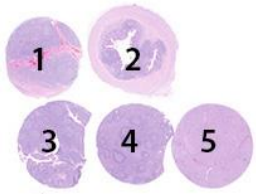
# Assessment Run 54 2018
# CD8

**Material**
The slide to be stained for CD8 comprised:




1: Tonsil (24h fixation), 2: Appendix, 3: Tonsil (72h fixation), 4: T-cell Lymphoma (CD8 negative), 5: T-cell Lymphoma (CD8 positive).

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing CD8 staining as optimal included:

- A strong, predominantly membranous staining reaction of virtually all normal suppressor/cytotoxic T-cells in the two tonsils, appendix and the T-cell Lymphoma (tissue core no. 4).
- An at least moderate, distinct membranous but also granular cytoplasmic staining reaction of virtually all the neoplastic T-cells in the T-cell Lymphoma (tissue core no. 5).
- No staining reaction in other cells including appendiceal columnar epithelial cells, B-cells and the neoplastic cells in the T-cell lymphoma (tissue core no. 4).

**Participation**

| | |
|---|---|
| Number of laboratories registered for CD8, run 54 | 282 |
| Number of laboratories returning slides | 262 (93%) |

**Results**
262 laboratories participated in this assessment. 172 (65%) 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:
- Less successful primary Ab (rmAb SP16 and rmAb SP57).
- Too low concentration of the primary Ab
- Technical issues

**Performance history**
This was the second NordiQC assessment of CD8. The pass rate decreased significantly in this assessment compared to the previous run 14, 2005 (see table 2).

Table 2**. Proportion of sufficient results for CD8 in the two NordiQC run performed**

| | **Run 14 2005** | **Run 54 2018** |
|---|---|---|
| Participants, n= | 63 | 262 |
| Sufficient results | 92% | 65% |

**Conclusion**
The mAb clones **C8/144B**, **4B11, 1A5** and the rmAb clone **BSR5** could all be used to obtain optimal staining results for CD8. Irrespective of the clone applied, efficient HIER, careful calibration of the primary antibody and use of a detection system with appropriate sensitivity were the most important prerequisites for an optimal staining result. The mAb clones C8/144B and 4B11, and corresponding Ready-To-Use (RTU) systems (IS/IR650 and GA650) or (PA0183) from Dako and Leica, respectively, are very robust assays providing high proportions of sufficient and optimal results. The majority of assays based on the rmAb clones SP16 and SP57, both as concentrated formats or RTU systems, were challenged by false positive staining reaction hindering interpretation of the specific signal for CD8.
Tonsil and appendix are recommended as positive and negative tissue controls: Virtually all suppressor/cytotoxic T-cells must show an as strong as possible membranous staining reaction (granular cytoplasmic staining reaction can also be observed). No staining reaction of B-cells, squamous epithelial cells of the tonsil or columnar epithelial cells of the appendix must be seen.

