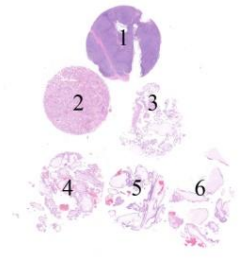
Assessment Run 41 2014
$p57^{Kip2}$ (p57)

Material
The slide to be stained for p57 comprised:



1. Tonsil, 2. Normal placenta, 3-4. Placenta, partial mole, 5-6. Placenta, complete mole

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a p57 staining as optimal were:

- A distinct, moderate to strong nuclear staining reaction of the majority of cytotrophoblastic cells of the normal placenta.
- An at least weak to moderate and distinct nuclear staining reaction of the majority of villous stromal cells in the normal placenta and the two partial moles.
- No nuclear staining reaction of syncytiotrophoblastic cells of the normal placenta.
- No nuclear staining reaction of any cells in the two complete moles (except decidua cells).
- No nuclear staining reaction in the tonsil.

Participation

Number of laboratories registered for p57, run 41	129
Number of laboratories returning slides	121 (94%)

Results
121 laboratories participated in the p57 assessment, NordiQC run 41. Of these 95 (79%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:

- Too low concentration of the primary antibody
- Too high concentration of the primary antibody
- Insufficient heat induced epitope retrieval (HIER)

Performance history
This was the first NordiQC assessment of p57. Table 2 shows the proportion of sufficient results

Table 2. **Proportion of sufficient results for p57 in the NordiQC run performed**

	Run 41 2014
Participants, n=	121
Sufficient results	79%

Conclusion
The mAb clones **25B2**, **57P06**, **KP10** and **KP39** could all be used to obtain optimal staining result for p57. In general, the mAb clone **KP10** as concentrate provided the highest pass rate, whereas the ready-to-use (RTU) system from Ventana/Cell Marque based on the **KP10** clone gave the highest proportion of optimal results.
Irrespective of the clone applied, efficient HIER, preferable in an alkaline buffer and careful calibration of the primary antibody are the main prerequisites for optimal staining results.
Normal placenta is recommended as positive and negative tissue control to verify correct calibration of the immunohistochemical protocol for p57. Cytotrophoblastic and intermediate trophoblastic cells as well as decidual cells must show a moderate to strong nuclear staining reaction, while an at least weak to moderate nuclear staining reaction must be seen in the majority of villous stromal cells. No nuclear staining reaction must be seen in the syncytiotrophoblastic cells.

Table 1. **Antibodies and assessment marks for p57, run 41**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **25B2**	33 1	Leica/Novocastra Vector Labs	9	11	12	2	59%	70%
mAb clone **57P06**[3]	37 2	Thermo/NeoMarkers Diagnostics Biosystems	17	15	6	1	82%	85%
mAb clone **KP10**	11 2 1	Cell Marque Immunologic Zeta	6	8	0	0	100%	100%
mAb clone **KP39**	5 1	Thermo/NeoMarkers Abcam	2	3	1	0	-	-
rmAb clone **SP119**	1	Spring Biosciences	0	0	0	1	-	-
Unknown	1	Unknown	0	0	1	0	-	-
Ready-To-Use antibodies								
mAb clone **57P06 MAD-00576QD**	1	Master Diagnostica	0	1	0	0	-	-
mAb clone **KP10 760-4617**	20	Cell Marque/Ventana	14	4	2	0	90%	90%
mAb clone **KP10 457M-97/8**	4	Cell Marque	2	2	0	0	-	-
mAb clone **KP10 MON-RTU1169**	1	Monosan	0	1	0	0	-	-
Total	121		50	45	22	4	-	
Proportion			42%	37%	18%	3%	79%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3)Thermo Fisher Scientific lists in the package insert that mAb clone 57P06 is same as mAb clone KP10

Detailed analysis of p57, Run 41
Following central protocol parameters were used to obtain an optimal staining:

