Problems

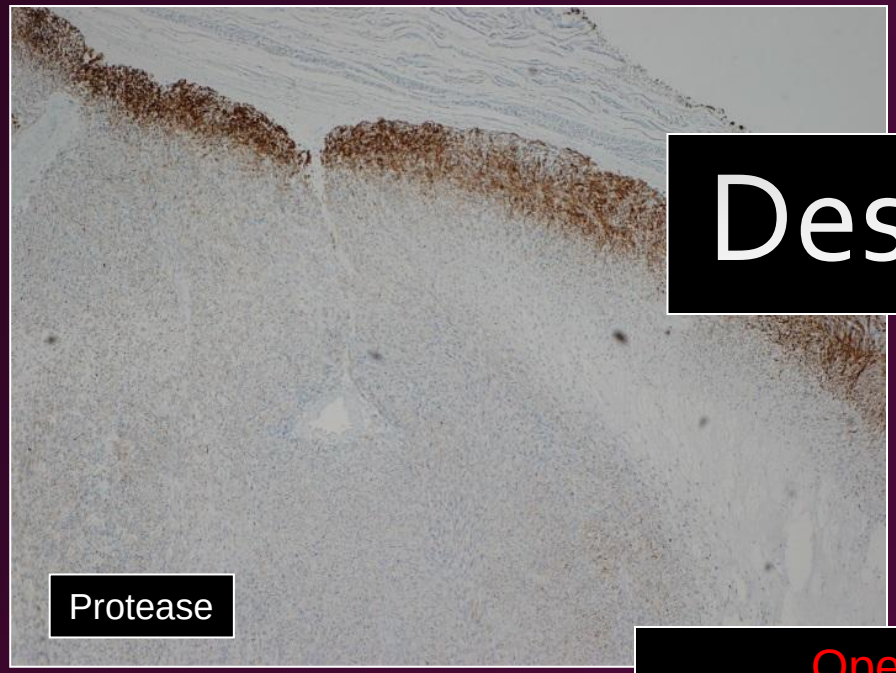
- Operating Room
- Accessioning

→ Delayed delivery to laboratory

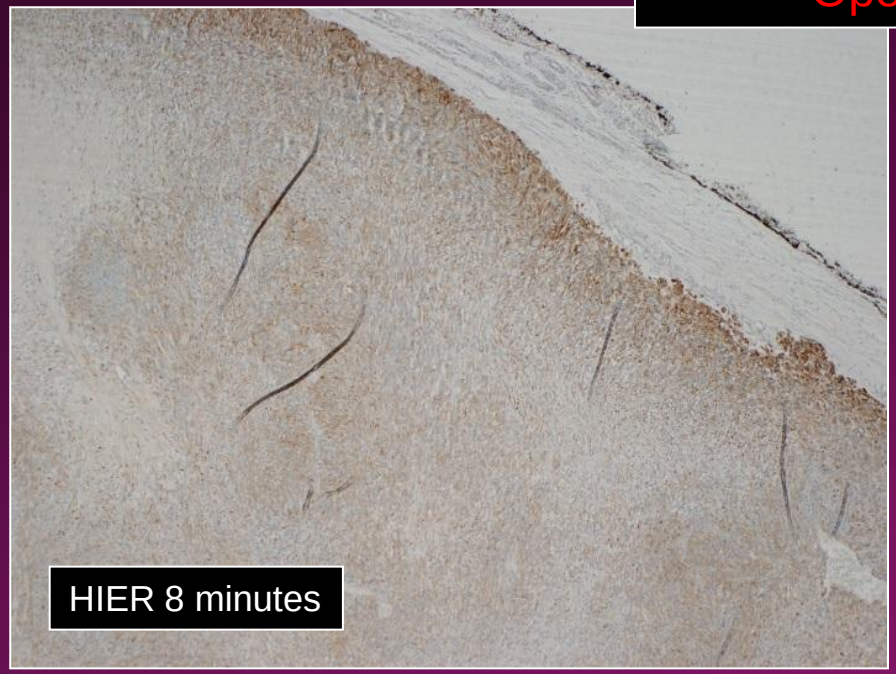
- Gross Room

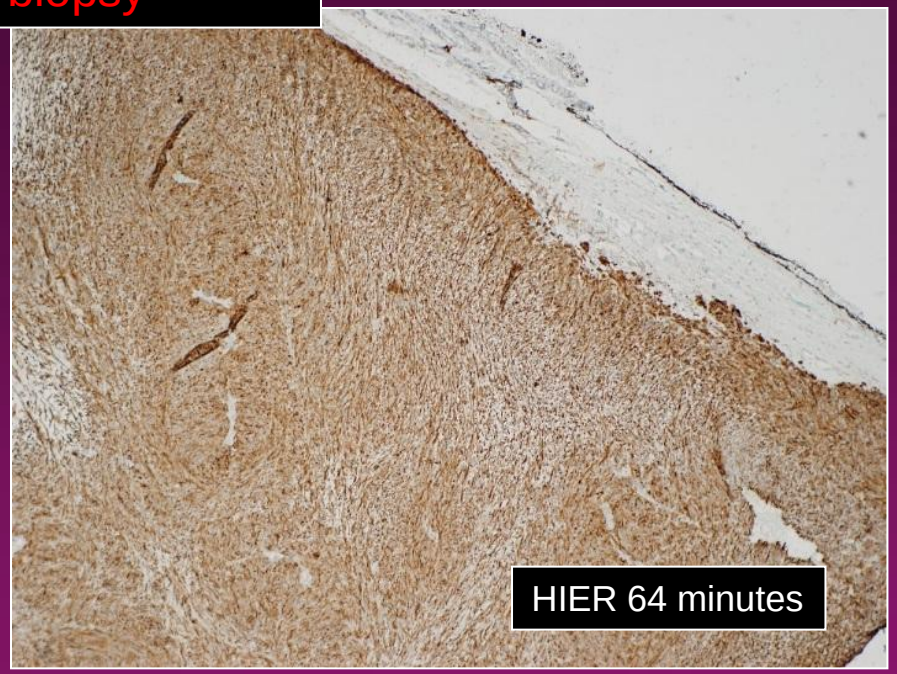
→ Stat processing
Delayed opening
Thick slices
Insufficient formalin
Small container

Desmin



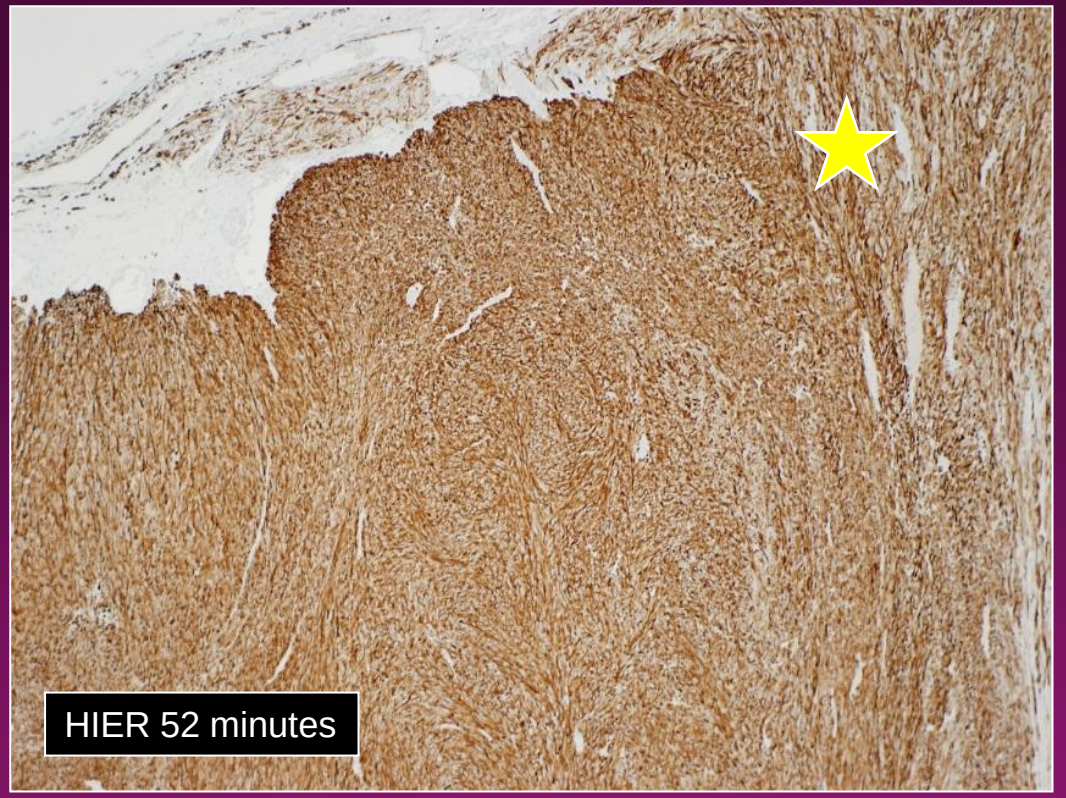
Open biopsy





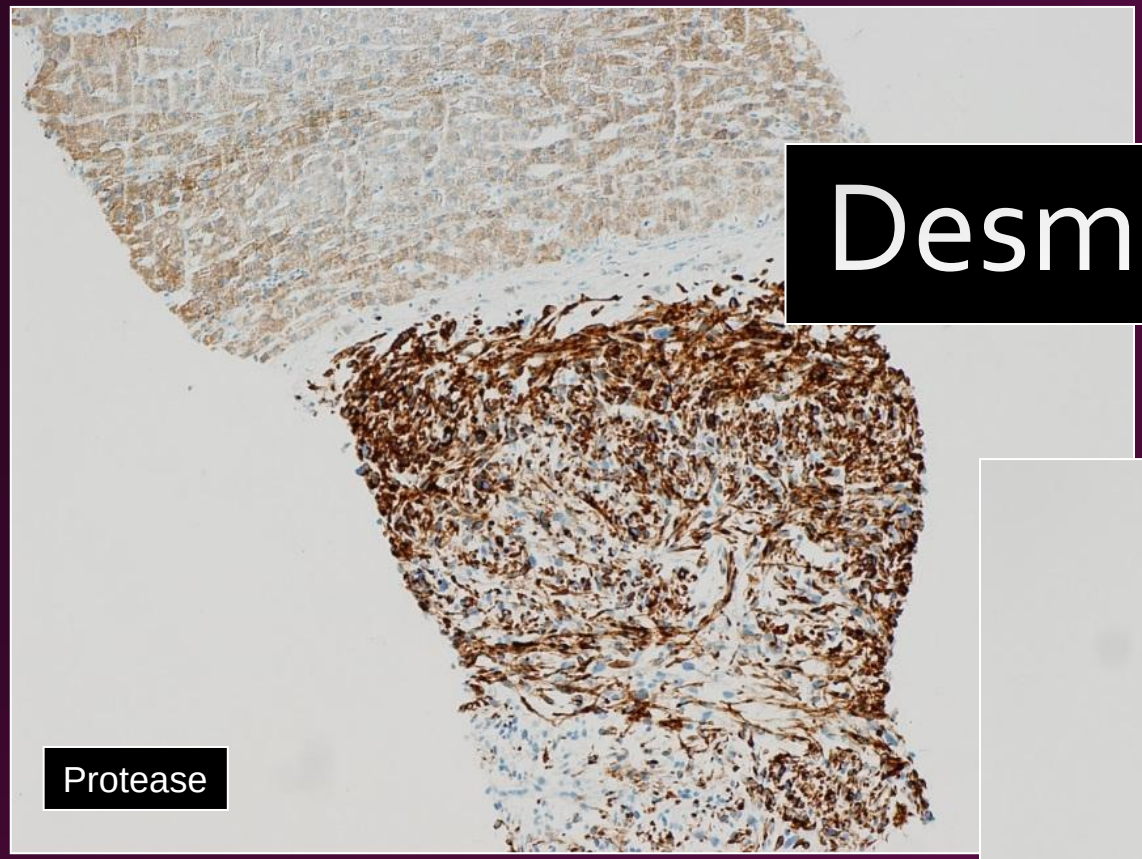
Desmin

Resection specimen

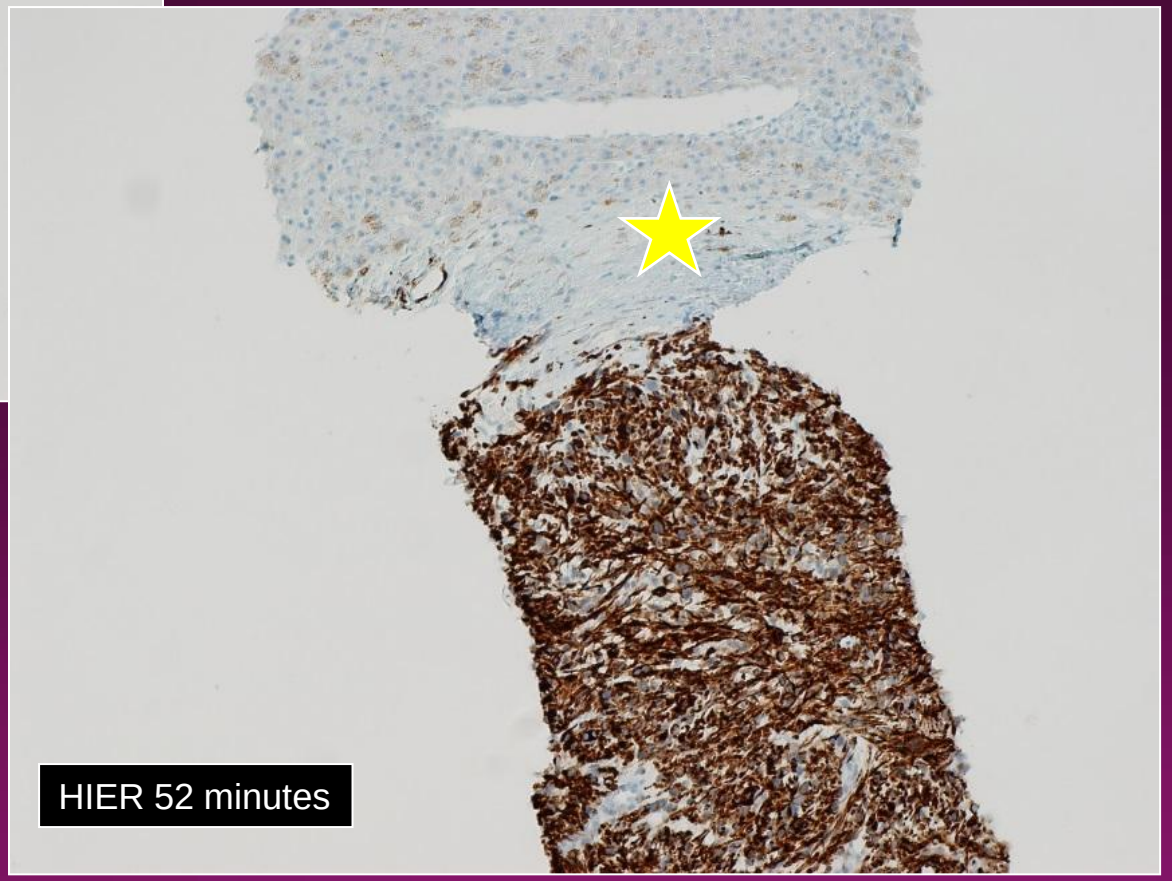


Desmin

Improved antigen retrieval and reduced background staining in hepatocytes!



Liver biopsy



Standard Terminology

- Incubation
  - The time during which the primary antibody is applied to the tissue after antigen retrieval.
  - The time required for incubation depends on the affinity of the antibody for the tissue.
  - The longer the incubation, the lower the required concentration.

- Amplification
  - The addition of a linking antibody that will provide additional binding sites for the detection system in order to amplify the signal and increase sensitivity of the test.