Table 1. **Antibodies and assessment marks for CD8, Run 54**

| Concentrated antibodies | N | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **4B11** | 19<br>1 | Leica/Novocastra<br>Monosan | 12 | 7 | 1 | 0 | 95% | 100% |
| mAb clone **C8/144B** | 70<br>4<br>3<br>1<br>1<br>1 | Agilent/Dako<br>Cell Marque<br>Zytomed Systems GmbH<br>Diagnostic Biosystem<br>Genemed<br>Biocare Medical | 58 | 20 | 2 | 0 | 98% | 97% |
| rmAb clone **SP16** | 4<br>3<br>1<br>1<br>1 | Cell Marque<br>Thermo Fisher Scientific<br>Spring Bioscience<br>Biocare Medical<br>DCS Diagnostics | 0 | 1 | 9 | 0 | 10% | - |
| rmAb clone **BSR5** | 1 | Nordic Biosite | 1 | 0 | 0 | 0 | - | - |
| Ready-To-Use antibodies | | | | | | | | |
| mAb clone **4B11, PA0183** | 8 | Leica/Novocastra | 7 | 1 | 0 | 0 | - | - |
| mAb clone **4B11, PA0183**[3] | 1 | Leica/Novocastra | 0 | 1 | 0 | 0 | - | - |
| mAb clone **1A5, AM422-10M**[3] | 1 | Biogenex | 1 | 0 | 0 | 0 | - | - |
| mAb clone **C8/144B, IS/IR623** | 28 | Agilent/Dako | 27 | 1 | 0 | 0 | 100% | 100% |
| mAb clone **C8/144B, IS/IR623**[3] | 10 | Agilent/Dako | 9 | 1 | 0 | 0 | - | - |
| mAb clone **C8/144B, GA623** | 22 | Agilent/Dako | 21 | 0 | 1 | 0 | 95% | 100% |
| mAb clone **C8/144B, GA623**[3] | 2 | Agilent/Dako | 1 | 0 | 0 | 1 | - | - |
| mAb clone **C8/144B, MON-RTU1030** | 1 | Monosan | 0 | 1 | 0 | 0 | - | - |
| mAb clone **C8/144B, MS-457-R7** | 1 | Thermo Fisher Sceintific | 0 | 1 | 0 | 0 | - | - |
| rmAb clone **SP16, MAD-000318QD-7/N** | 2 | Master Diagnostica | 0 | 1 | 1 | 0 | - | - |
| rmAb clone **SP16, PRM311** | 1 | Biocare Medical | 0 | 0 | 1 | 0 | - | - |
| rmAb clone **SP16, 108-18R** | 1 | Cell Marque | 0 | 0 | 1 | 0 | - | - |
| rmAb clone **SP57, 790-4460** | 73 | Ventana/Roche | 0 | 0 | 11 | 62 | 0% | - |
| Total | 262 | | 137 | 35 | 27 | 63 | - | |
| Proportion | | | 52% | 13% | 10% | 24% | 65% | |

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) Ready-to-use product developed for a specific semi/fully automated platform by a given manufacturer but inappropriately applied by laboratories on other non-validated semi/fully automatic systems or used manually.

## Detailed analysis of CD8, Run 54

The following protocol parameters were central to obtain optimal staining:

### Concentrated antibodies

mAb clone **C8/144B**: Protocols with optimal results were typically based on heat induced epitope retrieval (HIER) using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (12/15)*, TRS pH 9 (Dako) (1/1), Cell Conditioning 1 (CC1, Ventana) (32/46), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (7/9), TRIS-EDTA/EGTA pH 9 (1/2), DBS Montage EDTA Antigen Retrieval Solution (1/1), Bond Epitope Retrieval Solution 1 (BERS1, Leica) (2/2) or citrate buffer pH6 (2/2) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:200. Using these protocol settings, 71 of 73 (97%) laboratories produced a sufficient staining result (optimal or good).
*(number of optimal results/number of laboratories using this HIER buffer)

mAb clone **4B11**: Protocols with optimal results were based on HIER using CC1 (Ventana) (3/6), TRS pH 9 (3-in-1) (Dako) (2/3), BERS2 (Leica) (6/9) or BERS1 (Leica) 1/1 as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:100. Using these protocol settings, 15 of 15 (100%) laboratories produced a sufficient staining result.

rmAb clone **BSR5**: One protocol with an optimal result was based on HIER using TRIS-EDTA/EGTA pH 9 as retrieval buffer. The mAb was diluted 1:100 and anti-Rabbit HRP polymer (Nordic Biosite) was applied as the detection system.

Table 3. **Proportion of optimal results for CD8 for the two most commonly used antibodies as concentrate on the four main IHC systems***

| Concentrated antibodies | **Dako/Agilent Autostainer** | | **Dako/Agilent Omnis** | | **Ventana/Roche BenchMark 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 | BERS2 pH 9.0 | BERS1 pH 6.0 |
| mAb clone **C8/144B** | 7/10** (70%) | - | 5/5 (100%) | - | 29/41 (59%) | 0/1 | 7/9 (78%) | 2/2 |
| mAb clone **4B11** | 1/2 | - | 1/1 | - | 3/5 (60%) | - | 4/6 (67%) | 1/1 |

