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. regularly measurement of ER results) is needed.

Material
The slide to be stained for ER comprised:

No.	Tissue	ER-positivity*	ER-intensity*
1.	Uterine cervix	80-90%	Moderate to strong
2.	Tonsil	1-5%	Weak to moderate
3.	Breast carcinoma	90-100%	Moderate to strong
4.	Breast carcinoma	40-80%**	Weak to moderate
5.	Breast carcinoma	70-95%	Weak to strong
6.	Breast carcinoma	Negative	-
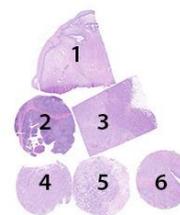
* ER-status and staining pattern as characterized by NordiQC reference laboratories using the rmAb clones EP1 & SP1.
**ER expression slightly heterogenous.

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 the columnar epithelial cells, 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[2] and squamous epithelial cells in the tonsil.
- An at least weak to moderate distinct nuclear staining in the appropriate proportion of the neoplastic cells in the breast carcinomas no. 3, 4 and 5.
- No nuclear staining of neoplastic cells in the breast carcinoma no. 6 and no more than a weak cytoplasmic reaction in cells with a strong nuclear staining.

A staining was classified as **good** if ≥10 % of the neoplastic cells in the breast carcinomas no. 3, 4 and 5 showed an at least weak nuclear staining reaction, but less than the reference range.
An at least week to moderate nuclear staining in the majority of the uterine columnar and squamous epithelial cells and in the dispersed cells expected to be positive in the tonsil.

A staining was assessed as **borderline** if ≥ 1 % but <10 % of the neoplastic cells in one of the breast carcinomas no. 3, 4 and 5 showed a nuclear staining reaction.

A staining was assessed as **poor** if a false negative staining reaction (<1%) was seen in one of the breast carcinomas no. 3, 4 and 5 or false positive staining reaction (≥1 %) in the breast carcinoma no. 6.

Participation

Number of laboratories registered for ER, B29	382
Number of laboratories returning slides	367 (96%)

Results
367 laboratories participated in this assessment. 345 of 367 (94%) achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks given (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Use of detection systems with low sensitivity
- Technical issues

Conclusion

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 ER. 83% of the participants used Ready-To-Use (RTU) systems for the demonstration of ER. The RTU systems from Ventana, Dako and Leica used as "plug-and-play" assays provided a pass rate of 96%. In this assessment, poor analytical sensitivity with too weak or false negative staining reaction was the predominant feature of insufficient results. Uterine cervix and tonsil can be recommended as positive tissue controls for ER. In uterine cervix, virtually all squamous and columnar epithelial cells must show a moderate to strong and distinct nuclear staining reaction. Endothelial cells and lymphocytes must be negative in this tissue.

Tonsil was especially found recommendable as a tool to monitor the level of analytical sensitivity for the demonstration of ER. Dispersed follicular dendritic cells[2] in germinal centers and squamous epithelial cells must show an at least weak but distinct nuclear staining reaction. In addition, tonsil can be used as negative tissue control, as B-cells in mantle zones and within germinal centers must be negative.

