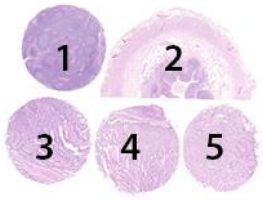
Assessment Run 57 2019

Mismatch Repair Protein MSH2 (MSH2)

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of IHC tests among NordiQC participants for MSH2 status in colon adenocarcinomas. Loss of MSH2 function due to gene mutation or epigenetic changes is characterized by absence of nuclear expression in neoplastic cells, whereas intact nuclear MSH2 expression indicates normal MSH2 function and no gene mutations.

Material
The slide to be stained for MSH2 comprised:

1. Tonsil, 2. Appendix, 3. Colon adenocarcinoma with normal MSH2 expression, 4-5. Colon adenocarcinoma with loss of MSH2 expression.



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a MSH2 staining as optimal were:

- An at least weak to moderate, distinct nuclear staining reaction of virtually all cells in the appendix.
- An at least weak to moderate, distinct nuclear staining reaction of virtually all mantle zone B-cells. and a moderate to strong, distinct nuclear staining reaction of the germinal centre B-cells in the tonsil.
- A moderate to strong, distinct nuclear staining reaction of virtually all neoplastic cells in the colon adenocarcinoma no. 3.
- No nuclear staining reaction of the neoplastic cells in the colon adenocarcinomas no. 4 and no. 5, but a distinct nuclear staining reaction in the vast majority of other cells (e.g. stromal cells).

A weak cytoplasmic staining reaction was accepted providing that it did not compromise the interpretation.

Participation

Number of laboratories registered for MSH2, run 57	280
Number of laboratories returning slides	258 (92%)

Results
258 laboratories participated in this assessment. Of these, 208 (81%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks given.

The most frequent causes of insufficient staining reaction were:
- Insufficient Heat Induced Epitope Retrieval (HIER)
- Use of less sensitive detection systems
- Use of OptiView with amplification kit for the mAb clone G219-1129

Performance history
This was the fourth NordiQC assessment of MSH2. A slightly increased pass rate has been observed compared to the first assessment runs for MSH2.

Table 2. **Proportion of sufficient results for MSH2 in four NordiQC runs performed**

	Run 22 2008	Run 41 2014	Run 50 2017	Run 57 2019
Participants, n=	51	143	231	258
Sufficient results	73%	67%	79%	81%

Conclusion
The mAb clones **FE11**, **G219-1129, 79H11, MX061** and the rmAb clone **RED2** could all be used to obtain optimal staining results for MSH2. Irrespective of the clone applied, HIER in an alkaline buffer and use of a sensitive and specific 3-step polymer/multimer based detection system gave the highest proportion of optimal results.
The Ready-To-Use (RTU) systems based on clone FE11 (Dako/Agilent IR085) and clone G219-1129

(Ventana/Roche 760-5093), provided the highest proportion of sufficient and optimal results.
For the laboratories using the Dako/Agilent RTU system IR085 following the recommended protocol settings, a pass rate of 100% was obtained and 86% being optimal. For the laboratories using the Ventana RTU system 760-5093 complying to the recommended protocol settings, a pass rate of 94% was seen and 75% received an optimal score.
In general, the mAb clone G219-1129 was observed to be slightly more challenging to provide an optimal result and required a carefully calibrated protocol to balance between a distinct nuclear demonstration of MSH2 and no cytoplasmic cross-reaction in epithelial cells. This was especially a problem in tissue core no. 4 and 5, where loss of MSH2 should be identified. A weak cytoplasmic cross-reaction was accepted, but moderate to strong cytoplasmic reaction compromised the interpretation and was therefore not accepted.
Tonsil is recommendable as external positive tissue control for MSH2 to monitor IHC test reproducibility focusing on the analytical sensitivity. However, for IHC for Mismatch Repair proteins (MMR) as MSH2 it has to be emphasized that internal positive tissue controls, e.g. normal stromal cells adjacent to the neoplastic cells, are preferred to external controls. An observed intact expression of MMR proteins in the internal normal cells together with loss of MMR proteins in the neoplastic cells is of diagnostic importance.[1]