- Detection
  - The detection system uses a secondary antibody to link an enzyme (horse radish peroxidase – HRP) to the primary antibody. The more enzyme sites available, the greater the sensitivity.
    - Avidin-Biotin Systems
    - Polymer Systems
  - The chromogen (3,3'-diaminobenzidinetetrahydrochloride - DAB) oxidizes in the presence of HRP and a hydrogen peroxide solution forming an insoluble precipitate that we visualize as the "brown stain"

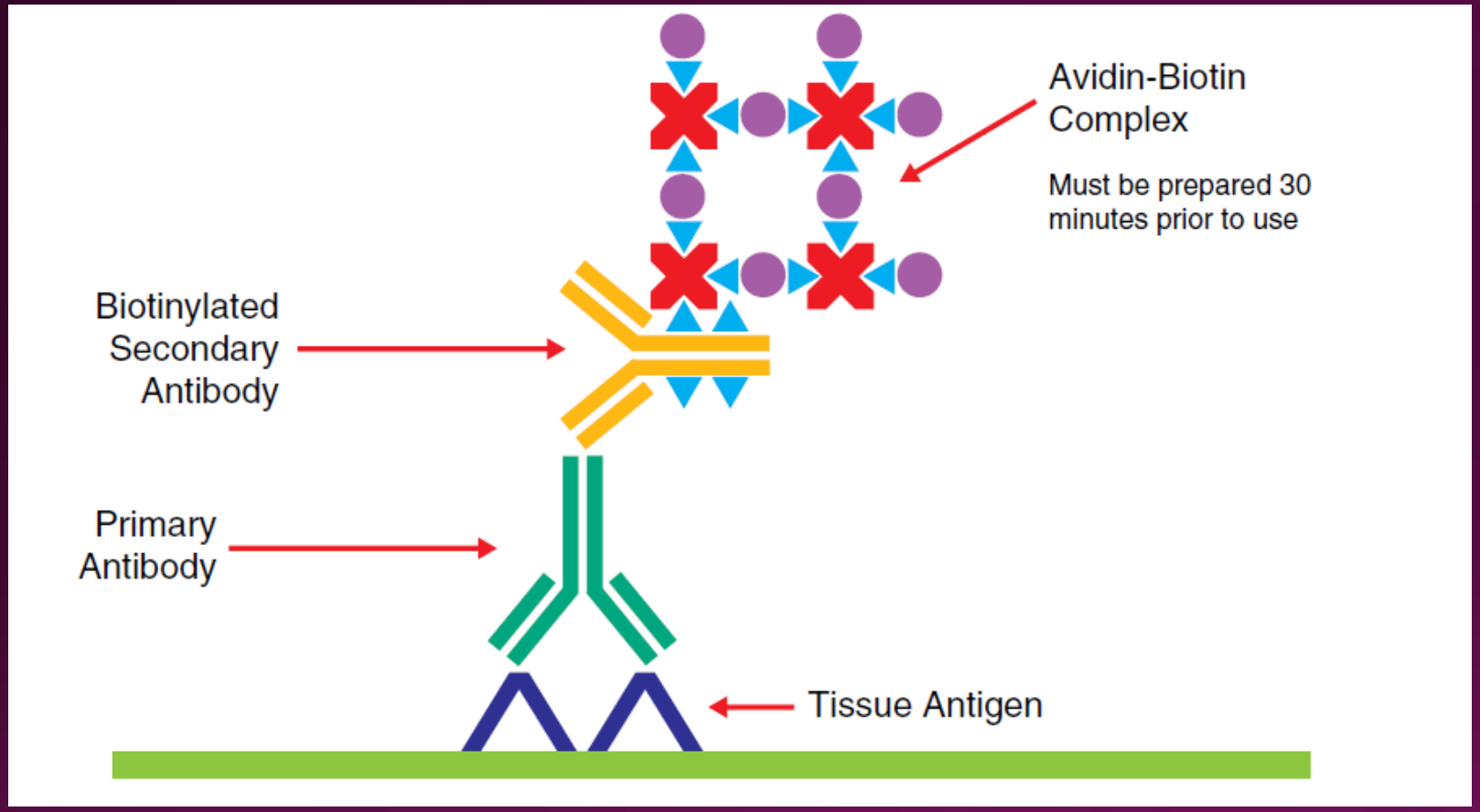
primary antibody

secondary antibody

HRP enzyme

biotin label

streptavidin



Avidin-Biotin Complex (ABC) Detection Method

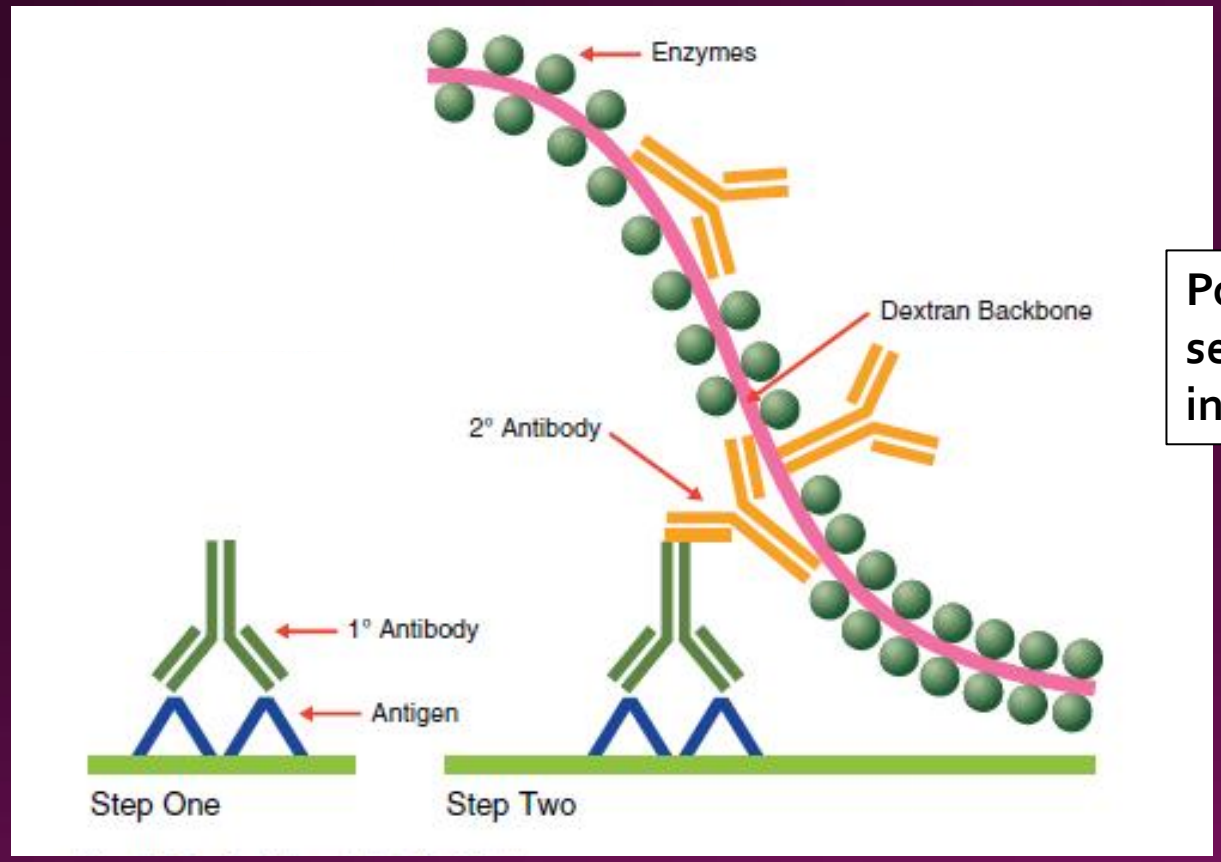
- **Primary antibody**
- **Secondary antibody with attached biotin**
- **Avidin-biotin complex with attached HRP**

primary antibody

secondary antibody

HRP enzyme

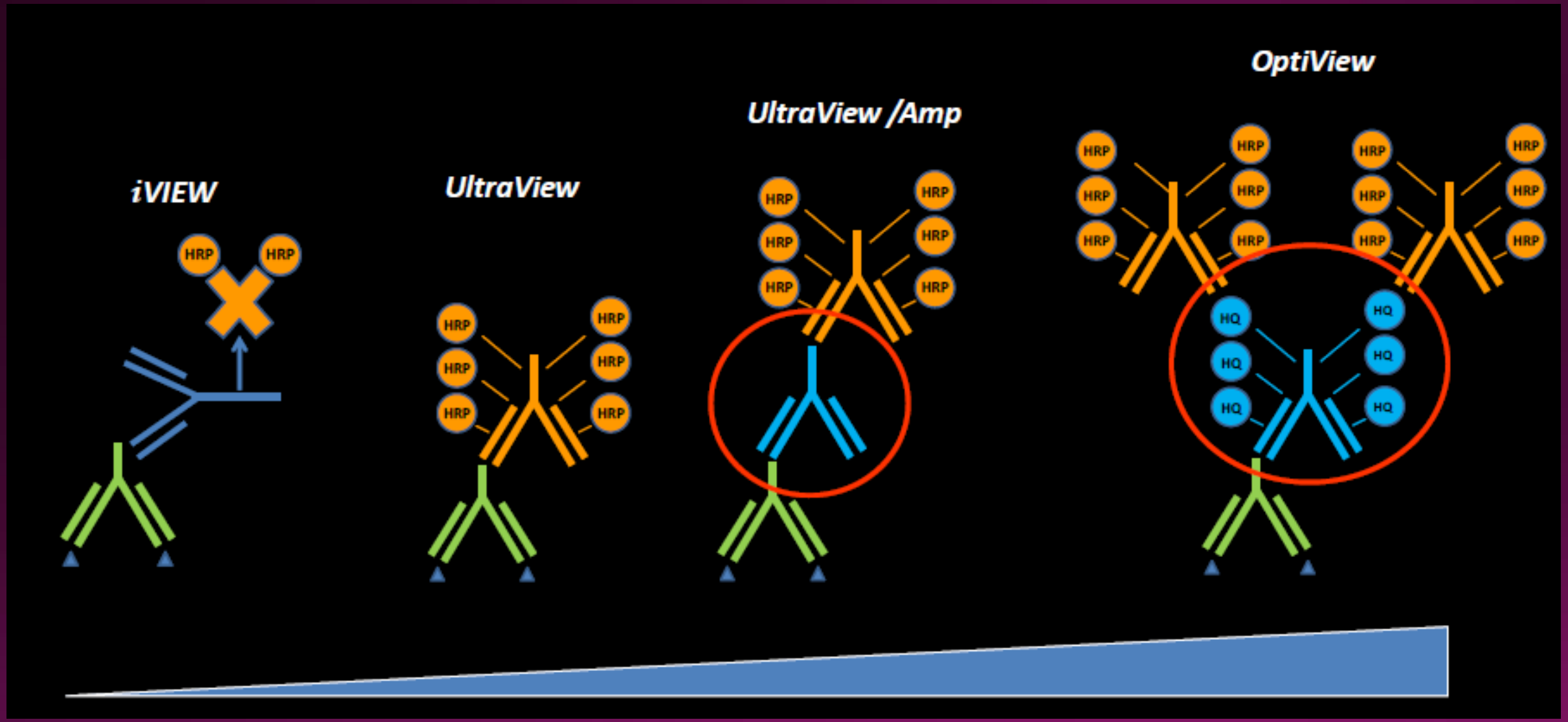
polymer



Polymer system increases sensitivity by markedly increasing the ratio of HRP

Two Step Polymer Detection System

Ventana / Roche Detection Systems



Increasing Sensitivity

Introducing New Antibodies

- Go to NordiQC website and check recommended clones for your platform
- Chose antibodies approved from in vitro diagnostic (IVD) use and avoid research antibodies which can vary significantly from lot to lot
- Start with a sensitive detection kit with long periods of HIER and test a range of dilutions then decrease HIER if necessary
- Use normal tissues with known high and low tissue expression to establish protocol

Standard Terminology

In order to hit the mark, you have to know which target to shoot!

- Calibration
  - Setting up the test achieve an appropriate level of sensitivity
  - Depends on selection of appropriate positive and negative control tissues to establish a target sensitivity

- Optimization
  - Altering parameters within the IHC test to establish a protocol for the primary antibody that reproducibly detects the target epitope at a desired sensitivity.
  - To optimize a protocol you have to know the target sensitivity
  - You optimize the protocol to achieve an appropriately calibrated test

Standard Terminology

- Validation
  - Establishing that the immunohistochemical test detects target antigen that it is designed to detect and achieves the expected test result
  - Achieved by performing the test on selected known positive and negative tissues

- Class I antibodies – minimum 20 cases (in-house)
  - 10 appropriate positives (minimum)
  - 10 appropriate negatives (minimum)
- Class II antibodies – minimum 40 cases (reference tested)
  - 20 appropriate positive (minimum)
  - 20 appropriate negative (minimum)
- ER, PR, HER2 antibodies – 50 to 100 cases (reference tested)
  - 25 to 50 positive
  - 25 to 50 negative

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Post-Analytical Phase

Technical Aspects of the Immunohistochemical Test

Standard Terminology

- Post-analytical phase interpretation errors can be avoided by ongoing verification of test performance

- Verification
  - An evaluation that establishes whether the immunohistochemical test is performing as expected

  - Performance of the immunohistochemical test can be verified for every case by evaluating the internal and external controls

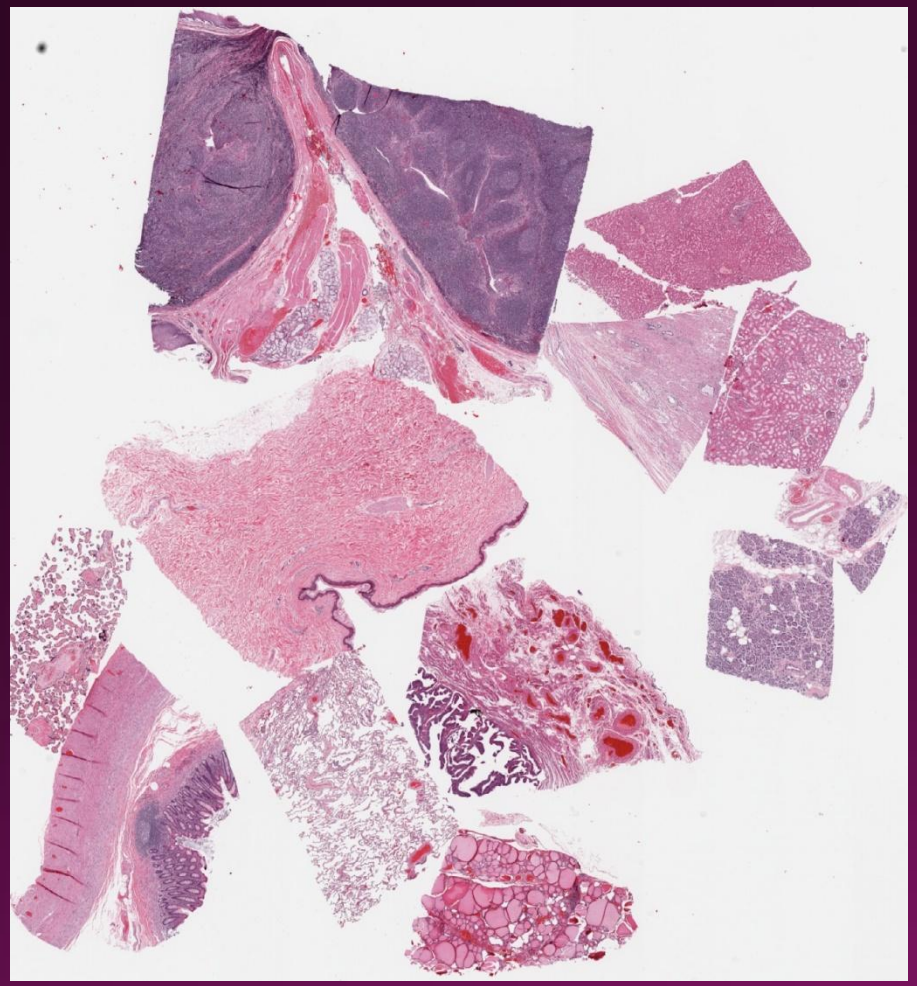
  - Is the test performing with expected sensitivity?
    - Assess by evaluating the appropriate positive tissue control.

  - Is the test performing with expected specificity?
    - Assess by evaluating the appropriate negative tissue control.