Table 1. **Antibodies and assessment marks for ER, B29**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **1D5**	1	Biocare Medical	-	-	1	-	-	-
mAb clone **6F11**	21	Leica/Novocastra	6	13	2	-	90%	29%
mAb clone **C6H7**	1	Cenovte	1	-	-	-	-	-
rmAb clone **EP1**	14 1	Dako/Agilent Cell Marque	7	4	4	-	73%	47%
rmAb clone **SP1**	15 6 1 1	Thermo Scientific Cell Marque Abcam Diagnostic Biosystems	14	6	3	-	87%	61%
Ready-To-Use antibodies							Suff.[1]	OR[2]
mAb clones **1D5 + ER-2-123 SK310**	1	Dako/Agilent	-	1	-	-	-	-
mAb clone **6F11 PA0009/PA0151 (VRPS[3])**	3	Leica	0	3	-	-	-	-
mAb clone **6F11 PA0009/PA0151 (LMPS[4])**	11	Leica	6	4	1	-	91%	55%
rmAb **EP1 IR/IS084 (VRPS[3])**	7	Dako/Agilent	4	3	-	-	100%	57%
rmAb **EP1 IR/IS084 (LMPS[4])**	27	Dako/Agilent	18	6	3	-	89%	67%
rmAb **EP1 GA084 (VRPS[3])**	18	Dako/Agilent	15	3	-	-	100%	83%
rmAb **EP1 GA084 (LMPS[4])**	21	Dako/Agilent	17	3	1	-	95%	81%
rmAb **EP1 8361-C010**	1	Sakura Finetek	1	-	-	-	-	-
rmAb **EP1 249R-27/28/29**	1	Cell Marque	1	-	-	-	-	-
rmAb **EP1 AN710-5M**	1	BioGenex	-	1	-	-	-	-
rmAb clone **SP1 790-4324/5 (VRPS[3])**	42	Ventana/Roche	33	6	2	1	93%	79%
rmAb clone **SP1 790-4324/5 (LMPS[4])**	165	Ventana/Roche	116	45	1	3	98%	70%
rmAb clone **SP1 249R-17/18**	4	Cell Marque	3	1	-	-	-	-
rmAb clone **SP1 RM-9101-R7**	1	Thermo Scientific	1	-	-	-	-	-
rmAb clone **SP1 MAD-000306QD**	1 1	Master Diagnostica Vitro SA	-	2	-	-	-	-
r/mAb clones **6F11 + SP1 PM308**	1	Biocare Medical	1	-	-	-	-	-
Total	367		244	101	18	4	345	
Proportion			66%	28%	5%	1%	94%	

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

Detailed analysis of ER, B29
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **6F11**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Cell Conditioning 1 (CC1, Ventana) (2/4)*, Target Retrieval Solution (TRS) High pH (Dako) (1/1), PT Module Buffer 1 (Thermo Scientific) (1/1), Bond Epitope Retrieval Solution 1 (BERS1, Leica) (1/5) or Bond Epitope Retrieval Solution 2 (BERS2, Leica) (1/7) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:100 and combined with either a 2- or 3-layer detection system. Using these protocol settings, 19 of 20 (95%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EP1**: Protocols with optimal results were based on HIER using CC1 (Ventana) (1/3) or TRS High pH (Dako) (6/10) as retrieval buffer. The rmAb was typically diluted in the range of 1:20-1:50 and combined with either a 2- or 3-layer detection system. Using these protocol settings, 7 of 8 (88%) laboratories produced a sufficient staining result.

rmAb clone **SP1**: Protocols with optimal results were based on HIER using CC1 (Ventana) (6/8), TRS pH 9 (Dako) (3/4), BERS2 (Leica) (3/5), Tris-EDTA pH 9 (1/1) or Trilogy pre-treatment solution (1/1) as retrieval buffer. The rmAb was typically diluted in the range of 1:30-1:200 and combined with either a 2- or 3-layer detection system. Using these protocol settings, 20 of 21 (95%) laboratories produced a sufficient staining result.

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. **Optimal results for ER using concentrated antibodies on the main IHC systems***

Concentrated antibodies	Dako/Agilent Autostainer		Dako/Agilent Omnis		Ventana BenchMark XT/Ultra/GX		Leica Bond III / Max	
	TRS High pH	TRS Low pH	TRS High pH	TRS Low pH	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **6F11**	-	-	1/1**	-	2/4	-	1/7	1/5
rmAb clone **EP1**	5/8	0/1	1/2	-	1/3	-	-	-
rmAb clone **SP1**	-	-	3/3	-	6/8	-	3/5	-

* 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).