Table 1. **Antibodies and assessment marks for MSH2, run 57**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **25D12**	1 1	Leica/Novocastra Diagnostic Biosystem	0	2	0	0	-	-
rmAb Clone **BSR77**	1	Nordic Biosite	0	0	1	0	-	-
mAb clone **FE11**	15 6 3 3 1 1	Dako/Agilent BioCare Biocare Medical Calbiochem Menarini Zytomed	10	8	9	2	62%	70%
mAb clone **G219-1129**	16 5 1	Cell Marque BD Biosciences Monosan	11	2	7	2	59%	61%
mAB clone **GB12**	1	Calbiochem	0	0	1	0	-	-
rpAb Clone **RED2**	1 1	Abcam Epitomic	2	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **25D12 PA0048**	2	Leica/Novocastra	0	0	1	1	-	
mAb clone **79H11 PA0989**	2	Leica Biosystem	2	0	0	0	-	
mAb clone **FE11 IR085**	66	Dako/Agilent	41	18	7	0	89%	
mAb clone **FE11 MSG031**	3	Zytomed Systems	0	3	0	0	-	
mAb clone **FE11 MAD-00677QD**	2	Master Diagnostica	0	2	0	0	-	
mAb clone **FE11 PM219**	1	Biocare Medical	1	0	0	0	-	
mAb clone **G219-1129 760-5093**	110	Ventana	62	32	13	3	86%	
mAb clone **G219-1129 286M-18 or 286M-17**	11	Cell Marque	5	4	2	0	81%	
rmAb clone **RED2 8327-C010**	2	Sakura Finetek	1	1	0	0	-	
Clone **MX061 MAB 08-36**	1	Maixin	1	0	0	0	-	
rmAb clone **SP46**	1	BioGenex	0	0	0	1	-	

AN743								
Total	258		136	72	41	9	-	
Proportion			53%	28%	16%	4%	81%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Detailed analysis of MSH2, Run 57

The following protocol parameters were central to obtain an optimal staining:

Concentrated antibodies
mAb clone **FE11**: Protocols with optimal results were all based on HIER using Bond Epitope Retrieval Solution 2 (BERS2; Leica) (6/14)*, Target Retrieval Solution (TRS) High pH (Dako) (3/4) or TRS pH 9 (3-in-1) (Dako) (1/5) as retrieval buffer. The mAb was diluted in the range of 1:25-1:100 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 **G219-1129**: Protocols with optimal results were all based on HIER using Cell Conditioning 1 (CC1; Ventana) (7/12), TRS High pH (Dako) (2/3) or BERS2 (Leica) (1/3) as retrieval buffer. The mAb was diluted in the range of 1:100-1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings, 11 of 18 (61%) laboratories produced a sufficient staining result.

Table 3. **Proportion of optimal results for MSH2 for the most commonly used antibodies as concentrate on the 4 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Dako Omnis**		**Ventana BenchMark GX / XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	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 **FE11**	1/5** (20%)	-	3/4	-	0/4	-	6/14 (23%)	-
mAb clone **G219-1129**	0/1	-	2/2	-	7/12 (58%)	0/1	1/3	-

* 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)

Ready-To-Use antibodies and corresponding systems
mAb clone **FE11**, product no. **IR085**, Dako, /Autostainer Link 48:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 10-30 min. at 95-99°C), 20-30 min. incubation of the primary Ab and EnVision FLEX+ (K8002) with mouse linker (K8021) as detection system. Using these protocol settings, 24 of 27 (89%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **G219-1129,** product no. **760-5093**, Ventana, BenchMark GX/XT/Ultra:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min.), 12-32 min. incubation of the primary Ab and UltraView (760-500) +/- amplification kit (760-080) or OptiView (760-700) +/- amplification kit (760-099 / 860-099) as detection systems. Using these protocol settings, 82 of 93 (88%) laboratories produced a sufficient staining result.

rmAb clone **RED2,** product no. **8327-C010**, Sakura Finetek, Genie:
One protocol with an optimal result was based on HIER using Sakura Finetek Tissue-Tek Genie High pH Antigen Retrieval Buffer (efficient heating time 60 min. at 98°C), 30 min. incubation of the primary Ab and Tissue-Tek PRO DAB Detection Kit (8826-K250) as detection system.

mAb clone **79H11,** product no. **PA0989,** Leica Biosystem, Bond III:
Two protocols with optimal results used identical protocol settings based on HIER in BERS2 (efficient heating time 20 min.), 15 min. incubation of the primary Ab and Bond Refine (DS9800) as detection system.