Control Tissue

- Control tissue must be fixed and processed in the same fashion as patient material
- Tumor control tissue is not recommended for calibration or verification of immunohistochemical protocols since tumor antigen expression can be variable and unpredictable
- Control tissue should incorporate a high expressing (specificity), low expressing (sensitivity), and non-expressing tissues (specificity)
- Low expressing tissues serve as quality indicators to ensure appropriate test
- A database of recommended normal control tissue is established on the NordiQC website

Normal Control Tissue TMA



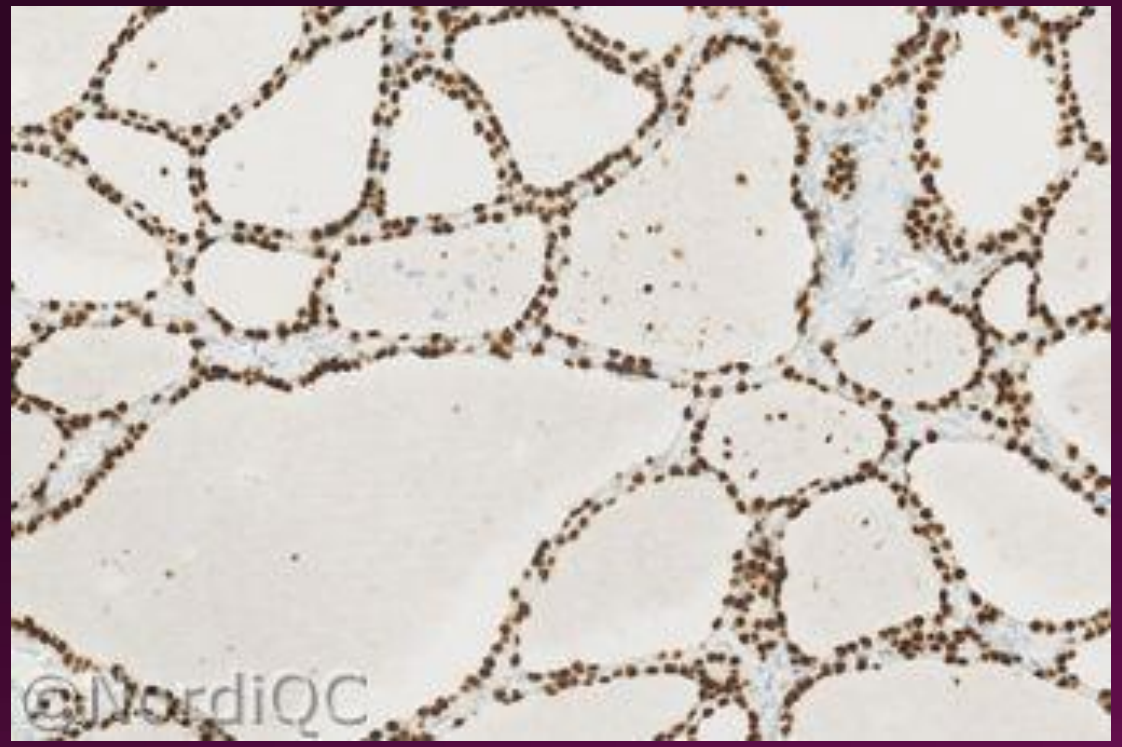
- Normal tissue microarrays can be constructed and should include tissues with high, low, and no expression of the target antigen
- The normal tissue TMA incorporates low level expressing tissues that serve as quality indicators for protocol sensitivity
- Absence of staining in quality indicators identifies protocols that lack sensitivity and require optimization

Normal Control Tissue TMA

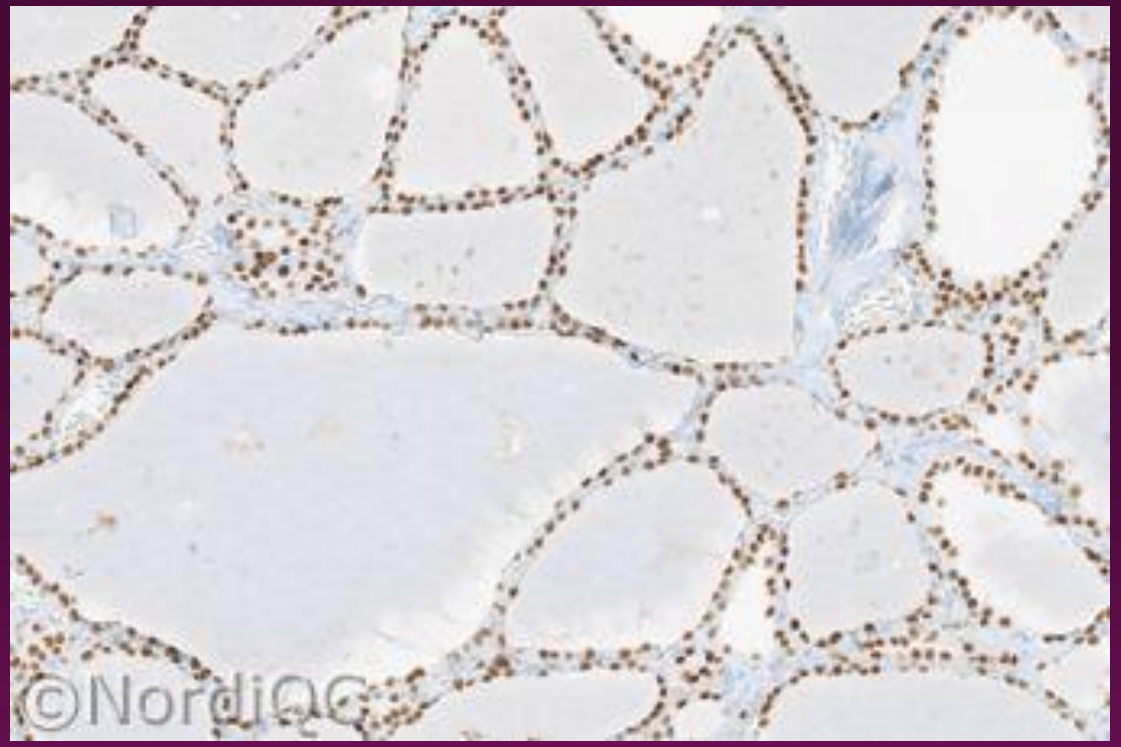
- Tissues selected for normal control tissue TMA are recommended normal tissue controls for majority of the antibodies utilized in surgical pathology and a minority of antibodies utilized in hematopathology
- Majority of antibodies utilized by hematopathology can be evaluated using only tonsil thus reducing the demand for the normal control tissue TMA
- The normal tissues are used to calibrate protocols (analytic phase) and verify test performance (post-analytic phase)

- Tonsil
- Skin
- Lung
- Appendix
- Pancreas
- Placenta
- Prostate
- Thyroid
- Fallopian tube
- Kidney
- Liver

TTF-1

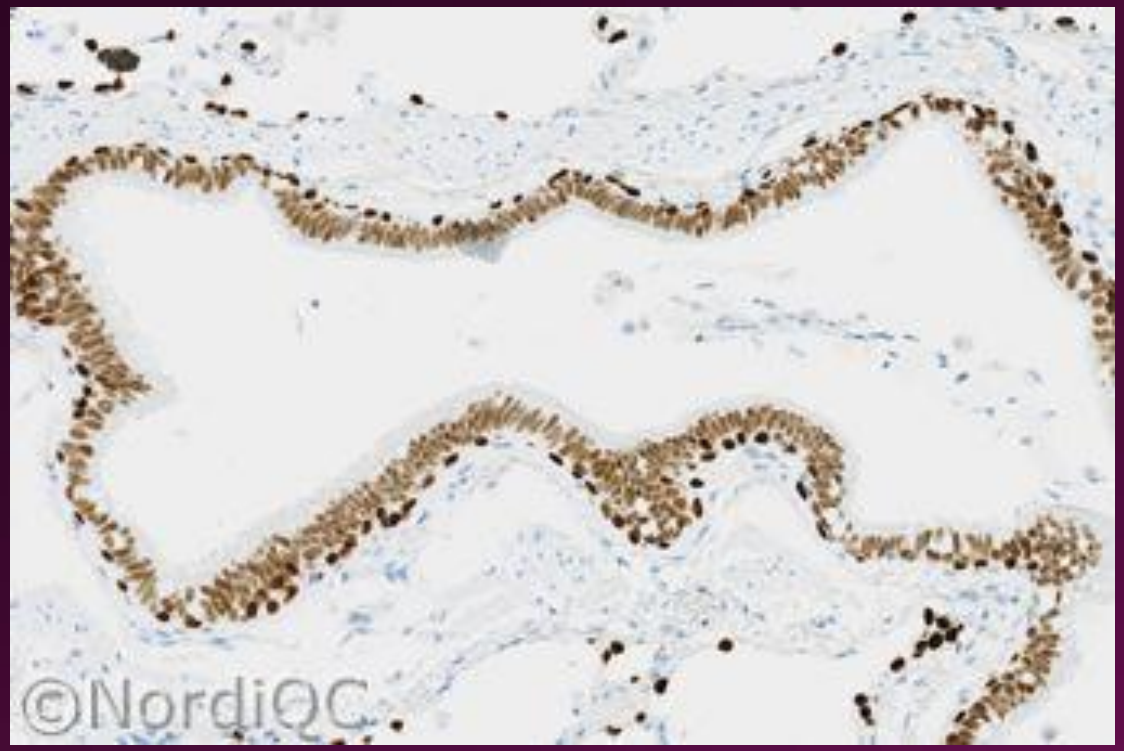


Optimal Protocol
Thyroid: Strong nuclear staining reaction is seen in virtually all follicular epithelial cells. No background staining is seen.

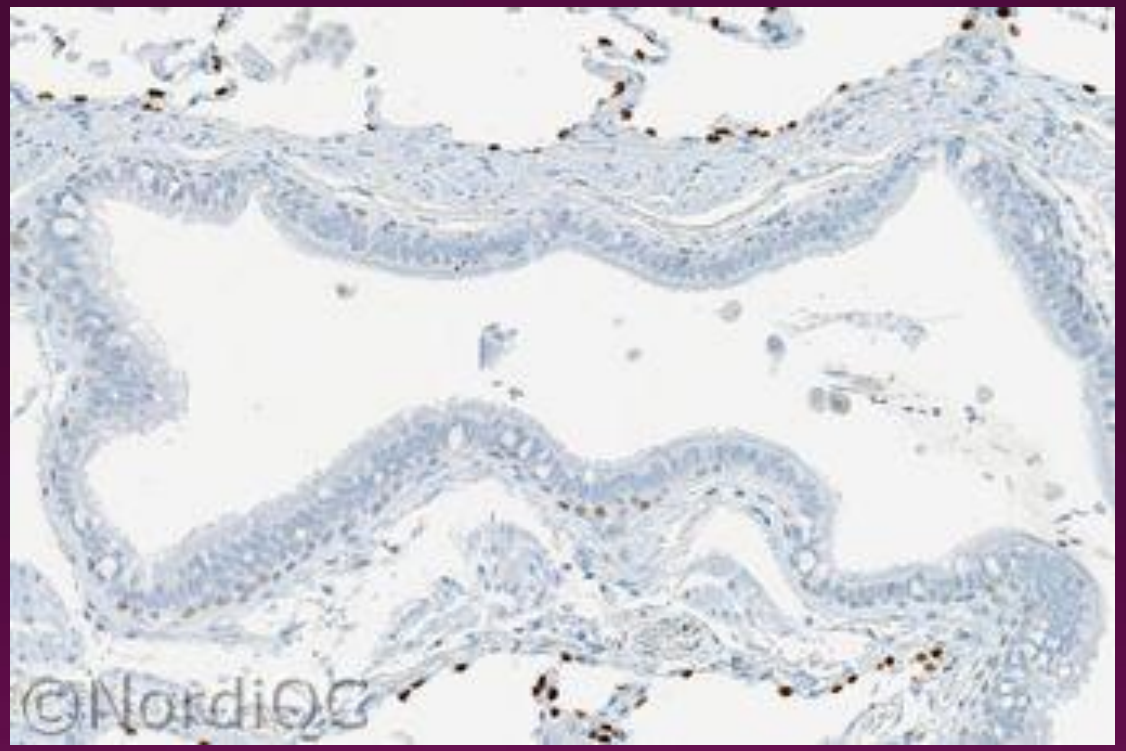


Insufficient Protocol
Thyroid: Moderate nuclear staining reaction is seen in the majority of follicular epithelial cells

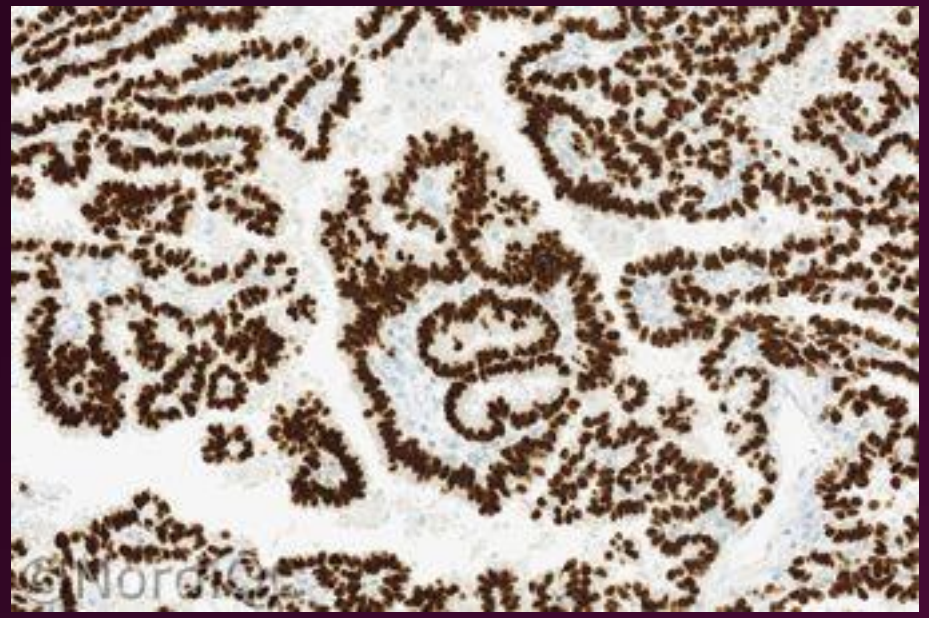
TTF-1



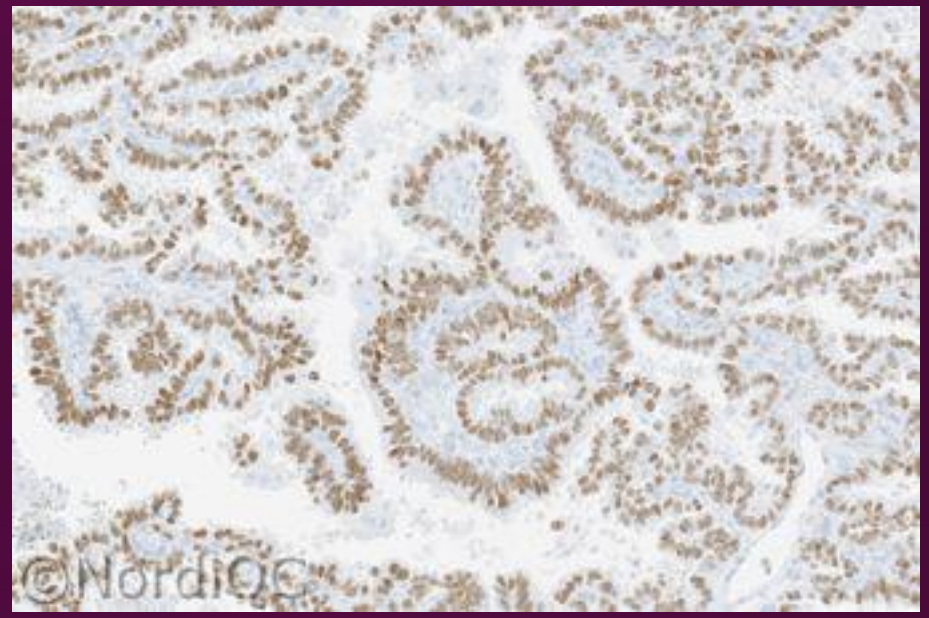
Optimal Protocol
Lung: Type II pneumocytes and basal epithelial cells lining the terminal bronchioles show a strong distinct nuclear staining reaction and columnar epithelial cells show a moderate nuclear staining reaction. No background staining is seen.

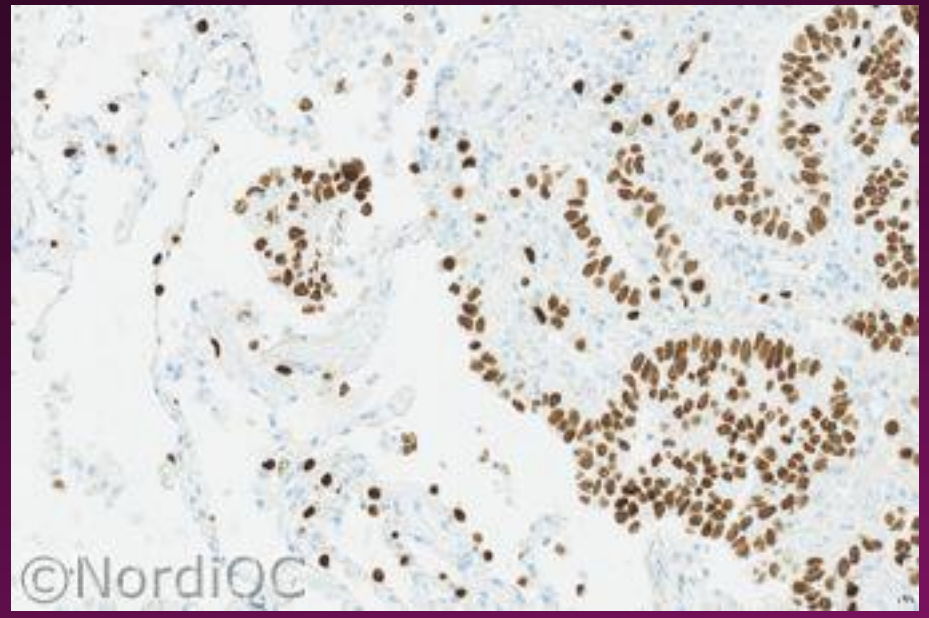


Insufficient Protocol
Lung: Type II pneumocytes and basal epithelial cells lining the terminal bronchioles show only a weak to moderate positive nuclear staining reaction and no reaction is seen in the columnar epithelial cells.