* 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 **C8/144B**, product no. **IS/IR623**, Dako, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 10-20 min. at 95-98°C), 20-30 min. incubation of the primary Ab and EnVision FLEX (K8000/K8002) as detection system. Using these protocol settings, 25 of 25 (100%) laboratories produced a sufficient staining result.

mAb clone **C8/144B**, product no. **GA623**, Dako, OMNIS:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 30 min. at 97°C), 10-20 min. incubation of the primary Ab and EnVision FLEX+ (GV800/GV823+GV821) as detection system. Using these protocol settings, 16 of 16 (100%) laboratories produced an optimal staining.

mAb clone **4B11**, product no. **PA0183**, Leica, Bond-max/Bond-III:
Protocols with optimal results were based on HIER using BERS2 pH 9 (efficient heating time 20 min. at 95-100°C), 15-20 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system.

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥10 asessed protocols). The performance was evaluated both as "true" plug-and-play systems performed strictly accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 4. **Proportion of sufficient and optimal results for CD8 for the most commonly used RTU IHC systems**

| RTU systems | **Recommended protocol settings*** | | **Laboratory modified protocol settings**** | |
|---|---|---|---|---|
| | Sufficient | Optimal | Sufficient | Optimal |
| Dako AS mAb C8/144B **IS/IR623** | 100% (13/13) | 92% (12/13) | 100% (12/12) | 100% (12/12) |
| Dako Omnis mAb C8/144B **GA623** | 100% (14/14) | 100% (14/14) | 88% (7/8) | 88% (7/8) |
| VMS Ultra/XT/GX rmAb SP57 **790-4460** | 0/5 | 0/5 | 0% (0/59) | 0% (0/59) |

* 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 were included.

**Comments**
In this second NordiQC assessment for CD8, the prevalent feature of an insufficient staining result was characterized by poor-signal-to noise ratio or false positive staining result. This pattern was observed in 93% of the insufficient results (84 of 90) and primarily related to the use of the rmAb clones SP16 and SP57. Virtually all laboratories applying one of these two clones, both within laboratory developed (LD) or Ready-To-Use (RTU) assays, demonstrated an aberrant membranous staining reaction of epithelial cells of the appendix (see fig. 4a and 4b). In the remaining 7% of the insufficient results, a too weak staining reaction for CD8 of low-level antigen expressing cells as the neoplastic T-cells of the T-lymphoma, tissue core no. 5, were seen or laboratories were challenged by technical issues. Virtually all laboratories were able to detect normal suppressor/cytotoxic T-cells (all cores) irrespectively of assay applied.

The mAb clone C8/144B was the most widely used antibody for demonstration of CD8 and provided optimal results on all four main IHC platforms, Omnis (Dako), Autostainer (Dako), Bond (Leica) and BenchMark (Ventana) respectively (see Table 3). Used within an LD assay, mAb clone C8/144B gave an overall pass rate of 98% (78 of 80) of which 73% were optimal (see Table 1). The antibody is robust and a sufficient result could be obtained applying several protocol parameters such as efficient HIER in either citric based buffers pH 6 or alkaline buffers pH 9 and use of both high and low sensitive polymer/multimer based detection systems as long as the titer of the primary Ab was carefully calibrated. However, it was observed that use of 3-step polymer/multimer based detection systems provided significantly higher proportions of optimal results, 39 of 49 (80%), compared to 2-step polymer/multimer based systems, 19 of 31 (61%).

The mAb clone 4B11 used within LD assays also provided a high pass rate of 95% (18 of 19) of which 63% (12 of 19) were assessed as optimal. The Ab provided optimal results on all main platforms (see Table 3). As for the mAb clone C8/144B mentioned above, no special requirements concerning HIER buffer and detection system were needed for optimal results, provided that careful calibration of the primary Ab titer was performed. The single protocol assessed as insufficient (see Table 1) used a too low concentration (1:400) of the primary Ab compared to optimal protocol settings as described above.