Concentrated antibodies
mAb clone **25B2**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) pH 6.1 (Dako) (1/1)*, Cell Conditioning 1 (CC1; Ventana) (1/9), Bond Epitope Retrieval Solution 2 (BERS2; Leica) (2/7), BERS 1 (Leica) (1/5), Diva Decloaker pH 6.2 (Biocare) (2/2) or Tris-EDTA/EGTA pH 9 (2/3) as retrieval buffer. The mAb was typically diluted in the range of 1:40-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 14 of 20 (70%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **57P06**: Protocols with optimal results were all based on HIER using TRS pH 9 (3-in-1) (Dako) (3/5), TRS pH 9 (Dako) (3/5), TRS pH 6.1 (Dako) (1/2), CC1 (Ventana) (2/13), CC2 (Ventana) (2/3), BERS2 (Leica) (1/2), Tris-EDTA/EGTA pH 9 (4/6) or Citrate pH 6 (1/2) as retrieval buffer. The mAb was typically diluted in the range of 1:75-1:2,000 depending on the total sensitivity of the protocol employed. Using these protocol settings 28 of 33 (85%) laboratories produced a sufficient staining result.

mAb clone **KP10**: Protocols with optimal results were all based on HIER using TRS pH 9 (3-in-1) (Dako) (3/4), TRS pH 9 (Dako) (1/2), CC1 (Ventana) (1/5) or Citrate pH 6 (1/2) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 11 of 11 (100%) laboratories produced a sufficient staining result.

mAb clone **KP39**: Protocols with optimal results were based on HIER using TRS pH 9 (3-in-1) (Dako) (1/1) or CC1 (Ventana) (1/3) as retrieval buffer. The mAb was diluted in the range of 1:200-1:800 depending on the total sensitivity of the protocol employed. Using these protocol settings 2 of 2 (100%) laboratories produced a sufficient staining result.

Table 3. **Proportion of optimal results for p57 for the two most commonly used antibodies as concentrate on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **25B12**	0/6** (0%)	1/1	1/9 (11%)	-	2/8 (25%)	0/4
mAb clone **KP10****	10/15 (67%)	1/2	3/13 (23%)	2/3	1/2	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** (number of optimal results/number of laboratories using this buffer)
*** 57P06 and KP10 pooled together

Ready-To-Use antibodies and corresponding systems
mAb clone **KP10**, product no. 760-4617, Ventana/Cell Marque, BenchMark XT/Ultra:
Protocols with optimal results were all based on HIER using mild or standard Cell Conditioning 1, 4-32 min. incubation of the primary Ab and IView (760-091), UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings 18 of 20 (90%) laboratories produced a sufficient staining result.

Comments
In this first NordiQC assessment of p57, the prevalent features of an insufficient staining result were either a generally too weak staining reaction of the cells expected to be demonstrated or poor signal-to-noise ratio.
Too weak staining results were seen in 73% of the insufficient results (19 of 26). Virtually all laboratories were able to demonstrate p57 in high-level p57 expressing cells (decidua and cytotrophoblastic cells), whereas low-level p57 expressing cells as villous stromal cells could only be demonstrated using an optimal and carefully calibrated protocol. Too low concentration of the primary Ab and/or insufficient HIER (e.g. too short efficient HIER time, usage of non-alkaline buffer) typically were reasons for too low sensitivity of immunohistochemical demonstration of p57.
In the remaining 27% of the insufficient results, poor signal-to-noise ratio and/or false positive staining reaction was seen. This pattern was typically characterized by a diffuse moderate background and/or cytoplasmic staining reaction in e.g. cytotrophoblasts and smooth muscle cells complicating the interpretation. In a few cases this staining pattern was accompanied by a weak to moderate aberrant nuclear staining reaction in cells not expressing p57 (syncytiotrophoblastic cells of the placenta and mantle zone B-cells of the tonsil). The poor signal-to-noise ratio was mainly seen for the mAb clone KP39 and mAb clone 57P06 (KP10) (Thermo Fisher Scientific (TFS)) and was typically enhanced using a too high concentration of the primary Ab. The mAb clone 57P06 (KP10) also showed a moderate to strong cytoplasmic staining reaction in squamous epithelial cells of the tonsil. This staining pattern was most likely due to MAG reaction (Mouse Ascites Golgi Reaction), as the Ab from TFS is provided as ascites format, while the clone from other vendors most typically is distributed as supernatant.