Ready-To-Use antibodies and corresponding systems
mAb clone **6F11,** product. no. **PA0009/PA0151**, Leica/Novocastra, Bond III/Bond Max:
Protocols with optimal results were based on HIER using either BERS2 or Novocastra™ Epitope Retrieval Solutions pH 6 for 20-40 min., 15-30 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) or Bond Polymer Refine Red (DS9390) as detection system. Using these protocol settings, 7 of 7 (100%) laboratories produced a sufficient staining result (optimal or good).

rmAb clone **EP1**, product no. **IR084/IS084**, Dako Agilent, Autostainer+/Autostainer Link:
Protocols with optimal results were based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 10-40 min. at 97-98°C), 20-45 min. incubation of the primary Ab and EnVision FLEX (K8000/K8002) or EnVision FLEX+ with rabbit linker (K8009/K8019) as detection system. Using these protocol settings, 23 of 23 (100%) laboratories produced a sufficient staining result.
10 laboratories used product no IR084/IS084 on other platforms. These were not included in the description above.

mAb clone **EP1**, product no. **GA084**, Dako, Dako Omnis:
Protocols with optimal results were based on HIER using TRS High pH (efficient heating time 30 min. at 97°C), 10-27 min. incubation of the primary Ab and EnVision FLEX (GV800) or EnVision FLEX+ with rabbit linker (GV800+GV809) as detection system. Using these protocol settings, 33 of 33 (100%) laboratories produced a sufficient staining result.
3 laboratories used product no. GA084 on other platforms. These were not included in the description above.

rmAb clone **SP1**, product no. **790-4324/4325**, Ventana, BenchMark XT, GX, ULTRA:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 8-76 min., 8-64 min. incubation of the primary Ab and UltraView (760-500) with or without UltraView/iView Amplification kit (760-080), iView (760-091) or OptiView (760-700) as detection system. Using these protocol settings, 189 of 195 (97%) laboratories produced a sufficient staining result.
Two 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 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/IS084**	7/7 (100%)	4/7 (57%)	16/17 (94%)	13/17 (76%)
Dako Omnis rmAb EP1 **GA084**	18/18 (100%)	15/18 (83%)	18/18 (100%)	15/18 (83%)
Leica Bond mAb 6F11 **PA009/PA0151**	3/3 (100%)	0/3 (0%)	10/11 (91%)	6/11 (55%)
VMS Ultra/XT rmAb SP1 **790-4324/4325**	39/42 (93%)	33/42 (79%)	161/165 (98%)	116/165 (70%)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and detection kit. Only protocols performed on the specified vendor IHC stainer are included.

Comments
In this assessment and as seen in previous NordiQC runs for ER, the most common feature of an insufficient staining result was inadequate protocol sensitivity, resulting in a weak or false negative staining reaction, with reduced detection of the ER epitope. This pattern was seen in 86% of the insufficient results (19 of 22). In the remaining 14% (3 of 22), poor signal to noise due to excessive cytoplasmic staining, impaired morphology or faulty counterstaining was seen.

Virtually all laboratories were able to demonstrate ER in the high-level ER-expressing breast carcinoma (tissue core no. 3), in which 90-100% of the neoplastic cells were expected to be demonstrated. By reference standard methods, this tumour showed a moderate to strong intensity. Demonstration of ER in the heterogeneous mid-level ER expressing breast carcinoma no. 4, in which an at least weak nuclear staining reaction of 40-80% of the neoplastic cells was expected, was much more challenging and required a carefully calibrated protocol. In contrast to Run B28, no false positive nuclear staining was seen in this run.

Ready-To-Use (RTU) Abs were used by 83% (306 of 367) of the participants. 94% (287 of 306) of these laboratories used a complete RTU system including the pre-diluted primary Ab, specified ancillary reagents and IHC stainer platform.