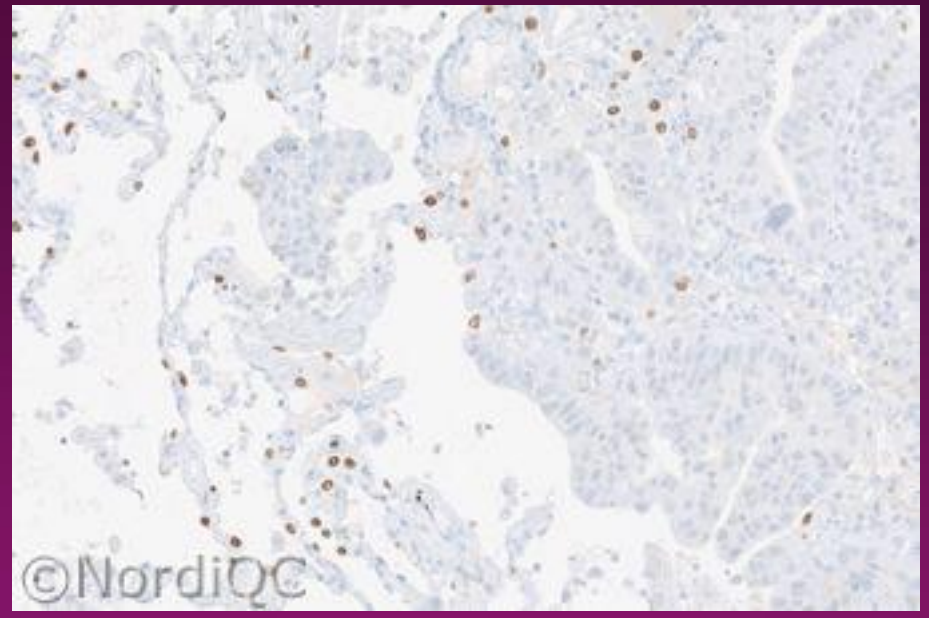


Lung Adenocarcinoma
L: Optimal
R: Insufficient

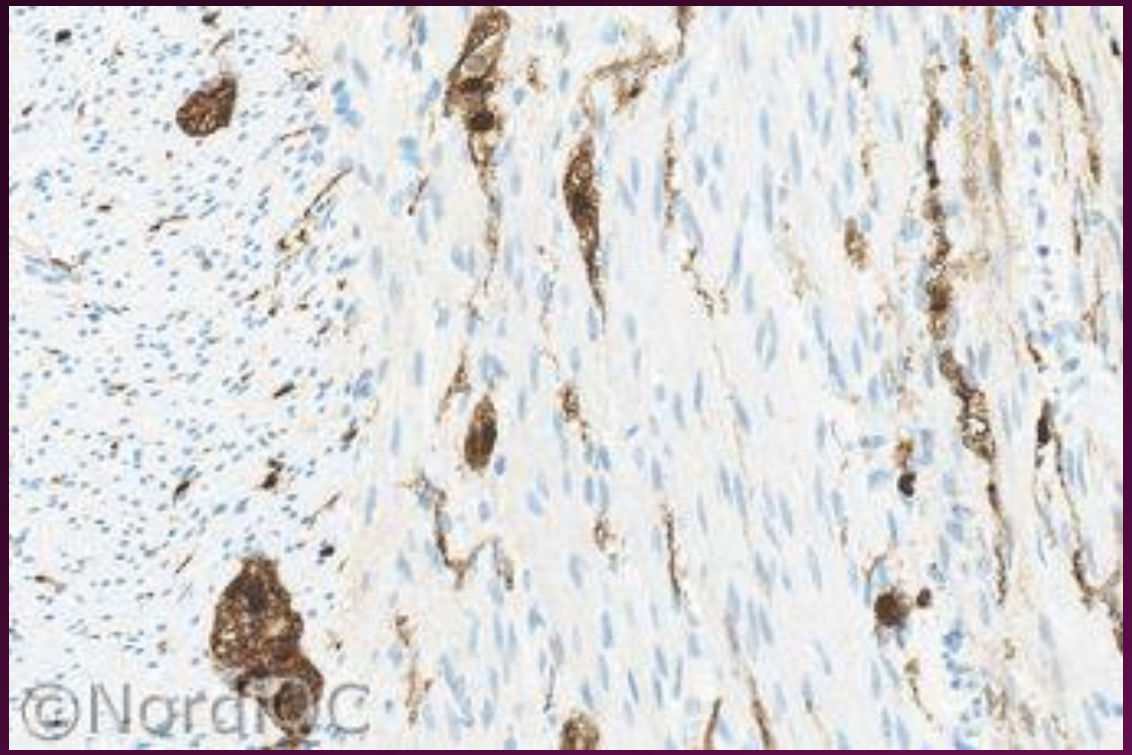




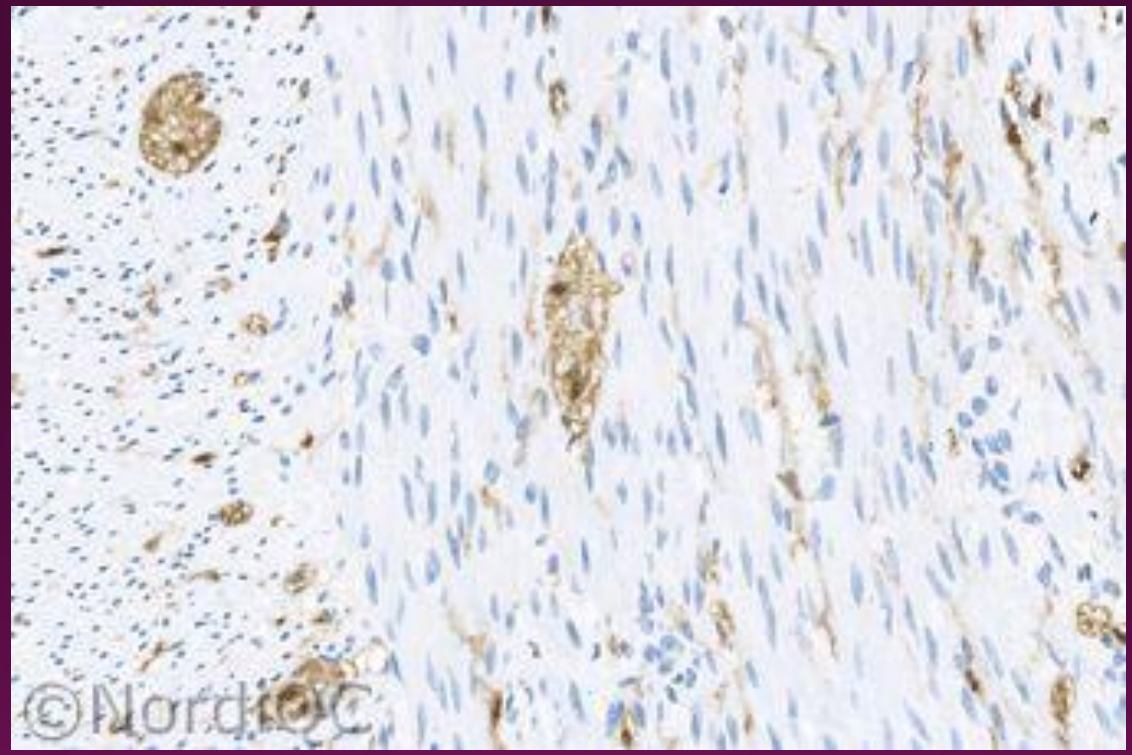
Lung Adenocarcinoma
L: Optimal
R: Insufficient



S100

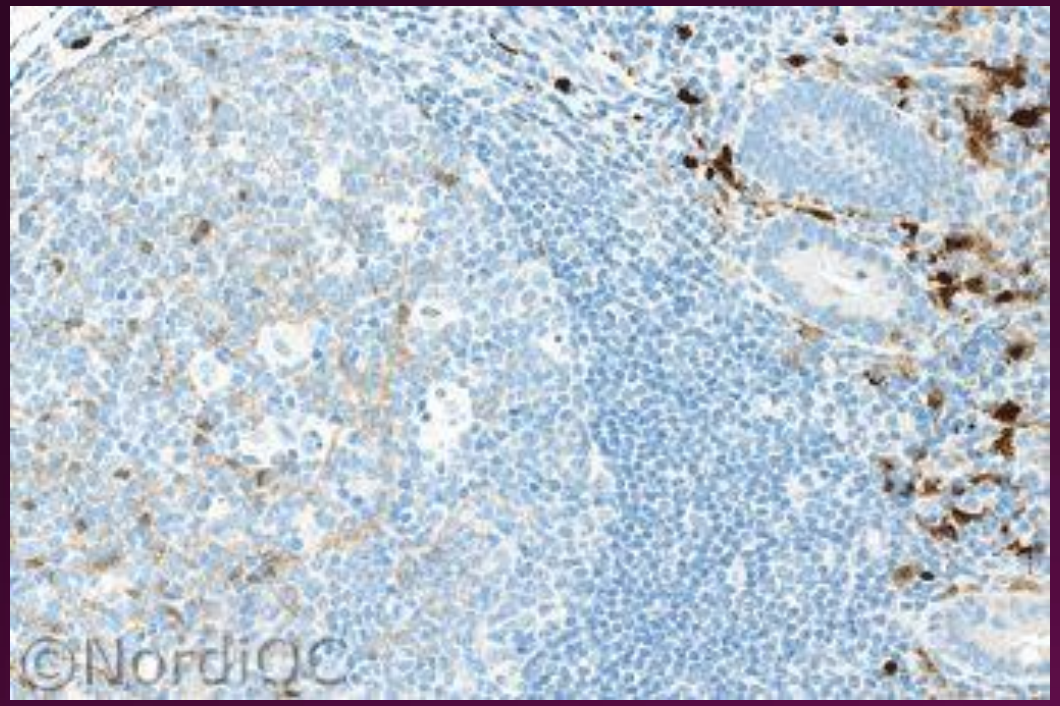


Optimal Protocol
Appendix: The Schwann cells and the satellite cells of the peripheral nerves show a moderate to strong staining reaction. No staining is seen in the smooth muscle cells.

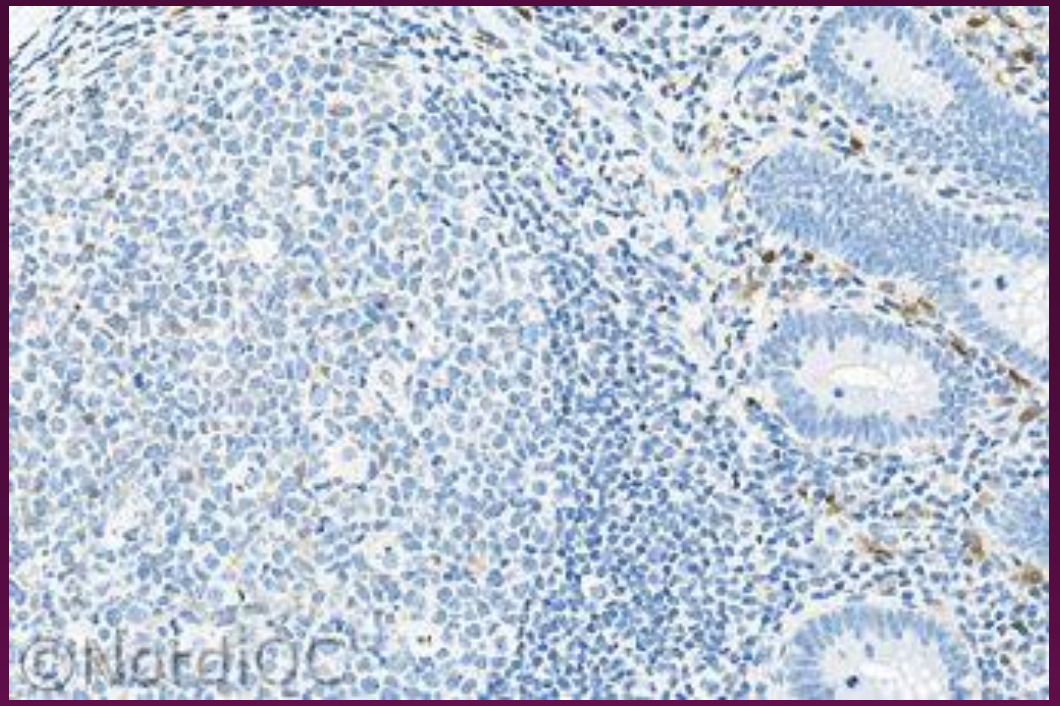


Insufficient Protocol
Appendix: The proportion and intensity of the positive cells is reduced. There is weak to moderate staining reaction, indicating that peripheral nerves can not reliably be used as positive control for S100

S100

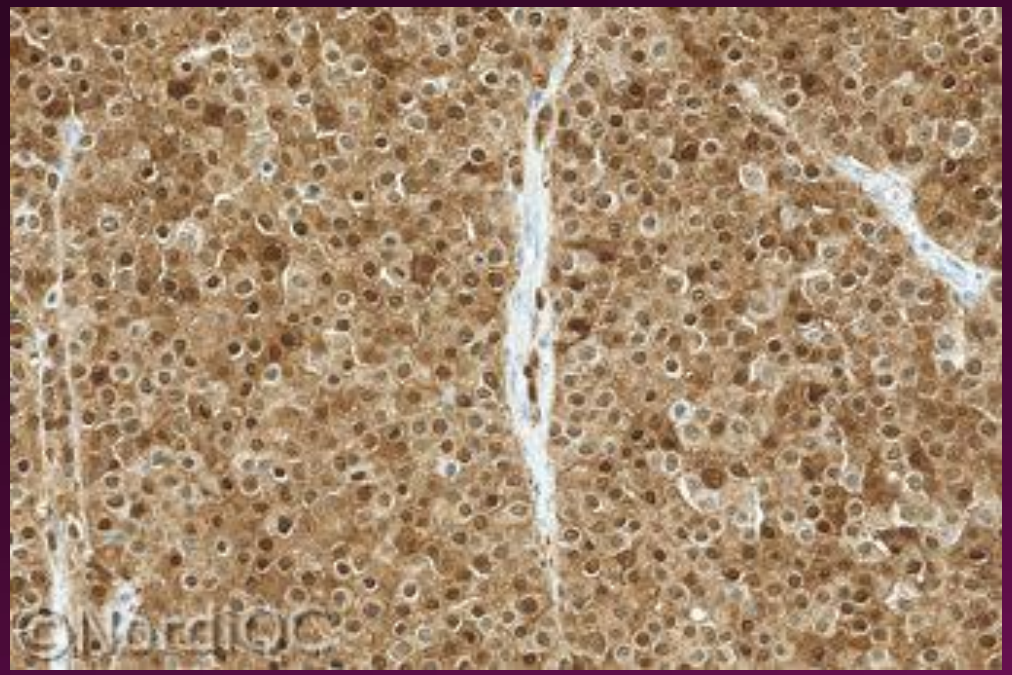


Optimal Protocol
Appendix: Peripheral nerves and macrophages in lamina propria show a strong cytoplasmic and nuclear staining reaction. Follicular dendritic cells of germina centre show a weak to moderate staining reaction. No background staining is seen.