Table 4 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 protocols performed on the specific IHC stainer device are included.

Table 4. **Proportion of sufficient and optimal results for MSH2 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS Link mAb FE11 **IR085**	100% (14/14)	86% (12/14)	87% (45/52)	56% (29/52)
VMS Ultra/XT mAb G219-1129 **760-5093**	94% (30/32)	75% (24/32)	86% (67/78)	53% (41/78)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments

In this assessment and in concordance with previous NordiQC MSH2 assessments, the prevalent feature of an insufficient result was a too weak or false negative staining reaction of cells expected to be demonstrated. This pattern was seen in 64% of the insufficient results (32 of 50 laboratories). The remaining 36% insufficient results were characterized by a poor signal-to-noise ratio and false positive staining reaction primarily in the cytoplasm of the neoplastic cells in the colon adenocarcinoma tissue core no. 4 and 5 compromising the interpretation.
The majority of laboratories were able to demonstrate MSH2 in cells with a high-level antigen expression as proliferating germinal centre B-cells in the tonsil, basal epithelial cells of the appendix and neoplastic cells in the colon adenocarcinoma tissue core no. 3 with normal MSH2 expression. Demonstration of MSH2 in low-level antigen expressing cells (as resting mantle zone B-cells, smooth muscle cells and stromal cells in the two colon adenocarcinomas with MSH2 loss) was more challenging and required an optimally calibrated protocol. In this context, it has to be emphasized that identification of loss of MSH2 expression in tumors is characterized by a negative nuclear staining reaction of the neoplastic cells. Consequently, it is decisive that normal cells within and around the neoplastic tissue show a distinct positive nuclear staining reaction, serving as reliable internal positive tissue control (Fig. 2a – Fig. 4a).

22% (57 of 258) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for MSH2. The mAb clones FE11 and G219-1129 were the most widely used antibodies (see Table 1) and could both be used to obtain an optimal staining result. 59% (13/22) of the laboratories using the mAb G219-1129 in a LD assay produced sufficient staining results of which 85% were optimal. The pass rate for the mAb clone FE11 within a LD assay was 62% (18/29) of which 56% were optimal. For both mAb clone FE11 and mAb clone G219-1129 and in concordance with previous MSH2 assessments, the hallmarks of LD-protocols with optimal results were use of HIER in an alkaline buffer (pH 8-9) in combination with a 3-step polymer/multimer based detection system as EnVision Flex+ (Dako), OptiView (Ventana) and Refine (Leica).
With mAb clone FE11, optimal results could be obtained on the most widely used IHC platforms, except for the Ventana BenchMark platform (see Table 3). The same pattern was also observed in the previous run 50 and, despite the number of laboratories are small, indicates, that the mAb clone FE11 might be difficult to optimize on the Ventana BenchMark platform. With mAb clone G219-1129, optimal results could be obtained on the main fully automated IHC platforms as Dako Omnis, Ventana BenchMark and the Leica Bond (see Table 3).

78% (201 of 258) of the laboratories used Abs in Ready-To-Use (RTU) formats. This was a significant increase compared to the two previous MSH2 assessments in 2014 and 2017, in which RTU formats were used by 60% and 69% of the participants, respectively. The two most widely used RTU systems for MSH2 were the Ventana/Roche **760-5093** assay based on the mAb clone G219-1129 and the Dako/Agilent assay **IR085** based on mAb clone FE11. Both RTU systems provided higher proportions of sufficient and optimal results compared to LD assays using the same clones (see Table 1). Optimal results could both be obtained by the official protocol recommendations given by the two companies for the RTU formats and by laboratory modified protocol settings.
21% of the laboratories using the Dako/Agilent IR085 system followed the recommended protocol settings and all 100% (14 of 14) achieved sufficient results with 86% being optimal (12 of 14). For the laboratory modified protocol settings the results were inferior: 87% (45 of 52) of the laboratories achieved sufficient results but only 56% (29 of 52) were optimal. The inferior performance was typically characterized by a reduced analytical sensitivity and a general too weak staining reaction primarily caused by omission of the mouse linker for the detection system.

