) assays were used by 6% (23 of 400) of the participants, continuing the downward trend from 11% in run B35. The three most commonly applied Abs mAb clone 6F11, rmAb clones EP1 and SP1 used in a LD assay could all provide an optimal result on the tissues supplied in this run, and sufficient results were obtained with all three Abs on the main IHC platforms (Dako/Agilent, Leica Biosystems and Ventana/Roche), see Tables 1b and 2. The overall pass rate for all laboratories using concentrated antibody formats in this run was 87% (20 of 23), with 70% (16 of 23) obtaining optimal results. Whilst the pass rate was lower than that seen for laboratories using RTU formats (87% vs 92%, respectively), the optimal rate was higher than that seen for RTU formats (70% vs 60%, respectively) indicating that concentrated formats may still be employed effectively.

The mAb clone 6F11 continues to be the most commonly used concentrated antibody (10 of 23, 44%): however, in this and previous runs, use of acidic retrieval buffer was associated with failure due to inadequate sensitivity resulting in a too weak staining reaction of the positive tumours. However, in previous runs, poor-signal-to-noise ratio and excessive background with a false positive staining reaction in the negative tumour were associated with prolonged HIER in an alkaline buffer. In addition, these conditions and/or too high a concentration of the primary antibody were seen to cause similar issues, with false positive staining of the negative tumour in the most extreme cases.

The rmAb clone SP1 was the most robust of the 3 most commonly used concentrated antibody formats with a pass rate of 100% (5 of 5), 80% optimal.

Worthy of mention, although used by fewer participants (3 of 23), **rmAb clone QR013** (Quartett), with overall pass and optimal rates of 100%, seems to be a very robust antibody. Protocols were only submitted on Leica Bond III (n=1) and Autostainer 48 (n=2, Dako/Agilent).

Irrespective of the clone applied, careful calibration of the primary Ab concentration in combination with efficient HIER, preferably in an alkaline buffer (with caveats for mAb clone 6F11 as discussed here, and in runs B39, B35, B28 and B15) were found to be the common core elements for an optimal performance.

Summary
In this run, 2-layer and 3-layer detection systems performed similarly overall.
Among laboratories using concentrated antibody formats, usage was almost evenly split: 52% (12/23) used 3-layer systems, while 48% (11/23) used 2-layer systems. However, performance differed markedly - 2-layer systems achieved a 100% pass rate (82% optimal), compared to 75% (58% optimal) for 3-layer systems.
For laboratories using RTU antibody formats, most (82%, 310/377) used 2-layer systems. Here, performance was comparable: 2-layer systems achieved a 92% pass rate (59% optimal), while 3-layer systems reached 90% (66% optimal).

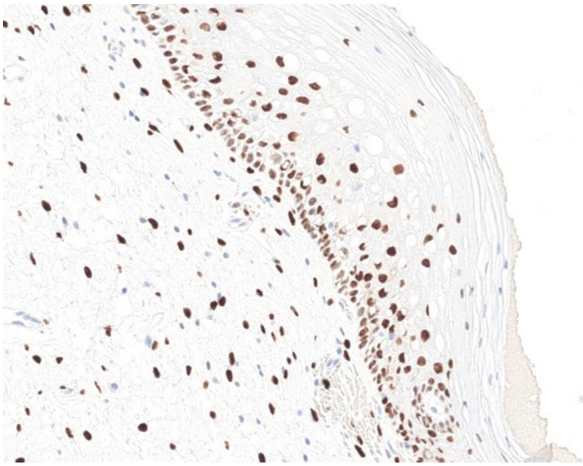


Fig. 1a
Optimal ER staining reaction of the uterine cervix using the rmAb clone SP1 as RTU format (790-4324) from Ventana/Roche, using HIER in CC1 (pH 8.5) for 64 min., incubation time 16 min. in primary Ab and visualized by OptiView and performed on BenchMark Ultra.
Virtually all squamous epithelial and stromal cells show a moderate to strong nuclear staining reaction.
Also compare with Figs. 2a – 5a, same protocol.