The corresponding RTU systems for the mAb clones C8/144B and 4B11 from Dako and Leica, respectively, also provided high proportions of sufficient and optimal results. For the Dako RTU systems (IS/IR623 and GA623), optimal results could be obtained both by the official recommendations and by modified protocol settings. Typically adjusting HIER time, incubation time of the primary Ab and/or detection system. Grouped together, 96% (48 of 50) of the protocols were assessed as optimal.

48% (73 of 151) of the laboratories used the RTU system based on the rmAb clone SP57 (Ventana) and all were assessed as insufficient. Although the assay provided strong staining of suppressor/cytotoxic T-cells and of the neoplastic T-cells in the T-lymphoma (tissue core no. 5) using either the official recommendation or laboratory modified protocol settings, the main problem was a false positive membranous staining reaction of epithelial cells of the appendix. According to the data sheet, it is recommended to use Antibody Diluent (REF251-018) at the ultraBlock step on the Benchmark XT/Ultra platforms. Four laboratories explicitly stated that they used this recommended procedure, but the blocking step had no or very little effect on the false positive staining reaction of appendiceal columnar cells. Therefore, and to avoid risk of misinterpretation of anaplastic tumors, laboratories should consider using a primary Ab not displaying this aberrant staining pattern (e.g., mAb clone C8/144B or 4B11).

This was the second assessment of CD8 in NordiQC (see Table 2). A significant decrease in pass rate was obtained compared to the latest run 14, 2005. The extended use of rmAb clones SP16 and SP57, both as concentrated format and RTU system accounted for the overall decrease in sufficient results. The overall pass rate for laboratories not using these rmAb clones was 97% (170 of 175) of which 78% (137 of 175) were assessed as optimal.

**Controls**
Tonsil and appendix are recommended as positive and negative tissue controls for CD8. In tonsil, the protocol must be calibrated to provide a distinct and strong membranous staining reaction of virtually all suppressor/cytotoxic T-cells. In appendix, dispersed suppressor/cytotoxic T-cells primarily located in lamina propria mucosa but also situated in the appendiceal epithelium must be strongly stained, whereas the epithelial cells should be negative.
No staining reaction must be seen in other cells including B-cells, stromal cells or squamous epithelial cells of the tonsil.

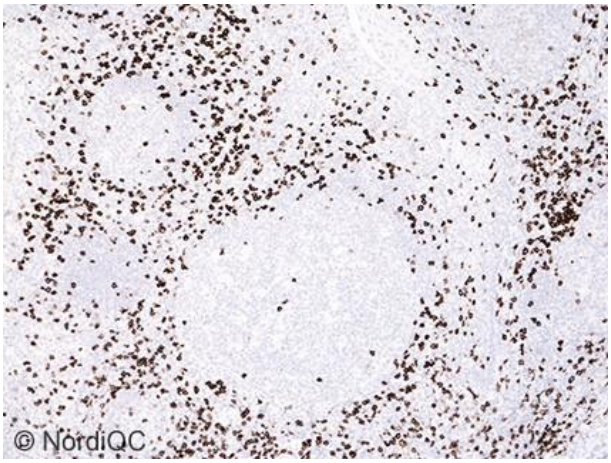



Fig. 1a (x100)
Optimal CD8 staining reaction of the tonsil (24h fixation) using the mAb clone 4B11 optimally calibrated, HIER in CC1 (64 min.) and UltraView with amplification as the detection system on the Benchmark Ultra, Ventana.
The vast majority of suppressor/cytotoxic T-cells show a strong and distinct membranous staining reaction. Same protocol used in Figs. 2a - 3a.