The Ventana/Roche RTU system, based on the rmAb clone SP1 (790-4324/4325) and performed on the BenchMark platform, was in this assessment the most widely applied assay being used by 56% of the participants and gave an overall pass rate of 97%. Optimal results could be obtained both by the vendor recommended protocol settings (16 min. incubation of the primary Ab, HIER in CC1 for 64 min. and UltraView or iView as detection kit) and by laboratory modified protocols adjusting incubation time of the primary Ab, HIER time and detection system as shown in Table 3. In this assessment, vendor recommended protocol settings were used by only 11% of the laboratories and provided a slightly reduced overall pass rate but a slightly increased proportion of optimal results compared to laboratory modified protocol settings. The use of UltraView detection kit with the addition of an amplification kit was found to be less likely to provide an optimal result, obtained by 53% (9 of 17) of users, although the pass rate of 94% was comparable to that obtained by the vendor recommended protocol. Use of UltraView with amplification resulted in a reduced signal-to-noise ratio for 29% (5 of 17) laboratories. Use of OptiView detection was observed to be a successful protocol modification, resulting in an optimal staining result for 94% (31 of 33) of users, compared to 79% for the manufacturer's protocol. Optiview detection gave a pass rate of 97% (32 of 33 users).

The Dako/Agilent RTU system GA084 for Omnis, based on rmAb clone EP1 was used by 10% of the participants and gave an overall pass rate of 100% when used on the Omnis platform. The proportion of sufficient and optimal results obtained by the vendor recommended protocol settings and by laboratory modified protocols were similar as shown in Table 3. The modified protocols either increased incubation time of the primary Ab and/or added a rabbit linker for the detection system.

The Dako/Agilent RTU system IR084/IS084 for Autostainer, also based on the rmAb EP1, provided an overall pass rate of 94%. As shown in Table 3, 71% (17 of 24) of the laboratories modified the protocol settings and obtained a higher proportion of optimal results compared to laboratories using the RTU system according to the Dako recommended protocol settings. The most common and successful modification observed was use of FLEX+ and rabbit linker as detection system, often combined with a reduced retrieval time. A modification including rabbit linker was used by 12 laboratories: 100% obtained sufficient and 83% optimal results.

The Leica RTU system PA009/PA0151 for BOND based on mab 6F11, was used by 4% of laboratories and gave an overall pass rate of 93%. In this assessment, vendor recommended protocol settings based on HIER in BERS1 for 20 min., 15 min. incubation of the primary Ab and Bond Refine as detection system provided a slightly superior pass rate compared to laboratory modified protocol settings (see Table 3), but the number of laboratories using this technique was low. No optimal results were obtained using vendor recommended protocols, where all users obtaining weak staining results. Laboratories using a protocol modification enhancing analytical sensitivity by using HIER in BERS2 showed a pass rate of 100% (6 of 6), 83% optimal. However high pH retrieval should be used with caution with this clone due to the concerning number of false positive staining reactions noted using this modification in run B28. Protocol modifications increasing incubation time of the primary antibody alone did not yield any optimal results and resulted in weak staining.

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 employed by 17% (61 of 367) of the participants. The three Abs, **mAb clone 6F11 and rmAb clones EP1 and SP1** used in a LD assay all could provide sufficient and optimal results on the main IHC platforms (Dako/Agilent, Leica and Ventana/Roche), see Tables 1 and 2. Irrespective of the clone applied, careful calibration of the primary Ab concentration in combination with efficient HIER, preferably in an alkaline buffer, and use of a sensitive 3-layer detection system were found to be the common core elements for an optimal performance.

Performance history
This was the twenty-second NordiQC assessment of ER. The proportion of sufficient results has been relatively stable in the recent runs from 2016-2020 (except for run B26) at a high and satisfactory level (see Graph 1).