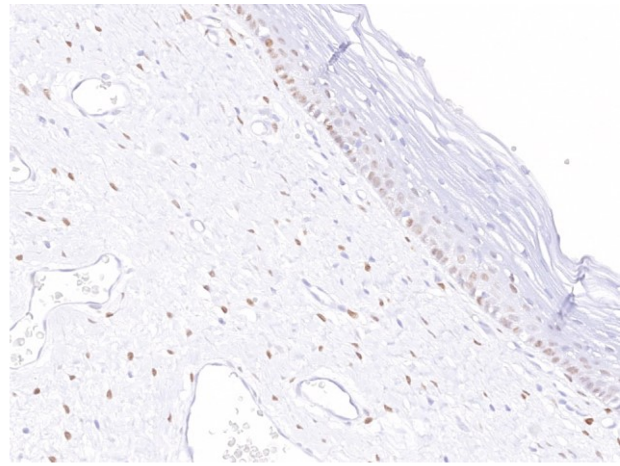


Fig 1b
ER staining reaction of the uterine cervix using the rmAb clone SP1 as RTU format (790-4324) from Ventana/Roche, using too short HIER in CC1 (pH 8.5) for 32 min., incubation time 16 min. in primary Ab and visualized by OptiView and performed on BenchMark Ultra.
A reduced intensity and proportion of squamous epithelial cells demonstrated is seen.
Also compare with Figs. 2b – 5b, same protocol.

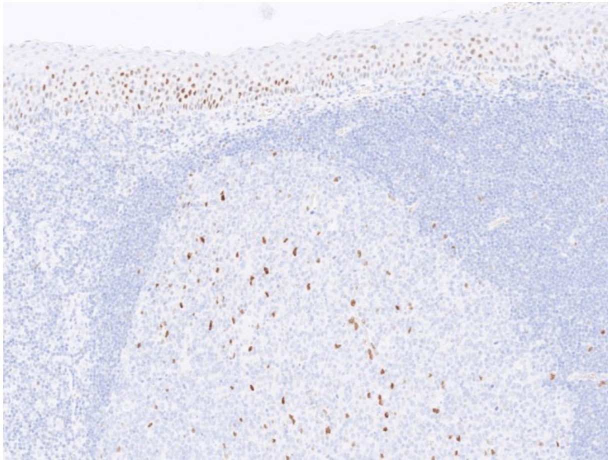


Fig. 2a
Optimal ER staining reaction of the tonsil using the same protocol as in Fig. 1a.
A distinct nuclear staining reaction in both dispersed follicular dendritic cells/T-cells in the germinal center and many squamous epithelial cells can be identified at even low magnification (10x).

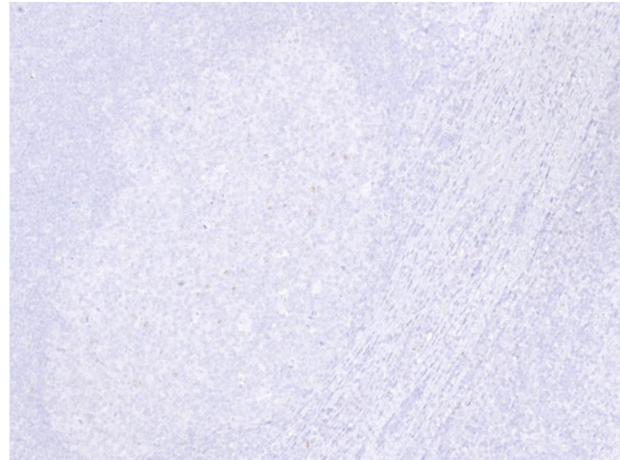


Fig. 2b.
Insufficient ER staining reaction of the tonsil using the same protocol as in Fig. 1b.
In a few squamous epithelial cells and follicular dendritic cells/T-cells only a faint equivocal staining reaction is observed. Compare with Fig. 1a same magnification.

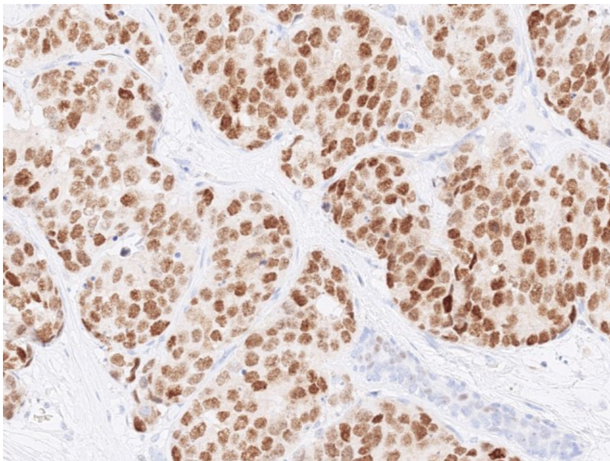


Fig. 3a
Optimal ER staining reaction of the breast carcinoma, tissue core no. 3, with 90-100% cells being positive (moderate to strong) using same protocol as in Figs. 1a – 2a.
The neoplastic cells display a moderate to strong and distinct nuclear staining reaction.