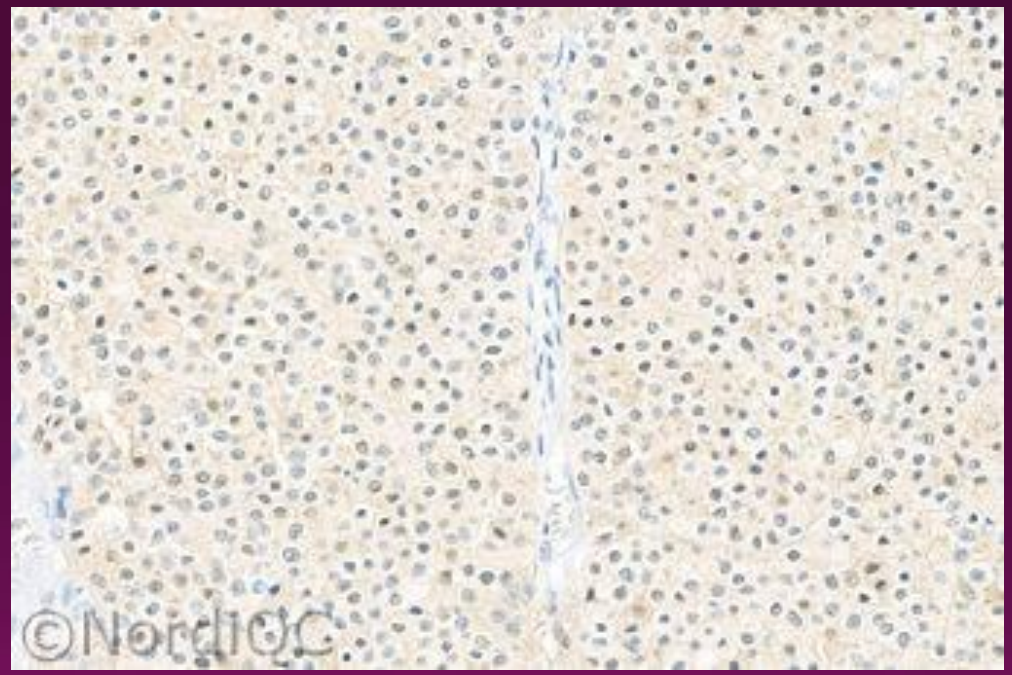


Insufficient Protocol
Appendix: Virtually no staining reaction is seen in the follicular dendritic cells in the germinal centre and the staining reaction in the macrophages and peripheral nerves is significantly reduced.

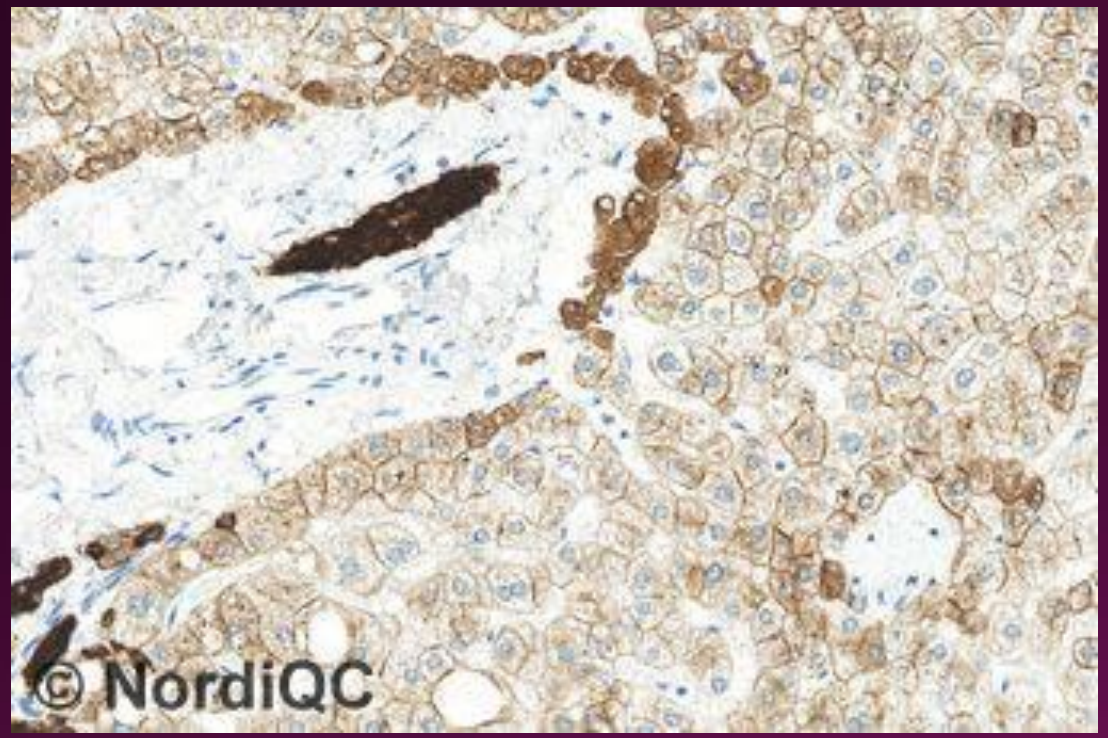
S100



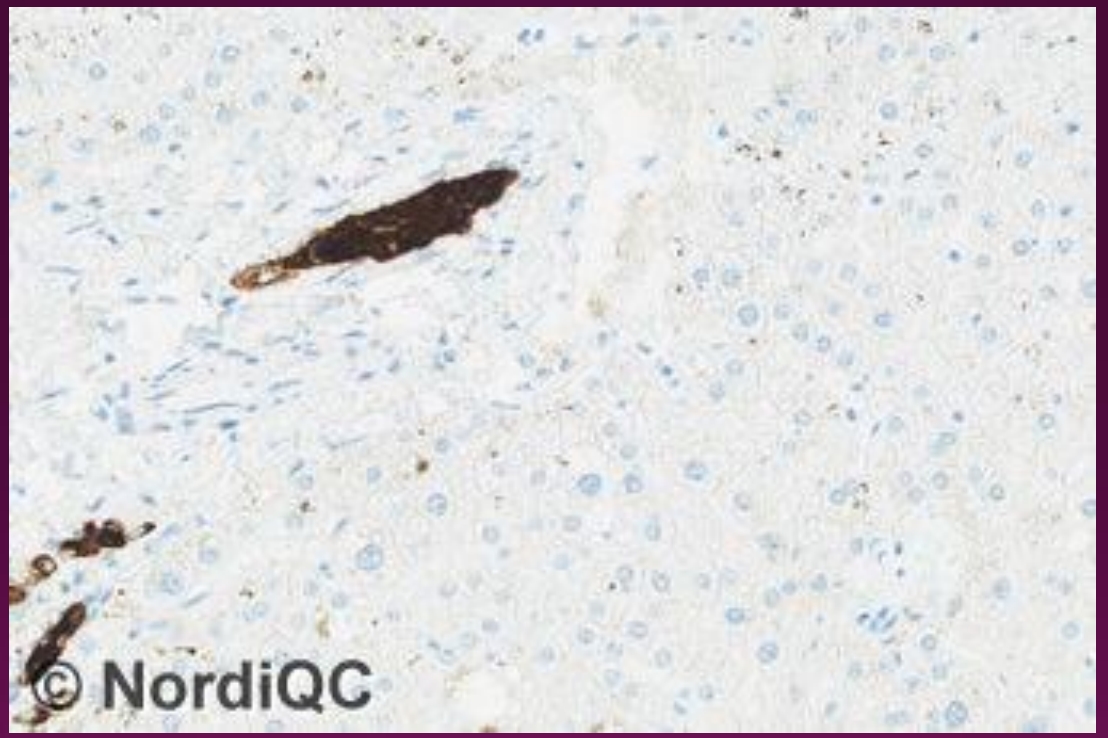
Melanoma:
L: Optimal
R: Insufficient



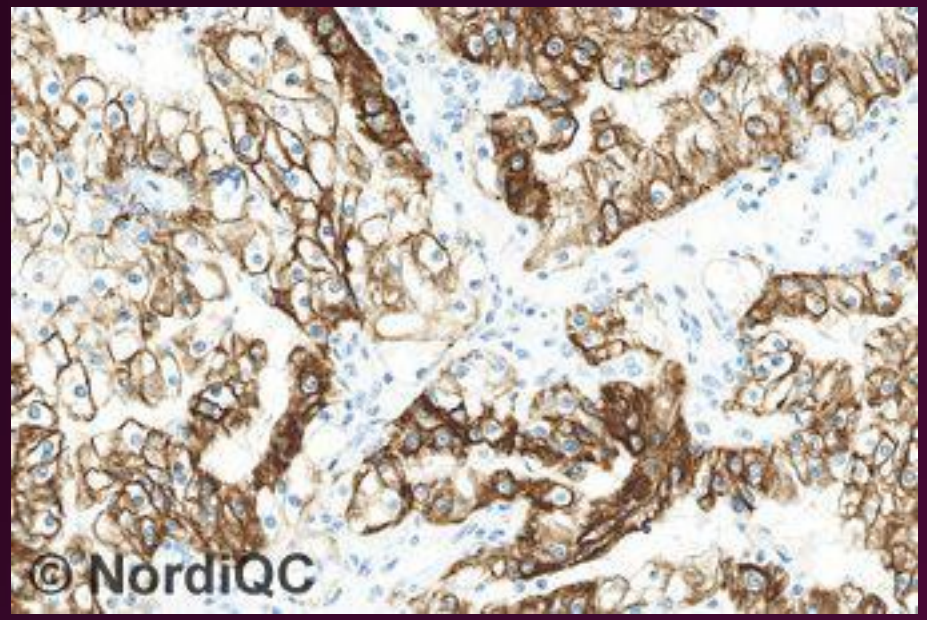
Pankeratin



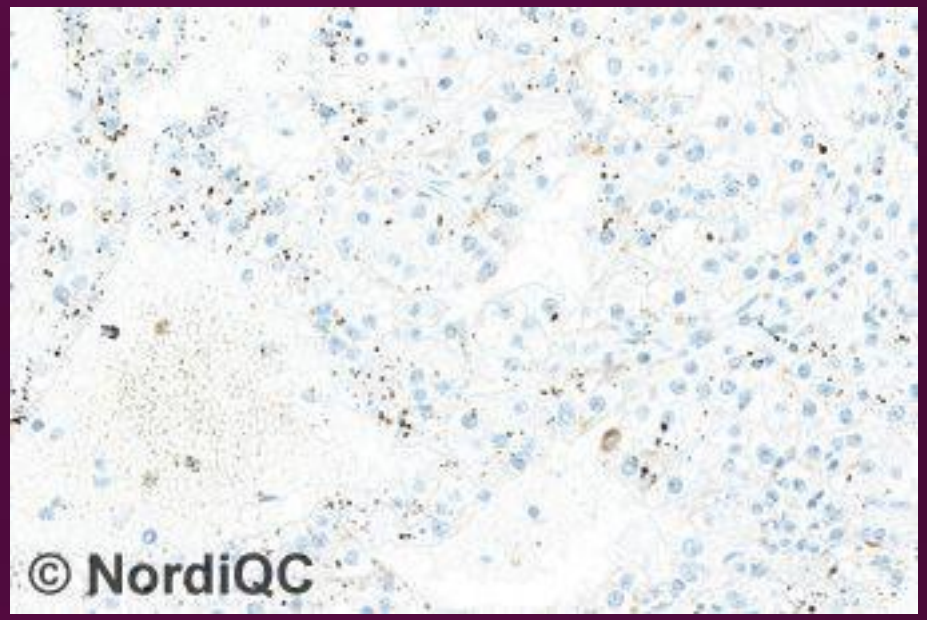
Optimal Protocol
Liver: Majority of hepatocytes show distinct, moderate reaction with membrane enhancement and bile duct epithelium shows strong cytoplasmic reaction

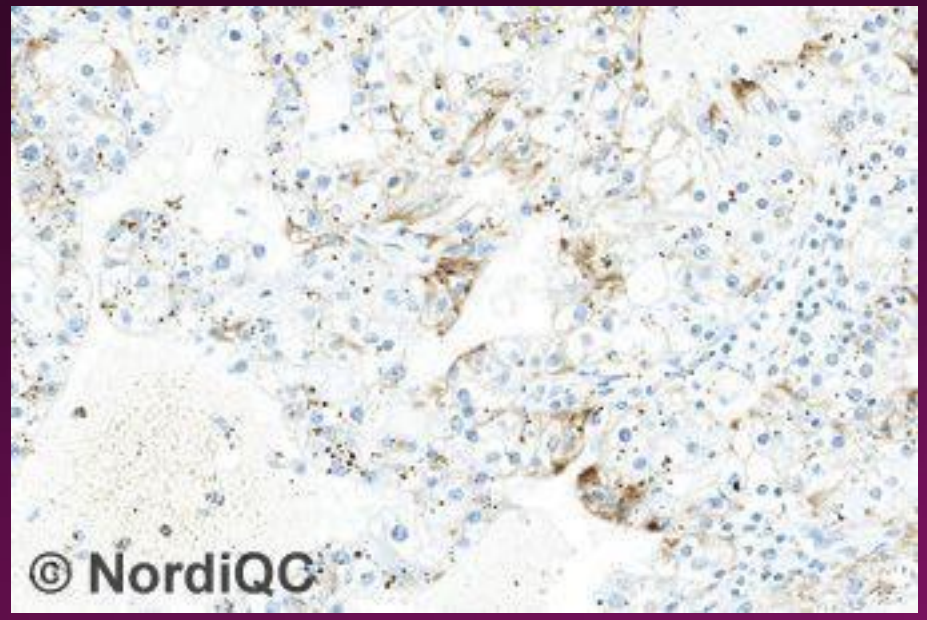


Insufficient Protocol
Liver: Epithelial cells of bile ducts are demonstrated, while hepatocytes are falsely negative