Graph 1. **Participant numbers and pass rates for ER during 22 NordiQC runs**
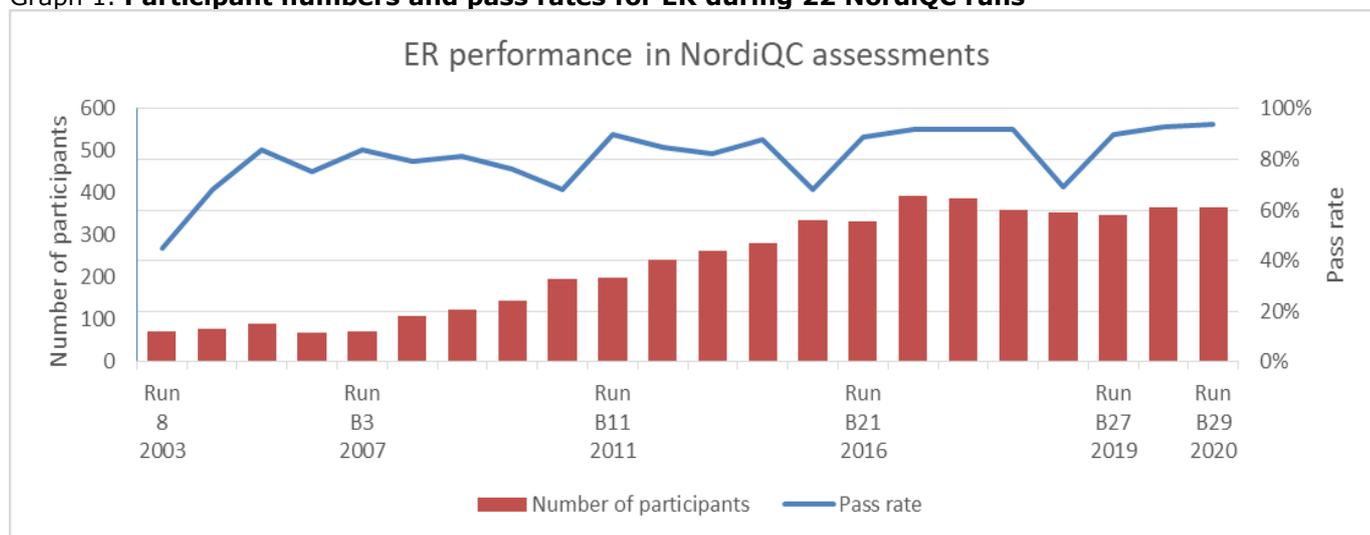
Fluctuations in pass rates, as seen in run B26, is likely caused by more challenging materiel circulated compared to other runs. In order to secure the consistency of the material circulated, NordiQC evaluates the material with two reference standard methods and monitor the ER expression levels throughout all TMAs used in the assessment.

Controls

In concordance with previous NordiQC runs, uterine cervix and tonsil was found to be valuable positive and negative tissue controls for ER staining: In the uterine cervix, optimal results were characterized by virtually all epithelial cells throughout the squamous epithelium and in the glands showing a moderate to strong and distinct nuclear staining reaction. 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 especially recommended as a tool to monitor the analytical sensitivity for the IHC demonstration of ER and was in fact superior to uterine cervix. It was observed, that dispersed cells (most likely follicular dendritic cells[2]) in germinal centers and squamous epithelial cells were distinctively demonstrated in virtually all protocols providing an optimal result. If the follicular dendritic cells were negative, a reduced proportion of ER positive cells were seen in the other tissues and most critically a too weak or even false negative staining was seen in breast carcinomas nos. 4 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 Jan 13. doi: 10.5858/arpa.2019-0904-SA