The mAb clone KP10 gave the highest proportion of sufficient results, and using the Ab as a concentrate, optimal staining could be obtained on all 3 main IHC systems (see table 3). Also the corresponding RTU systems based on the mAb clone KP10 provided a high proportion of sufficient and optimal results. The Ventana RTU (760-4617) gave a pass rate of 90% (18 of 20) of out which 78% were evaluated as optimal. Optimal staining results could be obtained by the officially recommended protocol based on HIER in CC1 for 32 min., 8 or 16 min. incubation of the primary Ab using UltraView or OptiView, but also by laboratory modified protocols typically with prolonged HIER and/or prolonged incubation time of the primary Ab.

Controls
Placenta is recommended as positive and negative tissue control. The protocol must be calibrated to give a moderate to strong nuclear staining reaction in the majority of cytotrophoblastic and intermediate trophoblastic cells, while an at least weak to moderate nuclear staining reaction must be seen in the majority of villous stromal cells. Decidua cells will show an intense nuclear staining reaction and cannot be used to calibrate the protocol or serve as a reliable internal positive tissue control due to high levels of p57 expression.
No nuclear staining reaction must be seen in syncytiotrophoblastic cells.

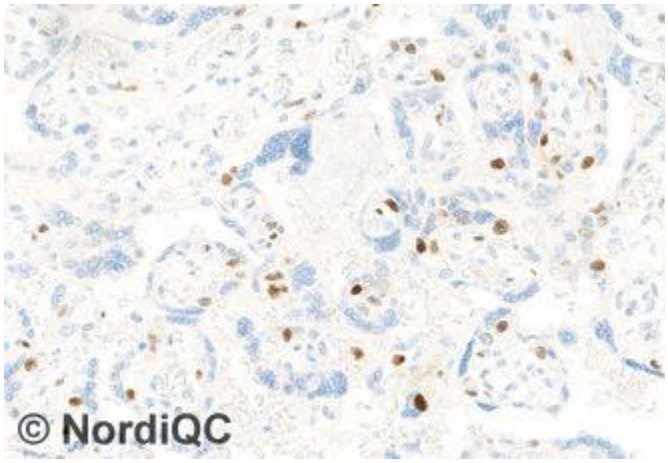


Fig. 1a (X200)
Optimal p57 staining of the normal placenta using the mAb clone KP10, using HIER in an alkaline buffer (CC1 pH 8.5, Ventana) and a 3-step multimer based detection system (OptiView, Ventana). A distinct, moderate to strong nuclear staining reaction in the majority of cytotrophoblastic cells is seen, while syncytiotrophoblastic cells are negative – also compare with Figs. 2a & 3a, same protocol

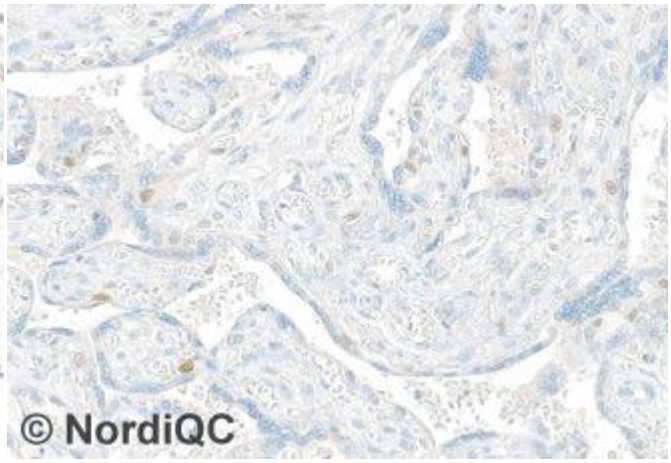


Fig. 1b (X200)
Insufficient p57 staining of the normal placenta using the mAb clone 25B2. The protocol provided a too low sensitivity most likely due to a too low titre of the primary Ab and use of a 2-step multimer based detection system (UltraView, Ventana). The intensity and proportion of cytotrophoblastic cells demonstrated is significantly reduced compared to the level expected and obtained in Fig. 1a – also compare with Figs. 2b & 3b, same protocol.