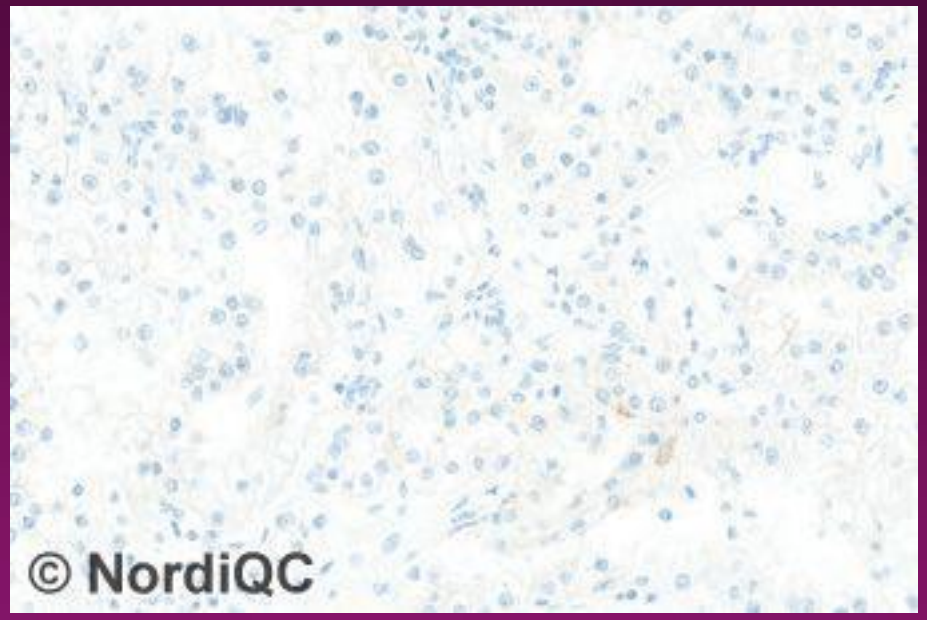


RCC:
L: Optimal
R: Insufficient

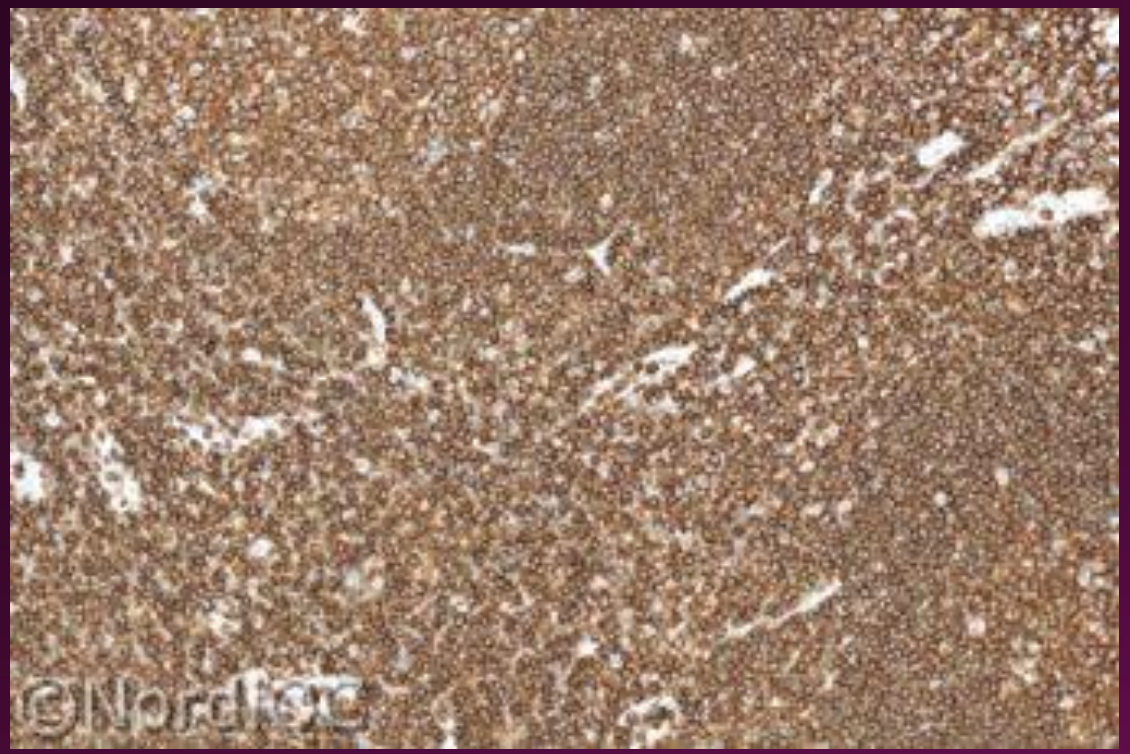




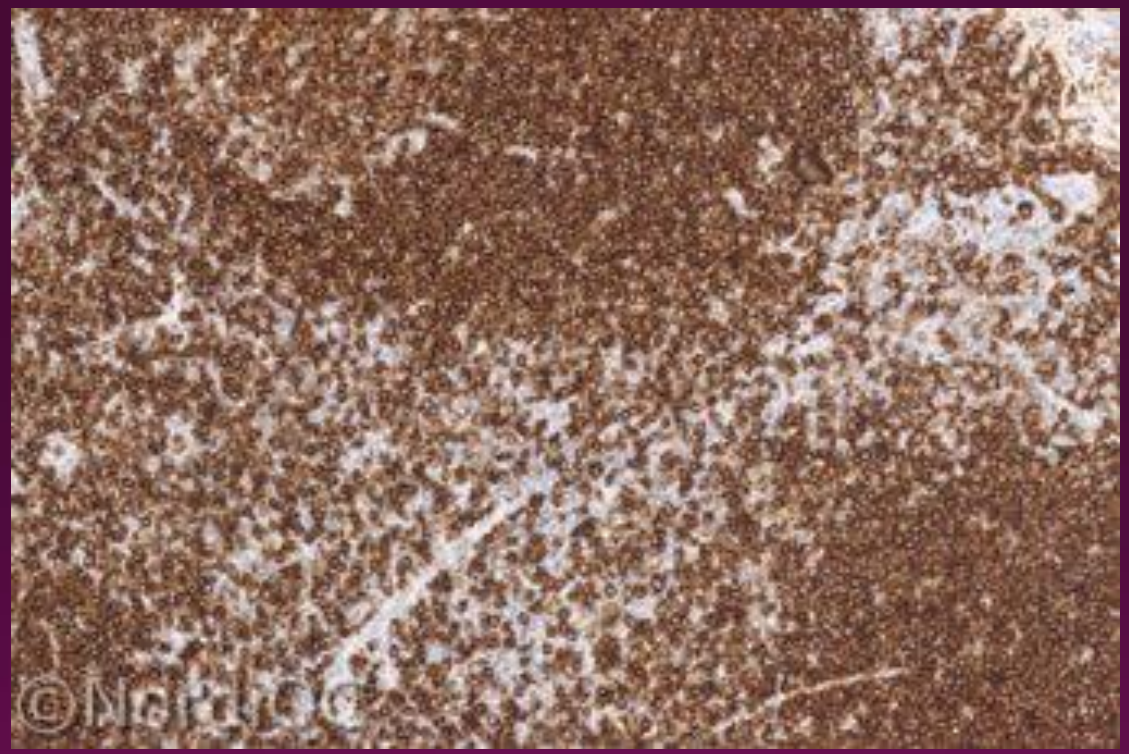
Low Expressing RCC:
L: Optimal
R: Insufficient



LCA

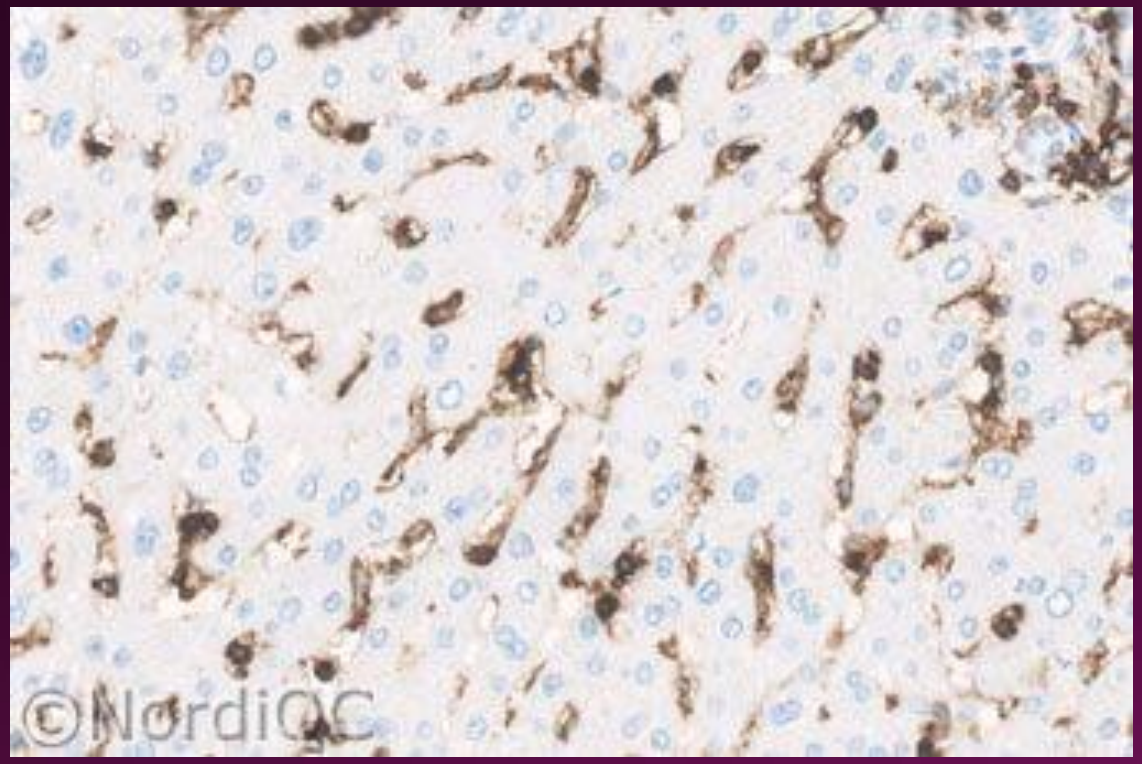


Tonsil Optimal Protocol
Tonsil: Virtually all the B- and T-lymphocytes show a strong and distinct membranous staining reaction. No background staining is seen.

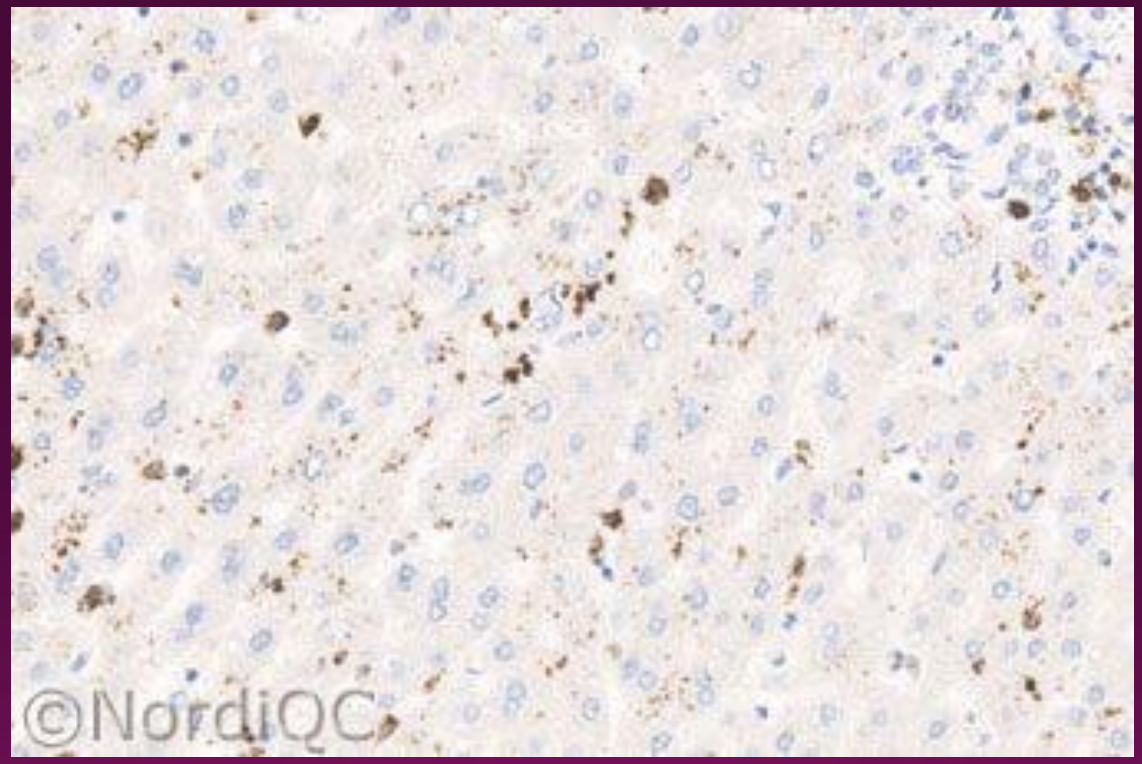


Tonsil Insufficient Protocol
Tonsil: The vast majority of the B- and T-lymphocytes are demonstrated. However, this protocol did not show Kupffer cell expression – next slide

LCA

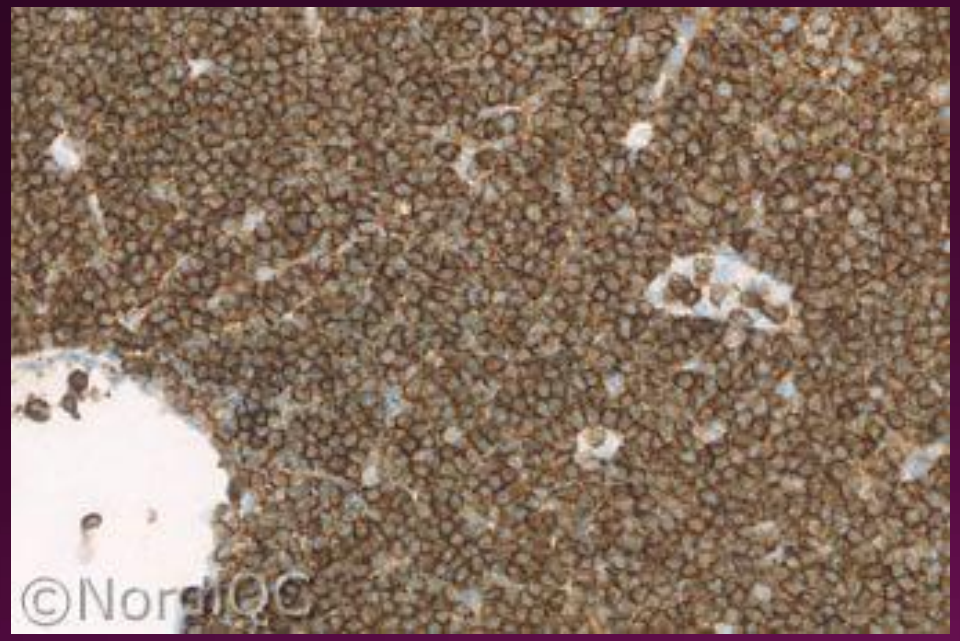


Optimal Protocol
Liver: Lymphocytes show a strong reaction, while the Kupffer cells display a weak to moderate reaction. The liver cells are negative and no background staining is seen.

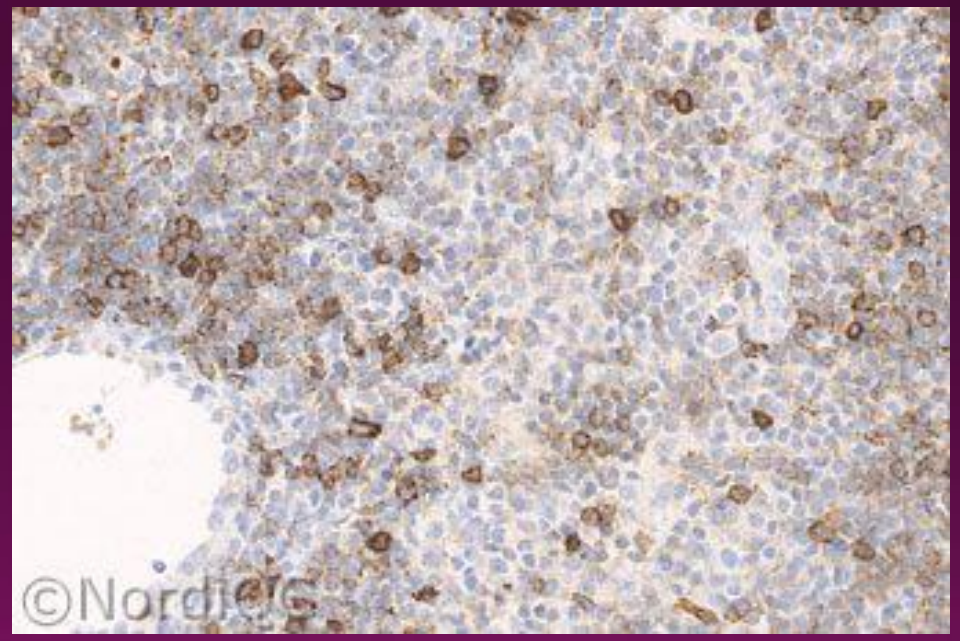


Insufficient Protocol
Liver: Only lymphocytes are demonstrated and the Kupffer cells with a low CD45 expression are falsely negative.