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
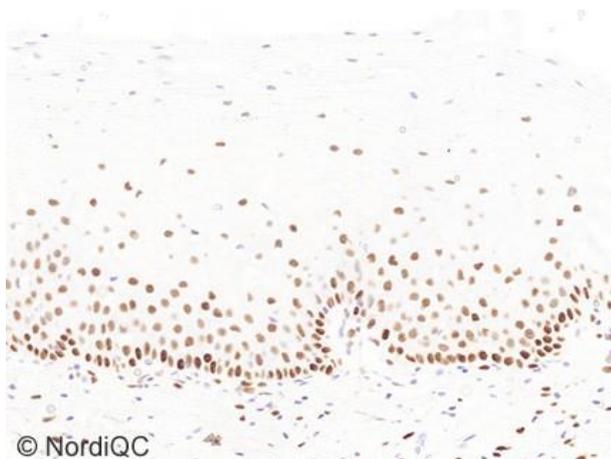
Fig. 1a
Optimal ER staining of the uterine cervix using the rmAb clone EP1 as RTU system (GA084, Dako Omnis) by vendor recommended protocol settings.
Virtually all squamous epithelial and stromal cells show a moderate to strong nuclear staining reaction.
Endothelial and lymphoid cells are negative.
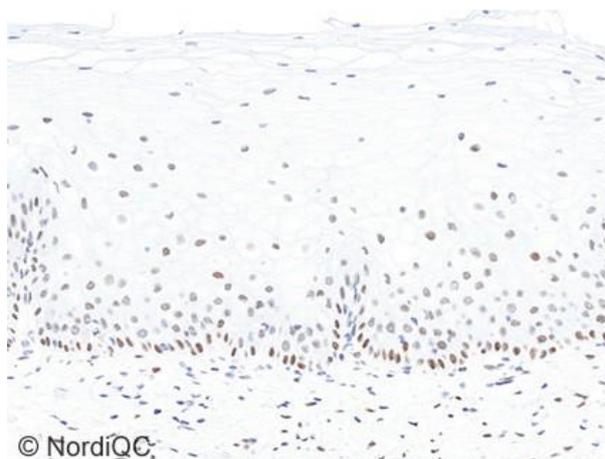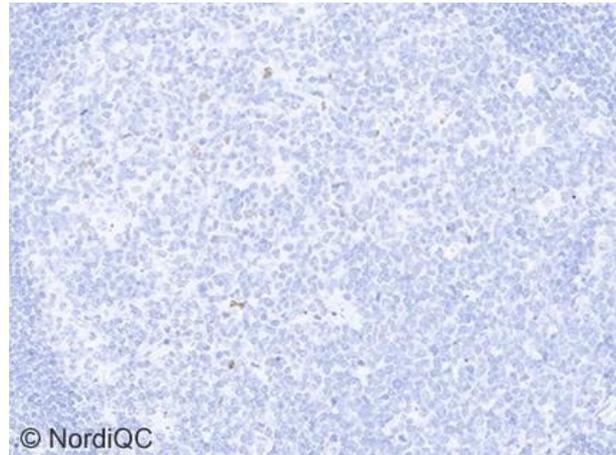Also compare with Figs. 2a-4a, same protocol.

Fig. 1b
ER staining of the uterine cervix using the rmAb clone EP1 by an insufficient protocol – same field as in Fig. 1a. The Dako/Agilent RTU format GA084 with intended use for Dako Omnis was applied on a Leica Bond stainer by protocol settings providing a too low analytical sensitivity.
The intensity and proportion of squamous epithelial and stromal cells demonstrated is reduced.
However, also compare with Figs. 2b- 4b, same protocol.
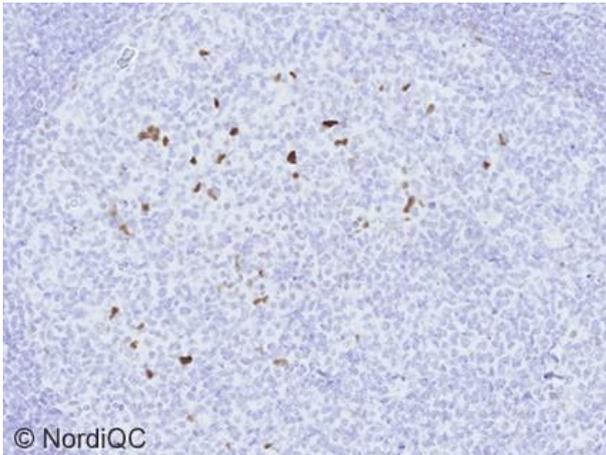
Fig. 2a
Optimal ER staining of the tonsil using same protocol settings as in Fig. 1a.
A moderate to strong, distinct nuclear staining reaction is seen in dispersed follicular dendritic cells in the germinal center.
No nuclear staining reaction is seen in the mantle zone B-cells and in general a high signal-to-noise ratio is seen.