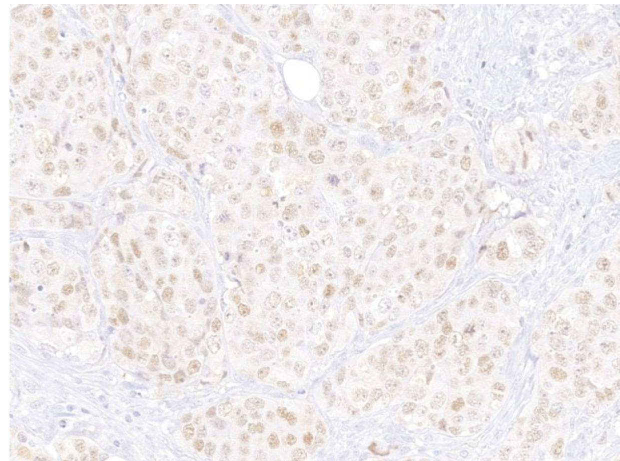


Fig. 3b.
ER staining reaction of the breast carcinoma, tissue core no. 3, with 90-100% cells being positive using the same protocol as in Figs. 1b – 2b.
More than 10% of the neoplastic cells display a positive staining reaction for ER, but the intensity and proportion of cells demonstrated is significantly reduced compared to the level expected and obtained in both Fig. 3a (Ventana/Roche RTU 790-4324 using Optiview) and Fig. 6b. (Dako/Agilent RTU GA084).
However also compare with Fig. 4b, same protocol.

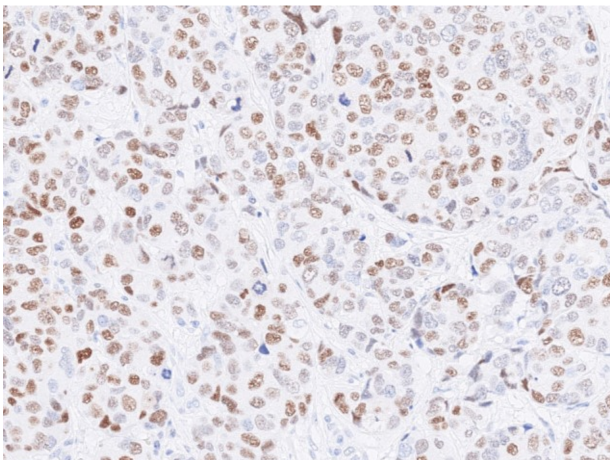


Fig. 4a
Optimal ER staining reaction of the breast carcinoma, tissue core no. 5, with 40-70% of the neoplastic cells expected to be positive (weak to moderate) using same protocol as in Figs. 1a – 3a.
About 50-60% of the neoplastic cells display a weak to moderate but distinct nuclear staining reaction.

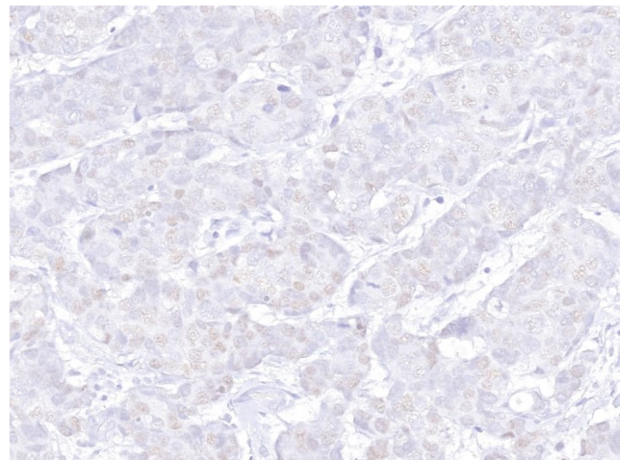


Fig. 4b
Insufficient ER staining reaction of the breast carcinoma, tissue core no. 5, with 40-70% of the neoplastic cells expected to be positive (weak to moderate) using same protocol as in Figs. 1b – 3b.
Only scattered (<5% overall) neoplastic cells show a weak and equivocal nuclear staining reaction for ER with a somewhat impaired morphology.