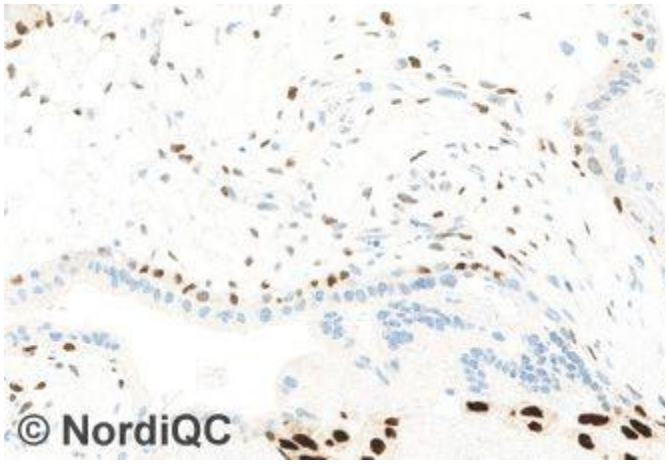


Fig. 2a (X200)
Optimal p57 staining of the partial mole, tissue core no. 3 using same protocol as in Fig. 1a. The majority of villous stromal cells show a distinct moderate nuclear staining reaction. A strong nuclear staining reaction in decidua cells is observed (bottom right corner).

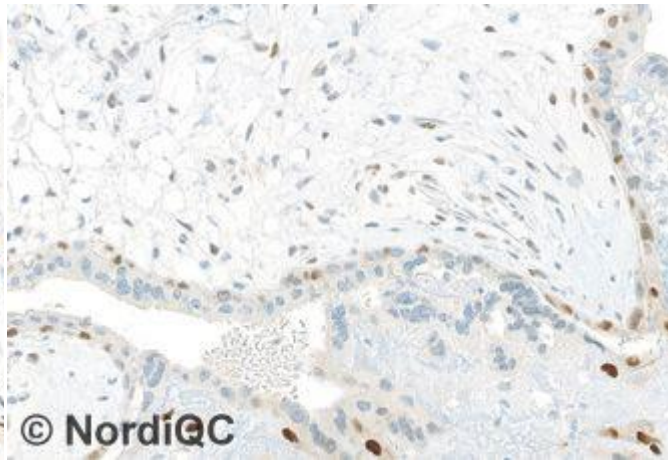


Fig. 2b (X200)
Insufficient p57 staining of the partial mole, tissue core no. 3 using same protocol as in Fig. 1b - same field as in Fig 2a. Only decidual cells show a distinct nuclear staining reaction, while the villous stromal cells virtually are negative.

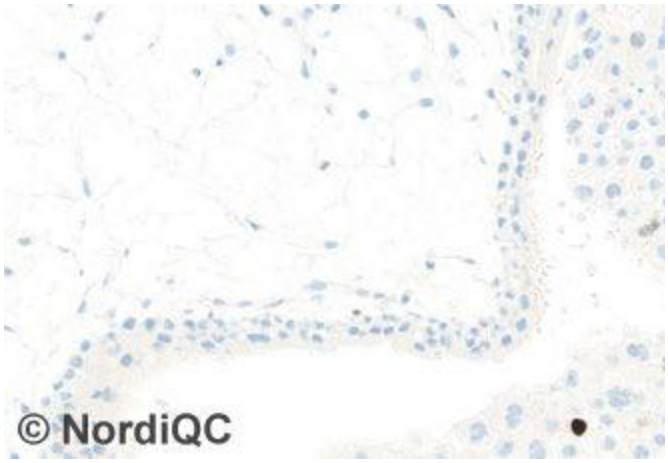


Fig. 3a (X200)
Optimal p57 staining of the complete mole, tissue core no. 5 using same protocol as in Figs. 1a & 2a. No nuclear staining reaction in the stromal cells is seen and only scattered decidua cells are demonstrated.