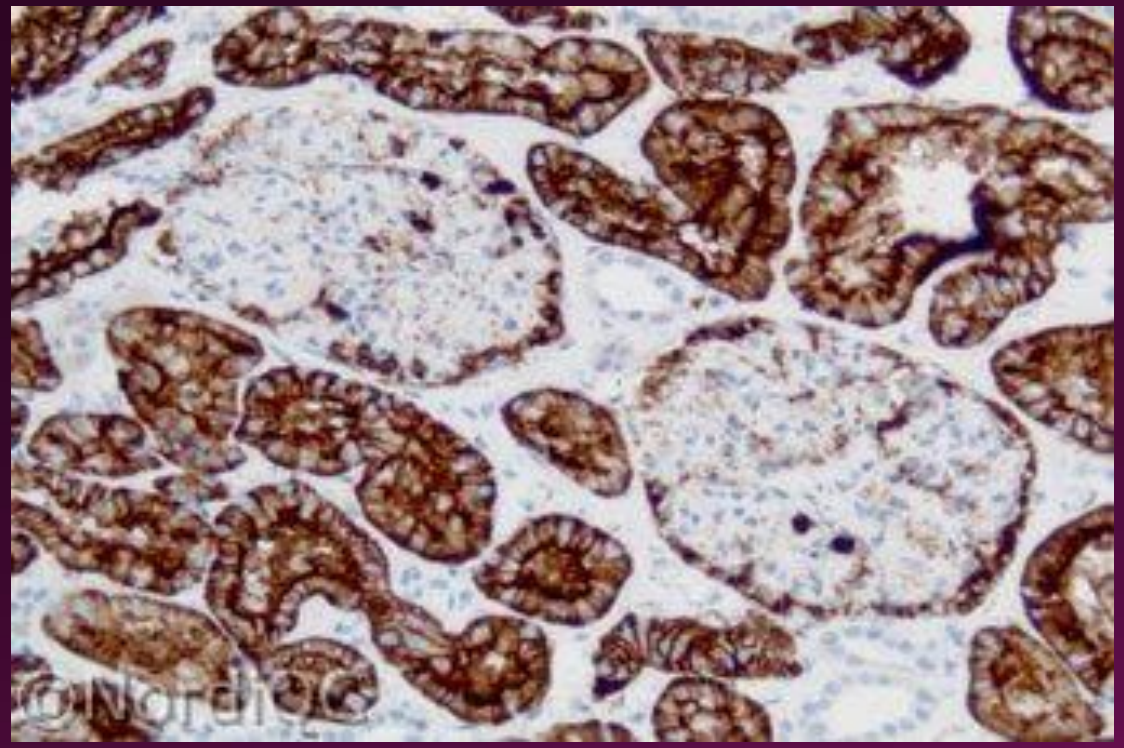
LCA



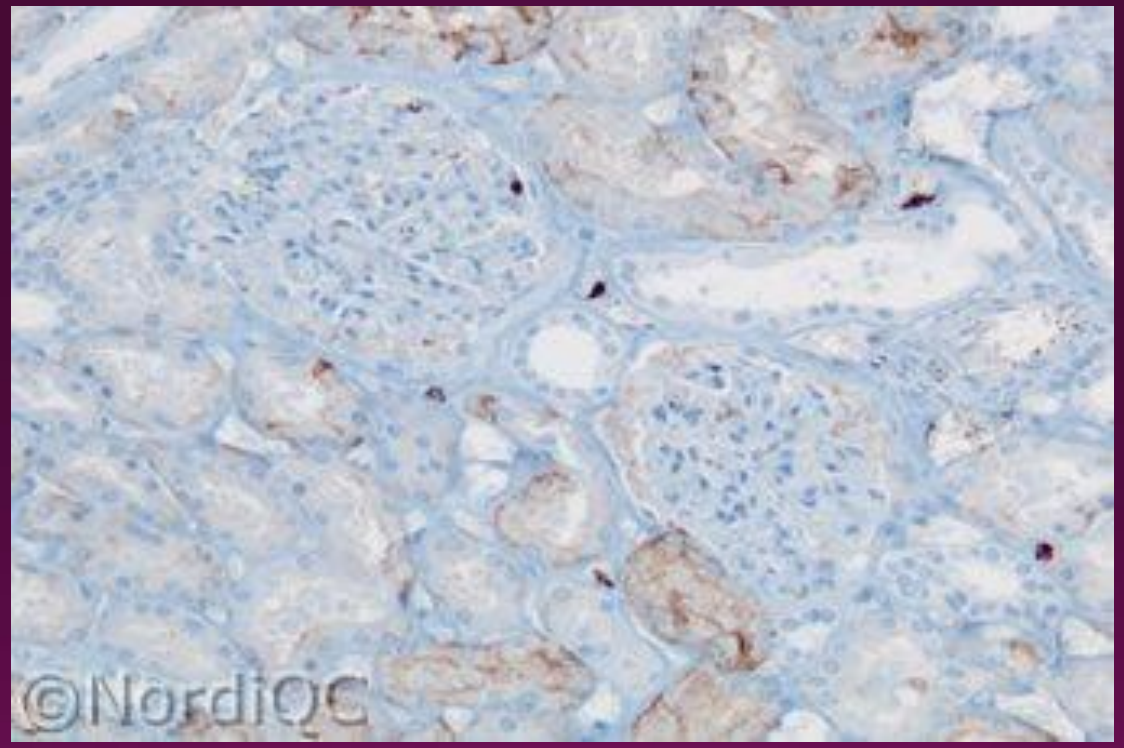
B-CLL:
L: Optimal Protocol
R: Insufficient Protocol



CD15

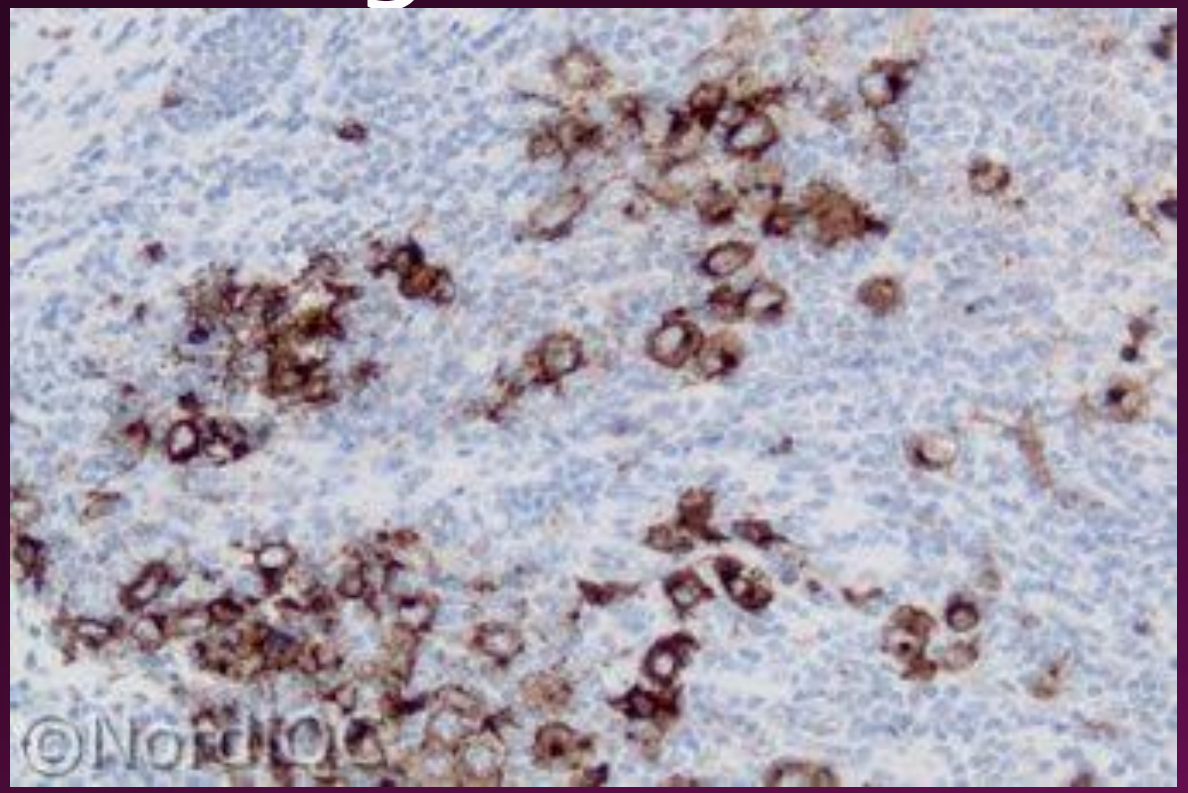


Optimal Protocol
Kidney: Epithelial cells lining the proximal tubules show a strong membranous and cytoplasmic reaction in virtually all the cells.

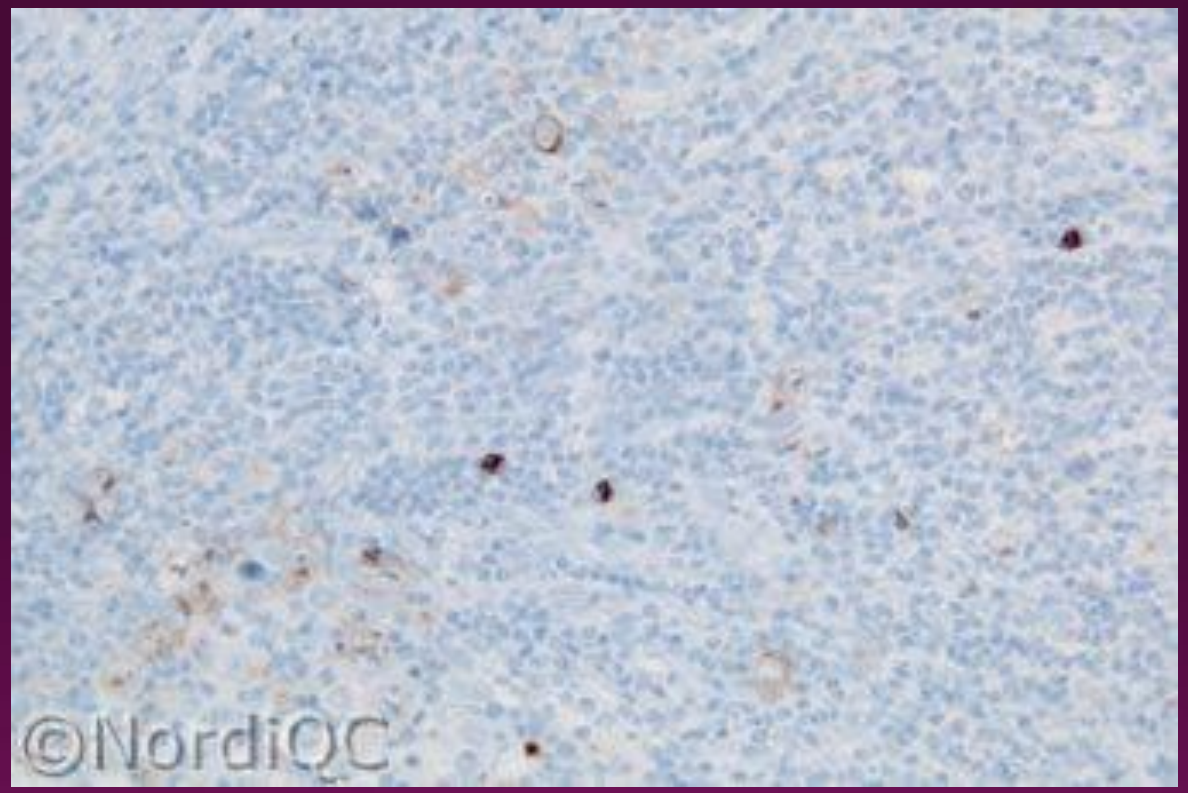


Insufficient Protocol
Kidney: Only scattered epithelial cells of the proximal tubules show a weak staining

CD15

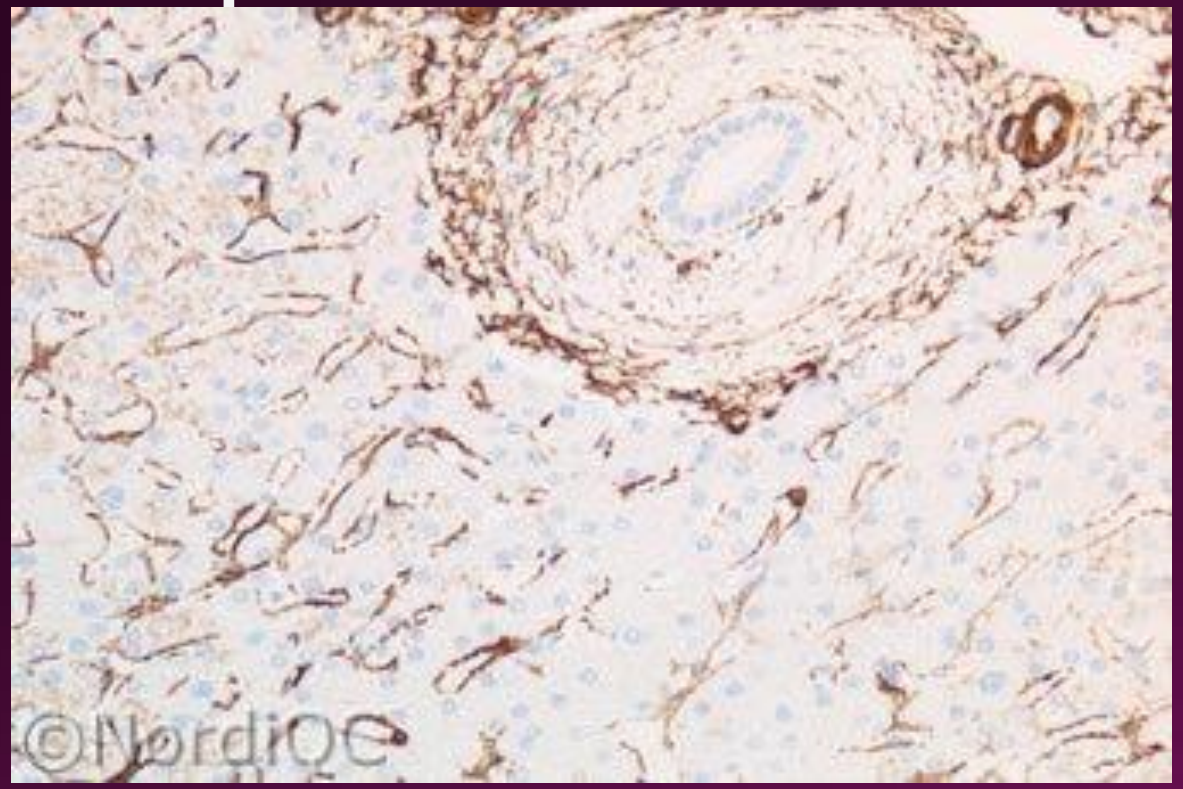


Optimal Protocol
Hodgkin Lymphoma: The Reed-Sternberg and Hodgkin cells show a strong membranous staining and a dot-like positivity.

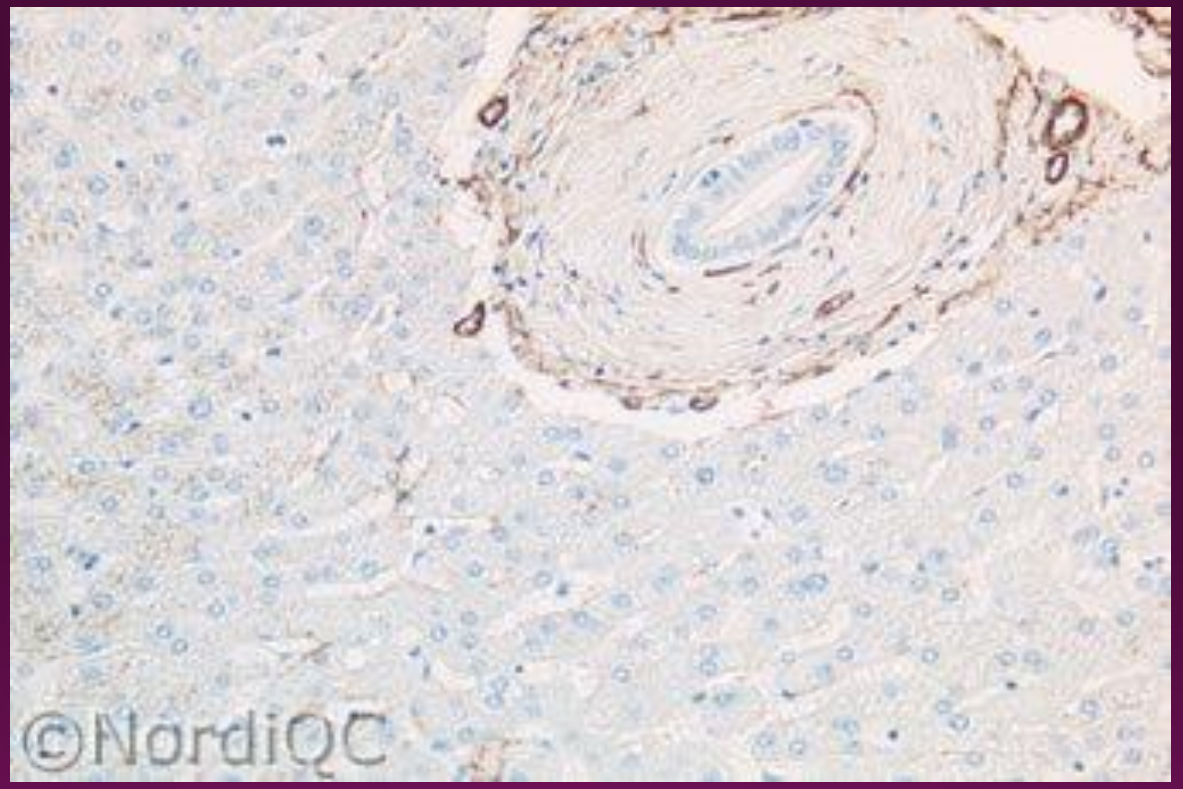


Insufficient Protocol
Hodgkin Lymphoma: Only few Reed-Sternberg and Hodgkin cells show a weak staining

Alpha Actin

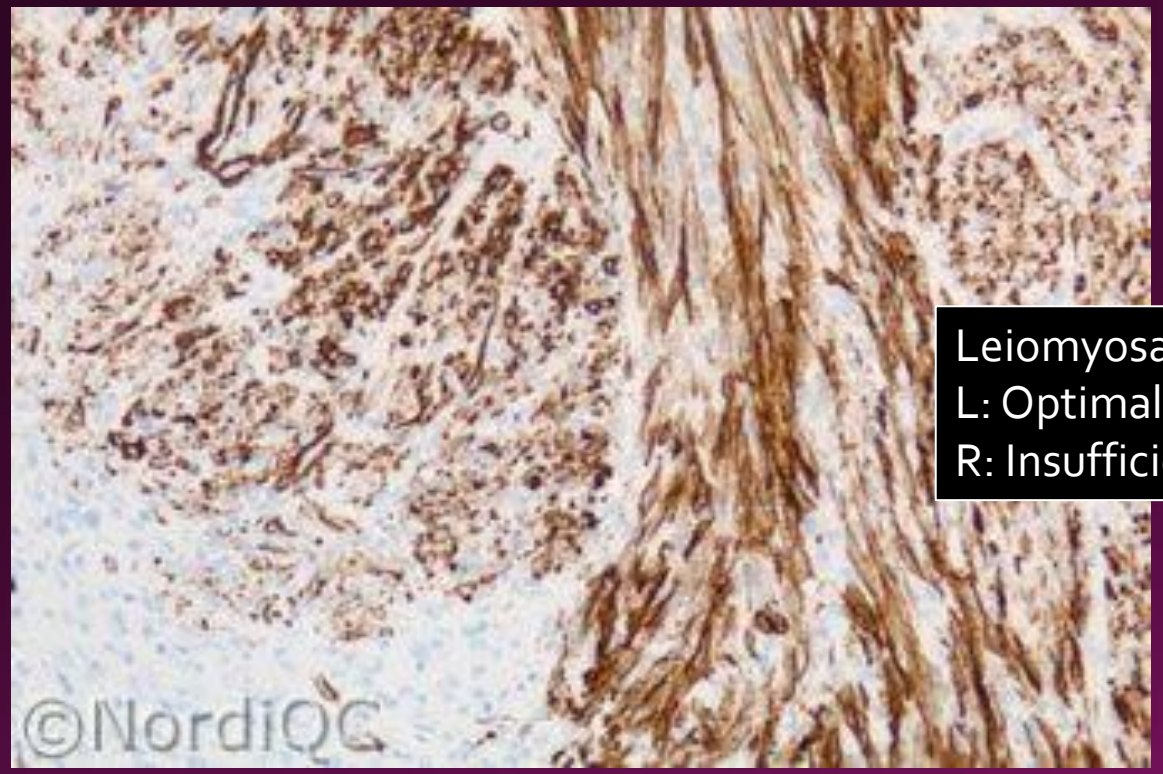


Optimal Protocol
Liver: Smooth muscle cells in the portal vessels as well as the perisinusoidal smooth muscle cells show a distinct staining. The liver cells are negative (weak granular staining is lipofuscin).