For the Ventana system based on 760-5093, 29% (32 of 110) followed the recommended protocol settings and 94% achieved sufficient results, 75% being optimal. Also for this RTU system, laboratory modified protocol settings provided inferior results, as a pass rate of 71% for this group was seen and only 53% was optimal (see Table 4).
The inferior performance for the laboratory modified protocols using the Ventana RTU system 760-5093 was predominantly characterized by a poor signal to noise ratio and an extensive granular cytoplasmic staining reaction in the neoplastic cells with nuclear loss of MSH2 in tissue core no 4 and 5 compromising interpretation in these carcinomas. The aberrant staining reaction was in particular seen with protocols increasing the analytical sensitivity compared to the level settled by the Ventana recommended protocol settings e.g. induced by prolonging HIER, Ab incubation time and/or use of amplification kit for OptiView.
Overall, the mAb clone G219-1129 was the most commonly used antibody both as concentrate and as RTU format and frequently showed an aberrant cross-reaction in the cytoplasm of the neoplastic cells in the two colon adenocarcinomas with loss of MSH2. When using this clone, it is very important to calibrate your protocol to avoid or reduce the cytoplasmic cross-reaction that can interfere with the interpretation of the staining.

Controls
Tonsil is a recommendable external positive tissue control for MSH2 (Fig 1.a) to monitor IHC test reproducibility focusing on the level of analytical sensitivity. Virtually all mantle zone B-cells must show an at least weak to moderate nuclear staining reaction, while a moderate to strong nuclear staining reaction must be seen in the proliferating germinal centre B-cells.
Colon adenocarcinoma with loss of MSH2 expression is recommended as external negative tissue control for MSH2 (see Fig. 3.a and Fig. 4.a) especially in the validation/verification process for the IHC assay. In tissues with MSH2 loss, no nuclear staining reaction should be seen in the neoplastic cells, whereas a distinct nuclear staining reaction must be seen in stromal cells.

For IHC for Mismatch Repair proteins (MMR) as MSH2 it has to be emphasized that internal positive tissue controls being e.g. normal stromal cells adjacent to the neoplastic cells are preferred to external controls. An observed intact expression of MMR proteins in the internal normal cells together with loss of MMR proteins in the neoplastic cells is of diagnostic importance.[1]

1Torlakovic EE, Nielsen S, Francis G, Garratt J, Gilks B, Goldsmith JD, Hornick JL, Hyjek E, Ibrahim M, Miller K, Petcu E, Swanson PE, Zhou X, Taylor CR, Vyberg M. Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee. Appl Immunohistochem Mol Morphol. 2015 Jan;23(1):1-18. doi: 10.1097/PAI.0000000000000163. Review. PubMed PMID: 25474126.

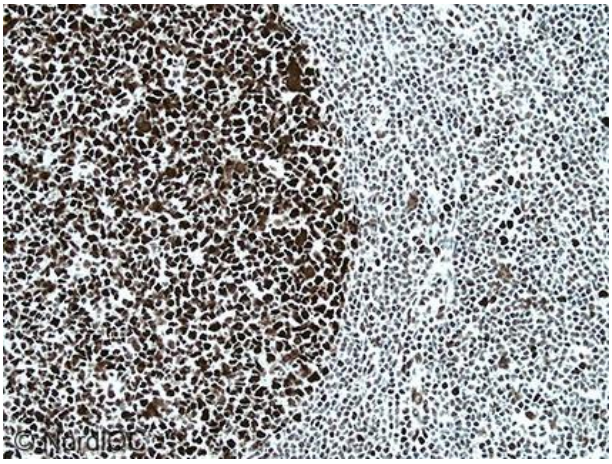


Fig. 1a
Optimal MSH2 staining of the tonsil using the mAb clone FE11, optimally calibrated, HIER in an alkaline buffer and a **3-step polymer** based detection system on the Dako Autostainer Link 48 platform.
Virtually all mantle zone B-cells show a moderate and distinct nuclear staining reaction, while the germinal centre B-cells show a strong nuclear staining reaction.
Also compare with Figs. 2a - 3a, same protocol.