Fig. 2b
Insufficient ER staining of the tonsil using same protocol settings as in Fig. 1b.
Only a faint equivocal staining reaction is observed in few follicular dendritic cells in the germinal center.
Compare with Fig. 2a – same field.
In this and previous NordiQC assessments, tonsil has been a valuable tool to identify protocols providing too low analytical sensitivity and risk of false negative results for ER. This is shown in Figs. 3b and 4b using same protocol.
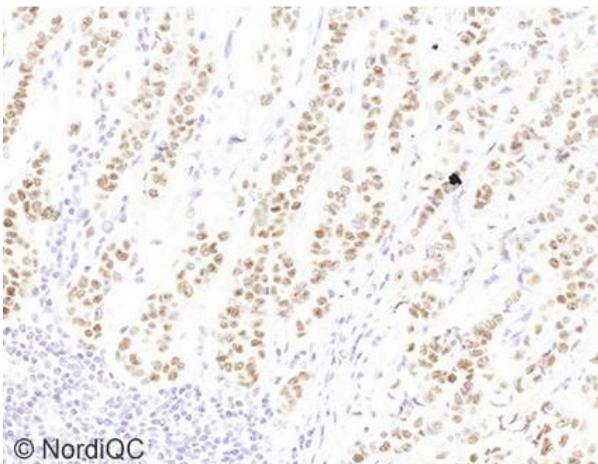
Fig. 3a
Optimal ER staining of the breast carcinoma no. 5 with 70-95% cells being positive using same protocol as in Figs. 1a-2a.
The neoplastic cells display a moderate and distinct nuclear staining reaction.
No background staining is seen.
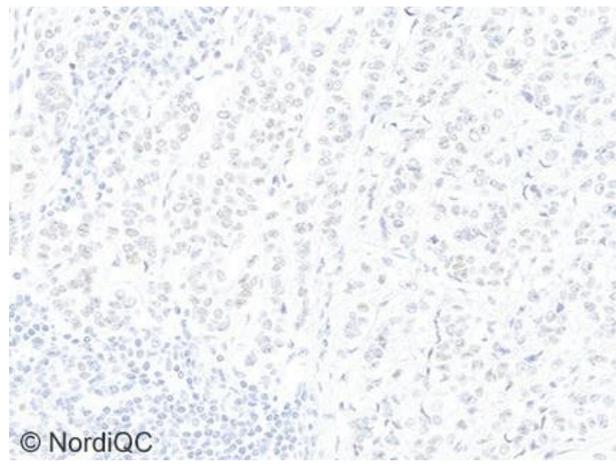
Fig. 3b
Insufficient ER staining of the breast carcinoma no. 5 with expected 70-95% cells being positive using same protocol as in Figs. 1b-2b.
Only scattered neoplastic cells are demonstrated showing an equivocal positivity.
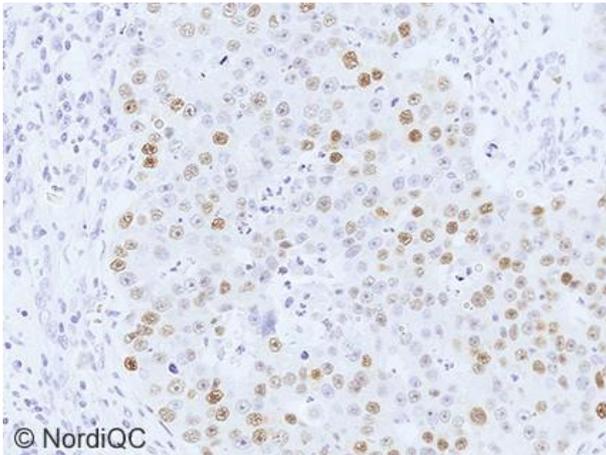
Fig. 4a
Optimal ER staining of the breast carcinoma no. 4 with minimum 40% of the neoplastic cells expected to be positive using same protocol as in Figs. 1a-3a.
About 50% of the neoplastic cells display a weak to moderate and distinct nuclear staining reaction.
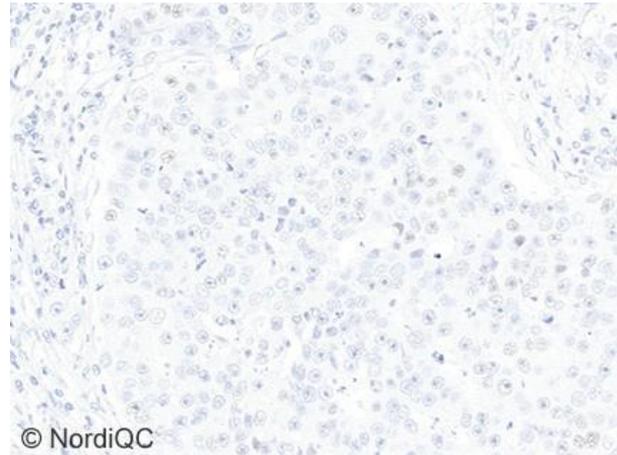No background staining is seen.