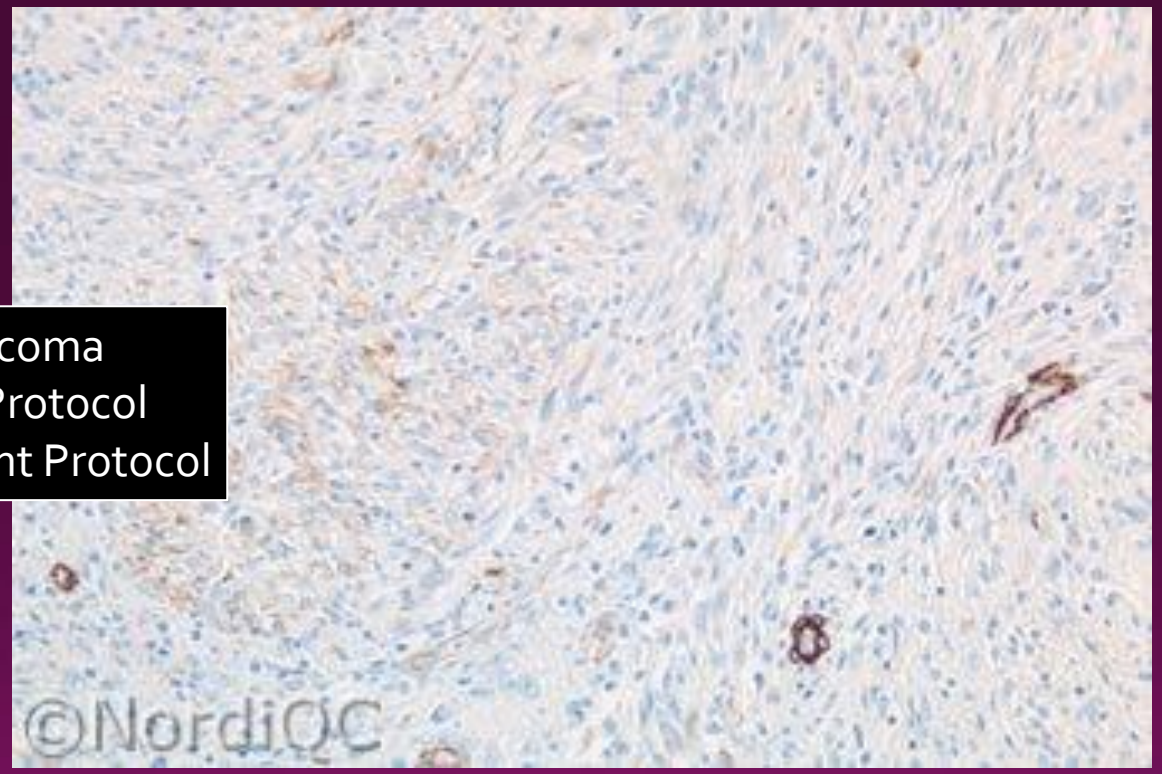


Insufficient Protocol
Liver: Smooth muscle cells in the portal vessels are demonstrated, while the perisinusoidal smooth muscle cells are virtually negative

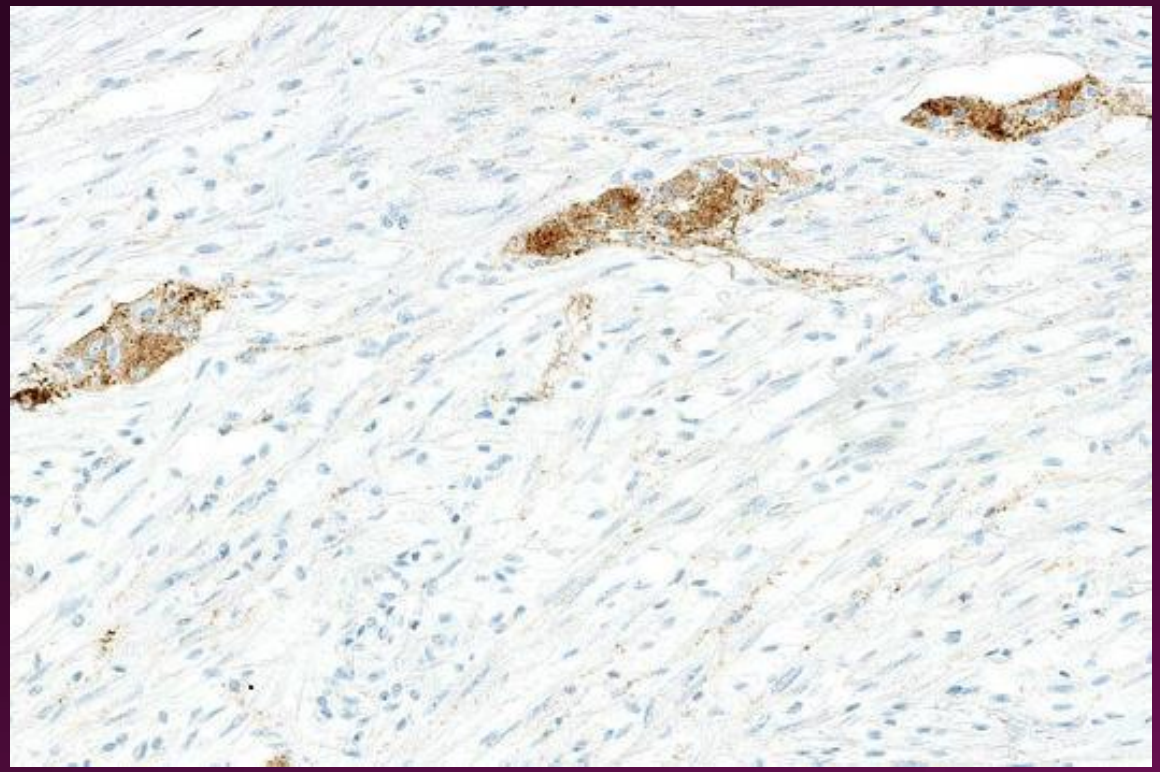
Alpha Actin



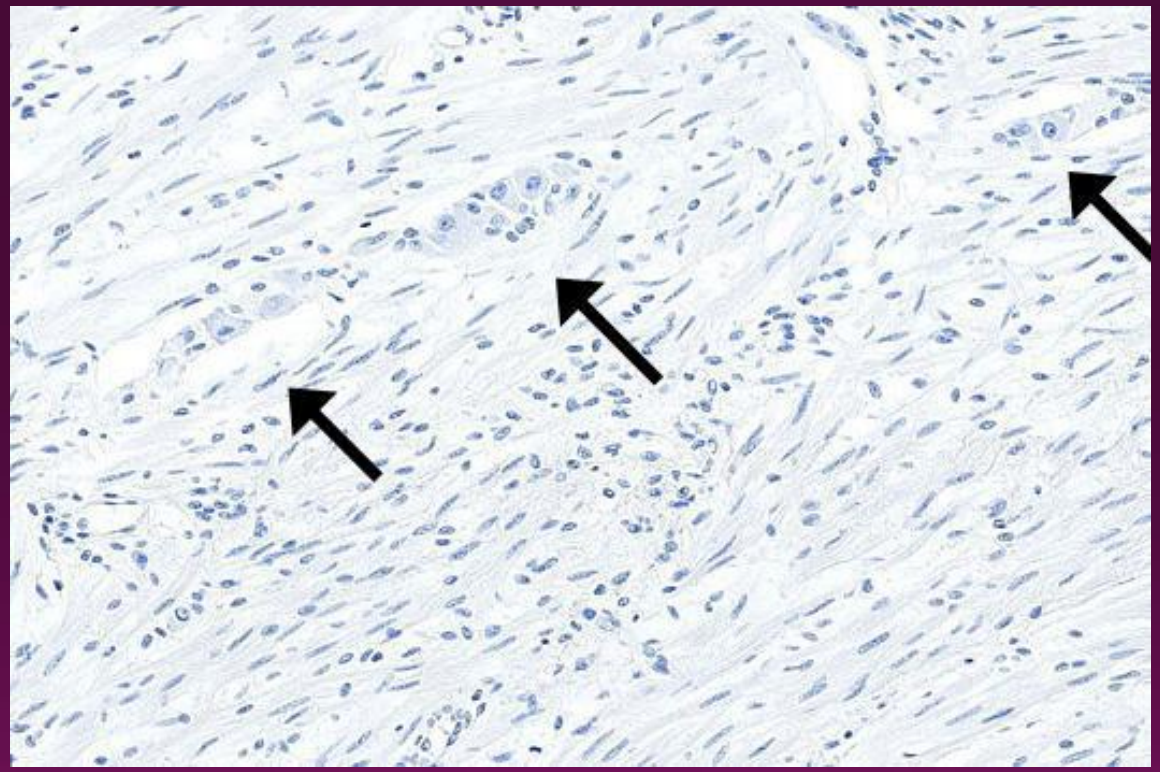
Leiomyosarcoma
L: Optimal Protocol
R: Insufficient Protocol



Lung ALK

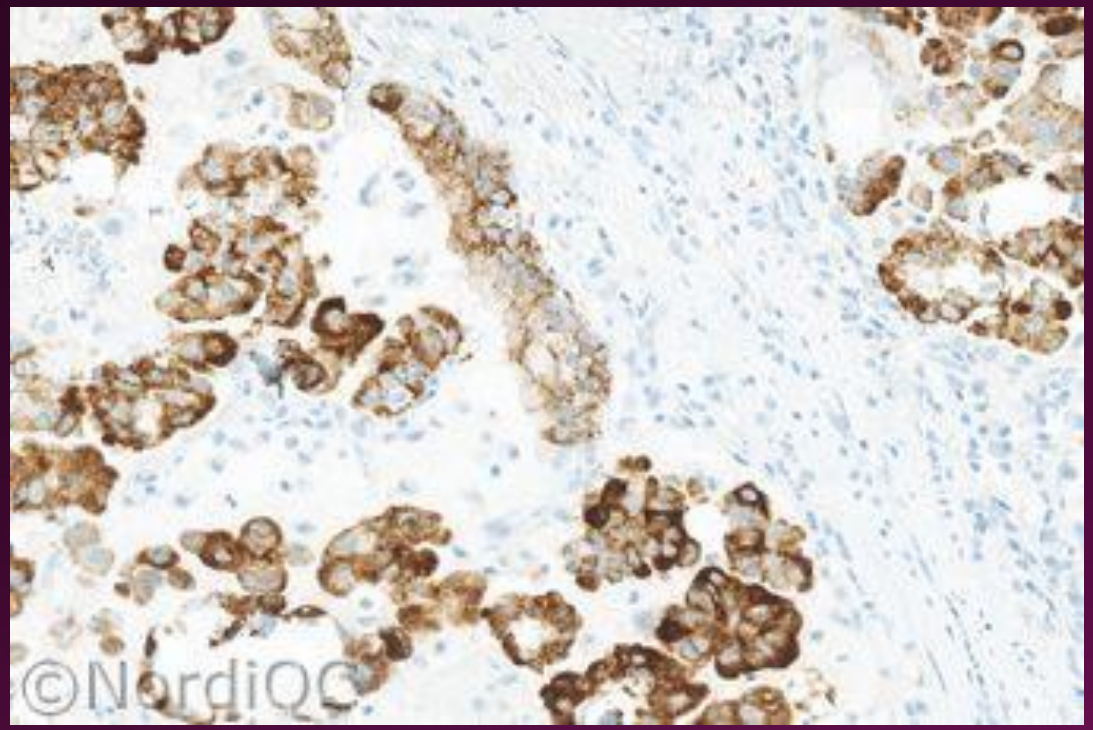


Optimal Protocol
Appendix: The ganglion cells of the myenteric plexus show a weak to moderate, distinct cytoplasmic staining reaction. The axons are faintly stained

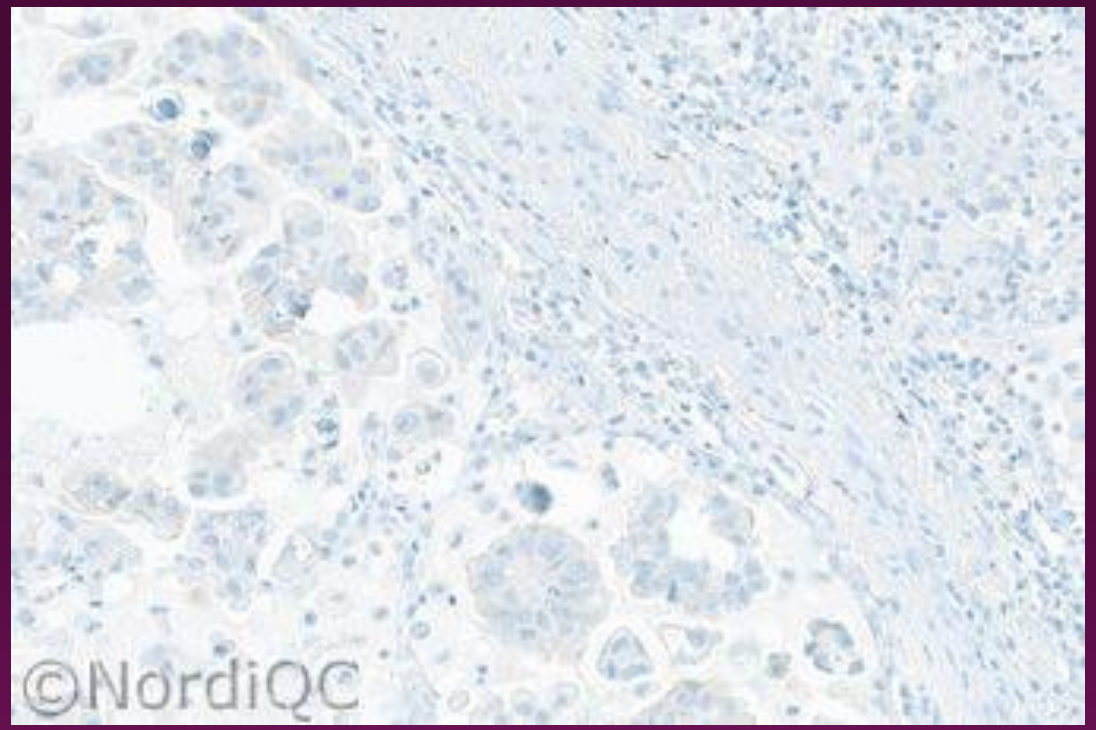


Insufficient Protocol
Appendix: The ganglion cells are unstained

Lung Adenocarcinoma with ALK gene Rearrangement



Optimal Protocol



Insufficient Protocol