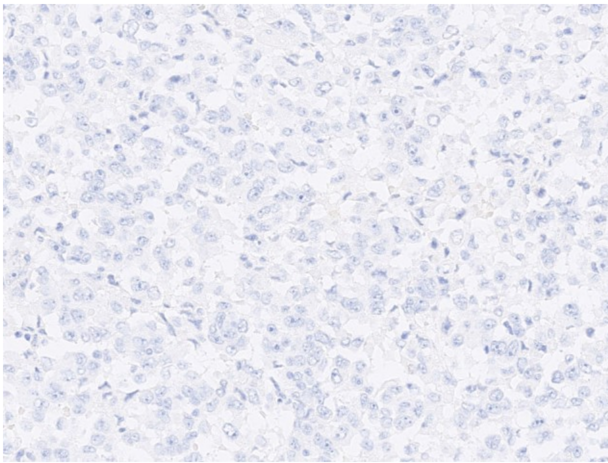


Fig. 5a
Optimal ER staining of the breast carcinoma expected to be ER negative, tissue core no. 4, using the same protocol as in Figs. 1a - 4a. No staining reaction in the neoplastic cells is seen.

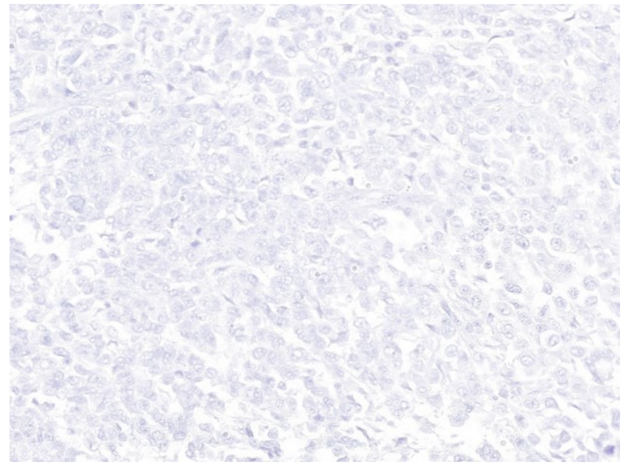


Fig. 5b
ER staining of the breast carcinoma expected to be ER negative, tissue core no. 4, using the same protocol as in Figs. 1b – 4b. As expected, no staining reaction in the neoplastic cells is seen.

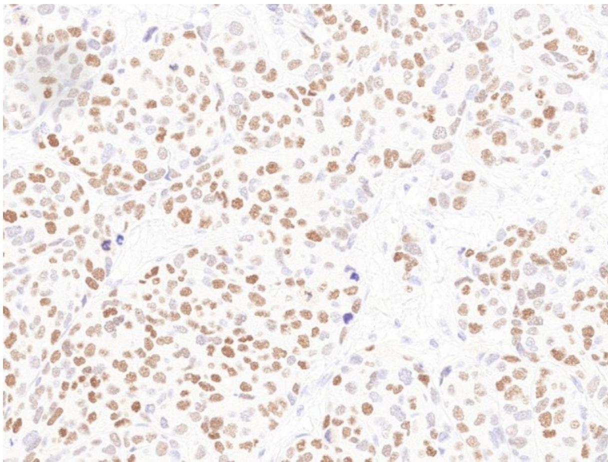


Fig. 6a
Optimal ER staining reaction of the breast carcinoma, tissue core no. 5, with 40-70% of the neoplastic cells expected to be positive (weak to moderate) using the Dako/Agilent ER RTU system for Dako Omnis based on the rmAb clone EP1. The protocol was applied in concordance to vendor protocol recommendations based on HIER in TRS High pH and EnVision FLEX as detection system.
About 50-60% of the neoplastic cells display a weak to moderate but distinct nuclear staining reaction.

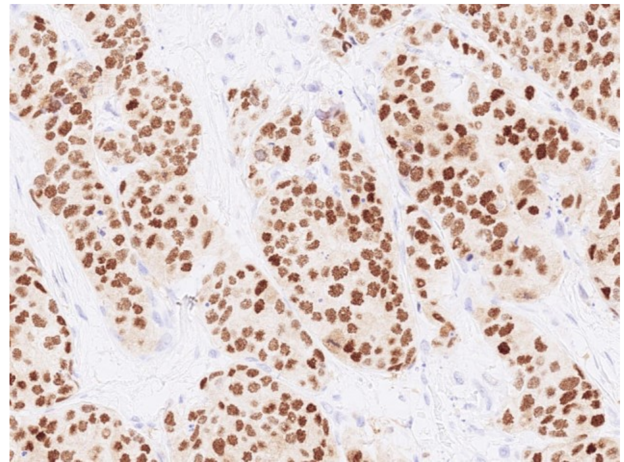


Fig. 6b
Optimal ER staining reaction of the breast carcinoma, tissue core no. 3, with 90-100% cells being positive (moderate to strong) using same protocol as in Fig. 6a. Virtually all neoplastic cells display a moderate to strong and distinct nuclear staining reaction.

NG/RR/KBA/LE/TJU 28.04.2026