Fig. 4b
Insufficient and false negative ER staining of the breast carcinoma no. 4 with expected 40-50% cells being positive using same protocol as in Figs. 1b-3b.
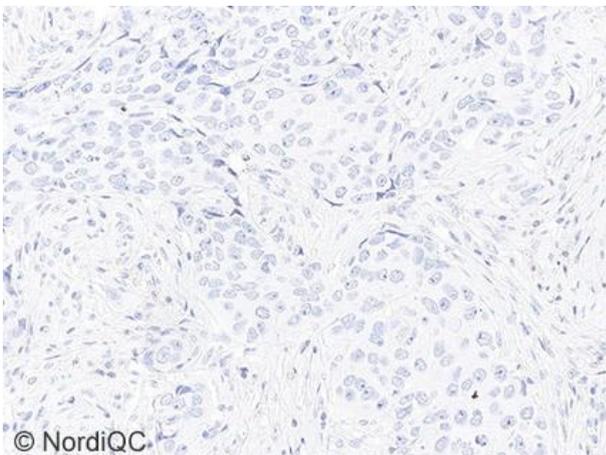<1% of the neoplastic cells are positive.

Fig. 5a
Optimal ER staining of the breast carcinoma no. 6 expected to be negative.
The protocol was based on the rmAb clone SP1 as RTU system (790-4324, Ventana BenchMark Ultra) by vendor recommended protocol settings using UltraView as detection system.
No nuclear staining reaction is seen and a high signal-to-noise ratio observed.
Compare with Fig. 5b, same tumour and protocol except Amplification kit being applied.
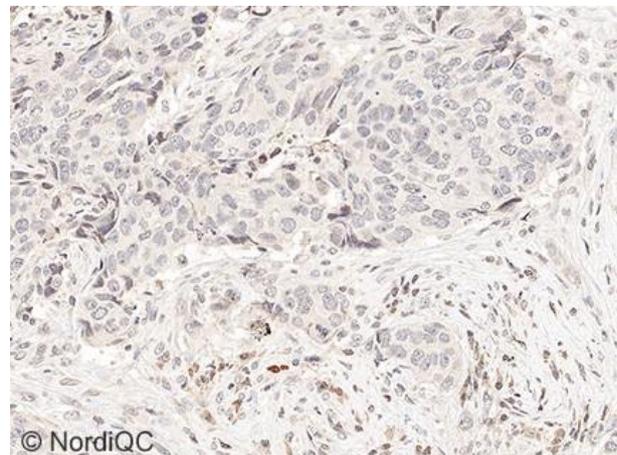

Fig. 5b
Insufficient ER staining of the breast carcinoma no. 6 expected to be negative.
The protocol was based on the rmAb clone SP1 as RTU system (790-4324, Ventana BenchMark Ultra) by laboratory modified protocol settings using UltraView+Amplification kit as detection system.
Plasma cells show an excessive staining reaction and a general poor signal-to-noise ratio is seen compromising the interpretation.
This aberrant pattern was frequently seen when Amplification kit was used to enhance the signal intensity for UltraView.

NG/RR/LE/SN 12.04.2020