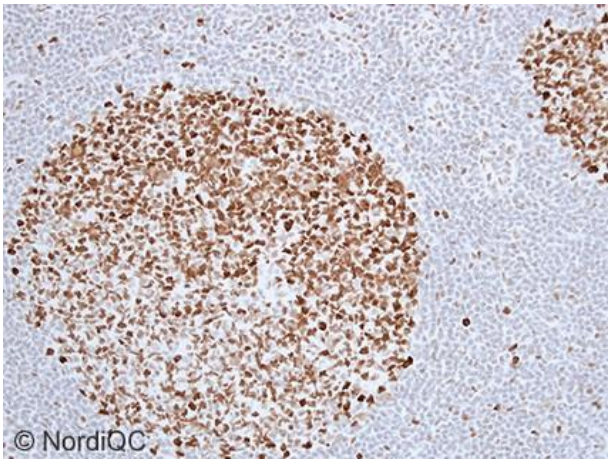


Fig. 1b
Insufficient MSH2 staining of the tonsil using the mAb clone FE11 with similar protocol settings as Figs 1a-3a, except for the use of **2-step polymer** based detection system on the Dako Autostainer Link 48 platform.
Only the germinal centre B-cells are demonstrated, while mantle zone B-cells expressing low level MSH2 are virtually unstained. Also compare with Figs. 2b-3b, same protocol.

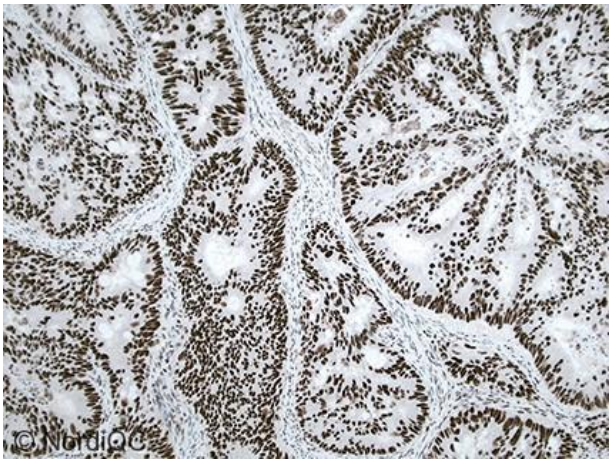


Fig. 2a
Optimal MSH2 staining of the colon adenocarcinoma tissue core no. 3 with normal MSH2 expression using same protocol as in Fig. 1a.
Virtually all neoplastic cells show a moderate to strong nuclear staining reaction. A high signal-to-noise ratio is obtained. No background staining is seen and a distinct nuclear staining reaction is seen in the stromal cells.

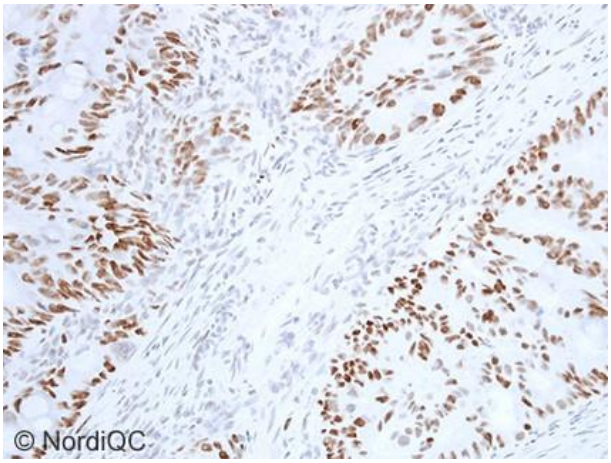


Fig. 2b
Insufficient staining reaction for MSH2 of the colon adenocarcinoma tissue core no. 3. Same protocol as Fig 1.b.
The proportion of positive cells and the intensity of the staining reaction is significantly reduced compared to the result expected and shown in Fig. 2.a. Especially note that the stromal cells are virtually negative.
Also compare with Figs. 3b and 4b, same protocol.

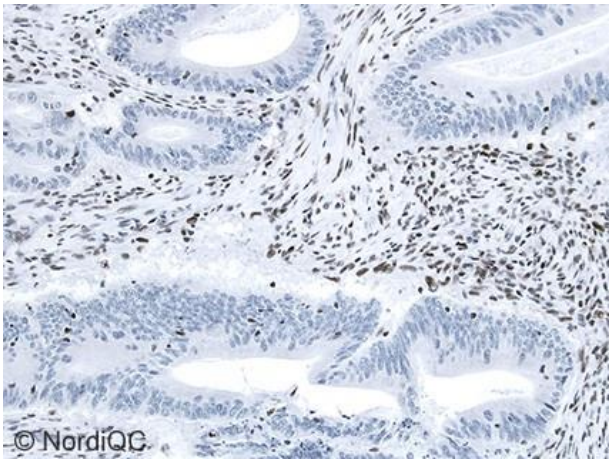


Fig. 3a
Optimal MSH2 staining of the colon adenocarcinoma no. 4 with loss of MSH2 expression using same protocol as in Figs. 1a - 2a.
The neoplastic cells are negative, while stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