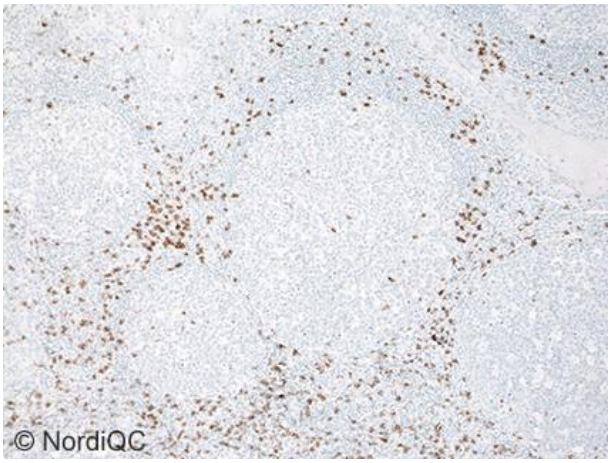



Fig. 1b (x100)
Insufficient CD8 staining of the tonsil (24h fixation) using the mAb clone 4B11, too diluted, but with exactly the same protocol settings as in Fig. 1a.
The suppressor/cytotoxic T-cells only display weak to moderate staining intensity. Same protocol used in Figs. 2b – 3b.

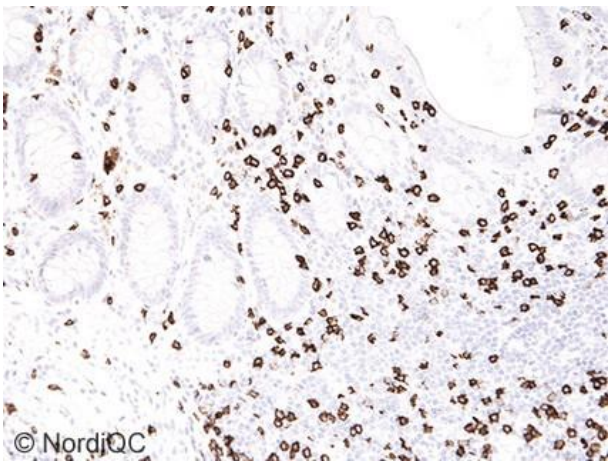



Fig. 2a (x200)
Optimal CD8 staining of the appendix using same protocol as in Fig. 1a. The suppressor/cytotoxic T-cells show a strong and distinct membranous staining reaction, whereas epithelial cells of the appendix display the expected negative staining pattern.

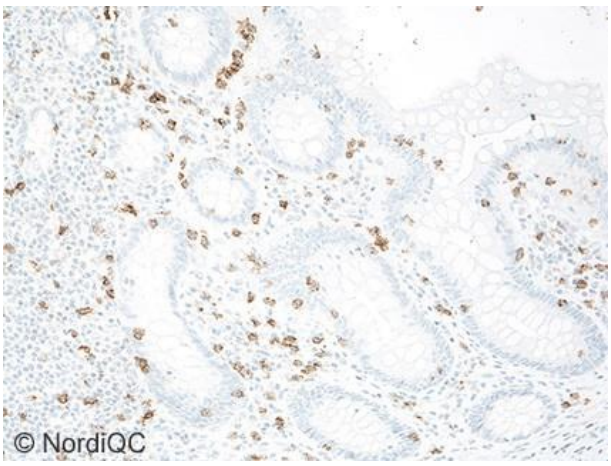



Fig. 2b (x200)
Insufficient CD8 staining of the appendix using same protocol as in Fig. 1b – same field as in Fig. 2a.
The suppressor/cytotoxic T-cells only show weak and granular to complete membranous staining reaction.

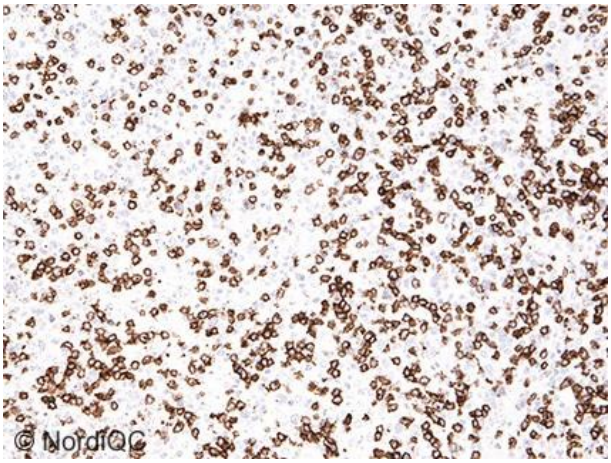



Fig. 3a (x200)
Optimal CD8 staining of the T-cell lymphoma (tissue core no. 5) using same protocol as in Figs. 1a and 2a. The vast majority of the neoplastic T-cells show a moderate to strong but distinct membranous staining reaction.