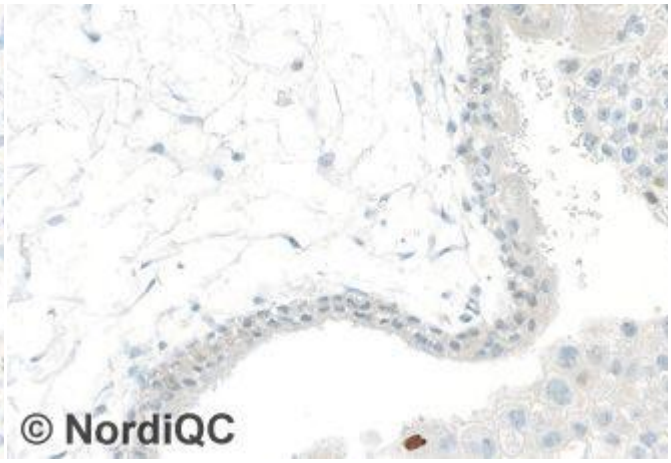


Fig. 3b (X400)
P57 staining of the complete mole, tissue core no. 5 using same protocol as in Figs. 1b & 2b. – same field as in Fig. 3a. No nuclear staining reaction in the stromal cells is seen – however as virtually no nuclear staining reaction in normal cytotrophoblastic cells is seen (Fig. 1b) the staining result cannot reliably be interpreted.

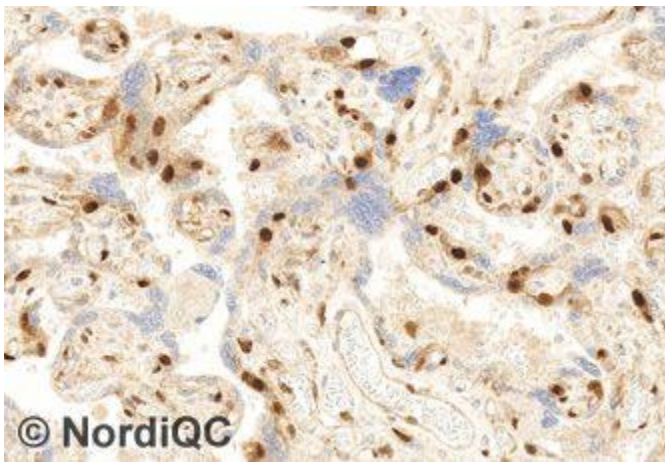


Fig. 4a
Insufficient p57 staining of the normal placenta using the mAb clone KP39. A general background and excessive cytoplasmic staining reaction of both cytotrophoblastic and endothelial cells is seen. The reduced signal-to-noise ratio most likely was caused by a too high concentration of the primary Ab. Also compare with Fig. 4b, same protocol.

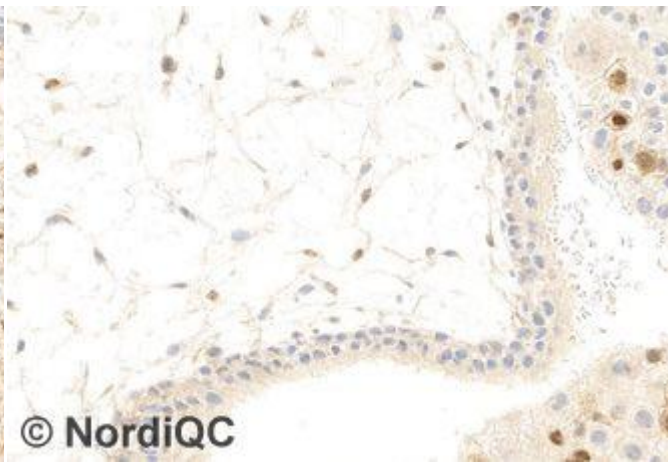


Fig. 4b
Insufficient p57 staining of the complete mole, tissue core no. 5 using same protocol as in Fig. 4a. An excessive cytoplasmic staining reaction and reduced signal-to-noise ratio complicates the interpretation and it is difficult to interpret the staining reaction in villous stromal cells expected to be negative (see Fig. 3a).

SN/RR/MV/LE 11-06-2014