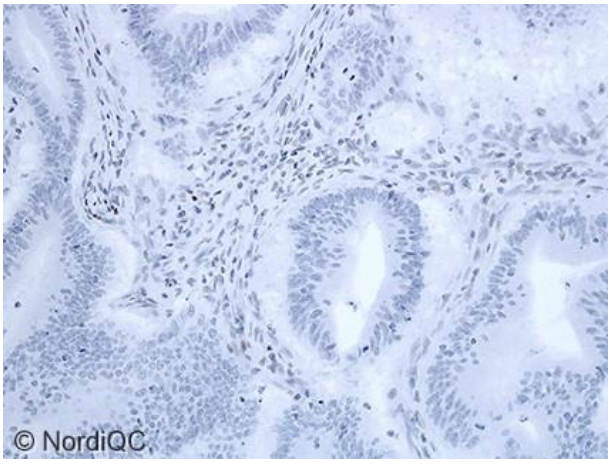


Fig. 3b
Insufficient MSH2 staining of the colon adenocarcinoma no. 4 with loss of MSH2 expression using same protocol as in Figs. 1b - 2b – same field as in Fig. 3a.
No staining reaction in the neoplastic cells is seen, but as also virtually no nuclear staining reaction is seen in the normal stromal cells, the staining pattern cannot reliably be interpreted.

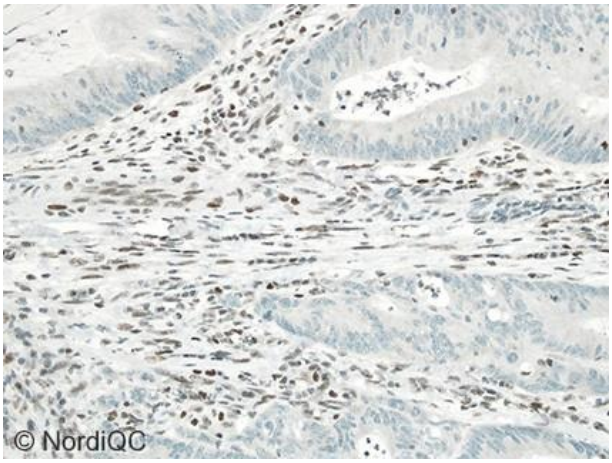


Fig. 4a
Optimal MSH2 staining of the colon adenocarcinoma no. 5 with loss of MSH2 expression using the Ventana RTU format (760-5093) of the mAb clone G219-1129 according to the recommended protocol settings for BenchMark using HIER for 40 min. in CC1, 12 min. in primary Ab and OptiView as detection system.
The neoplastic cells are negative, while stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control. Compare with Fig. 4b.

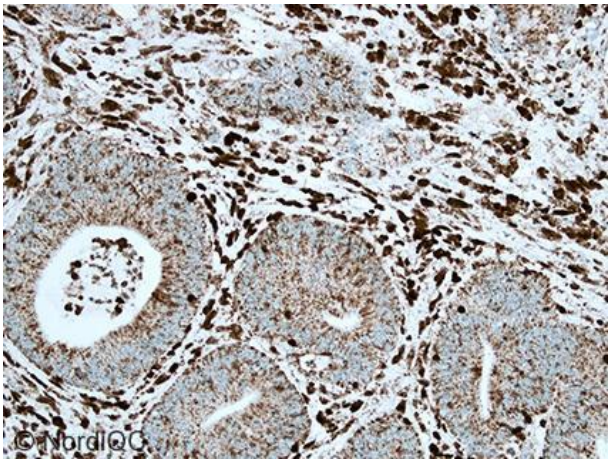


Fig. 4b
Insufficient staining reaction for MSH2 of the colon adenocarcinoma no. 5 with loss of MSH2 expression using the Ventana RTU format (760-5093) of the mAb clone G219-1129 with modified protocol settings using OptiView with amplification kit as detection system (otherwise same settings as in Fig. 4a).
The extensive cytoplasmic staining reaction in the neoplastic cells complicates the interpretation. Compare with Fig. 4a.

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