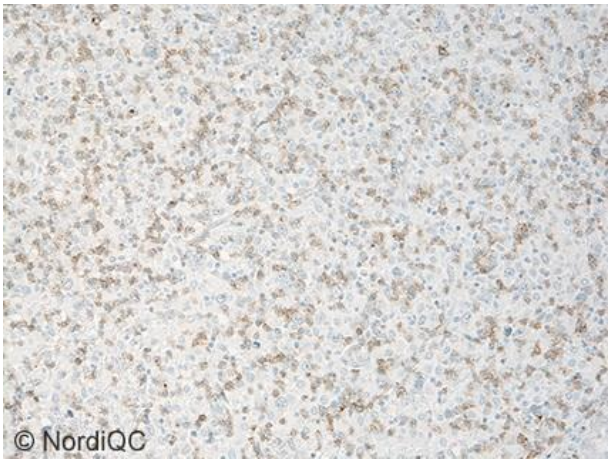



Fig. 3b (x200)
Insufficient CD8 staining of the T-cell lymphoma (tissue core no. 5) using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a. The neoplastic T-cells only display faint to weak staining intensity and the proportion of positive cells is significantly reduced.

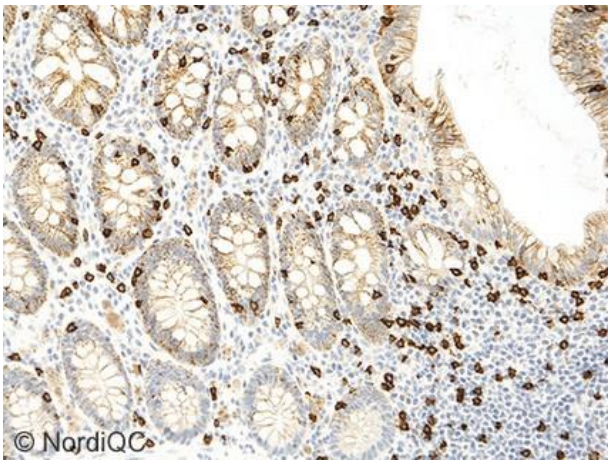



Fig. 4a (x200)
Insufficient CD8 staining of the appendix using a protocol based on the rmAb SP16. The suppressor/cytotoxic T-cells show the expected staining reaction but the epithelial cells of the mucosa also display an aberrant membranous staining reaction (false positive) – compare with optimal staining in Fig. 2a.

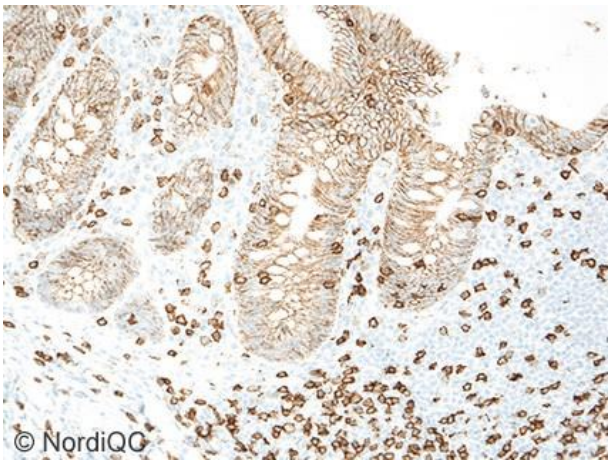



Fig. 4b
Insufficient CD8 staining of the appendix using the RTU system 790-4460 based on the rmAb SP57 (Ventana), applying an ultraBlock step and following the protocol setup strictly as recommended given by the vendor. The suppressor/cytotoxic T-cells show the expected staining reaction but epithelium of the appendiceal mucosa is false positive. No significant difference in the staining pattern was seen between applying an ultraBlock step or not – compare with optimal staining in Fig. 2a.

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