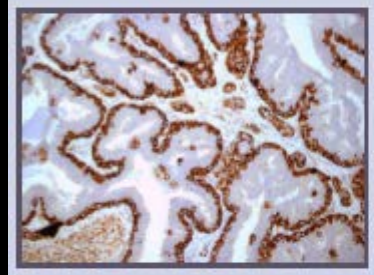

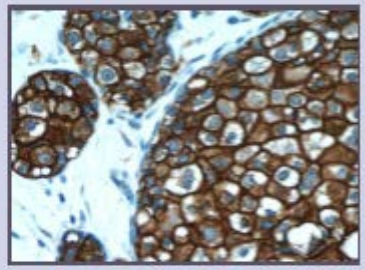

NordiQC TATA MEDICAL CENTER ISIMm International Society for IHC and Molecular Morphology

International Symposium on Immunohistochemistry

January 4th - 7th, 2018

# QA of IHC

# Breast pathology

Søren Nielsen
Global Pathology Manager
Agilent Technologies

(Former Scheme Manager, NordiQC)

# IHC - Protocols and controls for Breast tumours

Breast panel:

- GCDFP-15
- Mammaglobin
- Gata 3
- Smooth MHCM
- ASMA
- (p63)
- E-cadherin
- p120
- ER
- PR
- HER-2

- Is it primary breast ?
- Is it invasive ?
- Is it lobular or ductal ?
- Which teraphy ?

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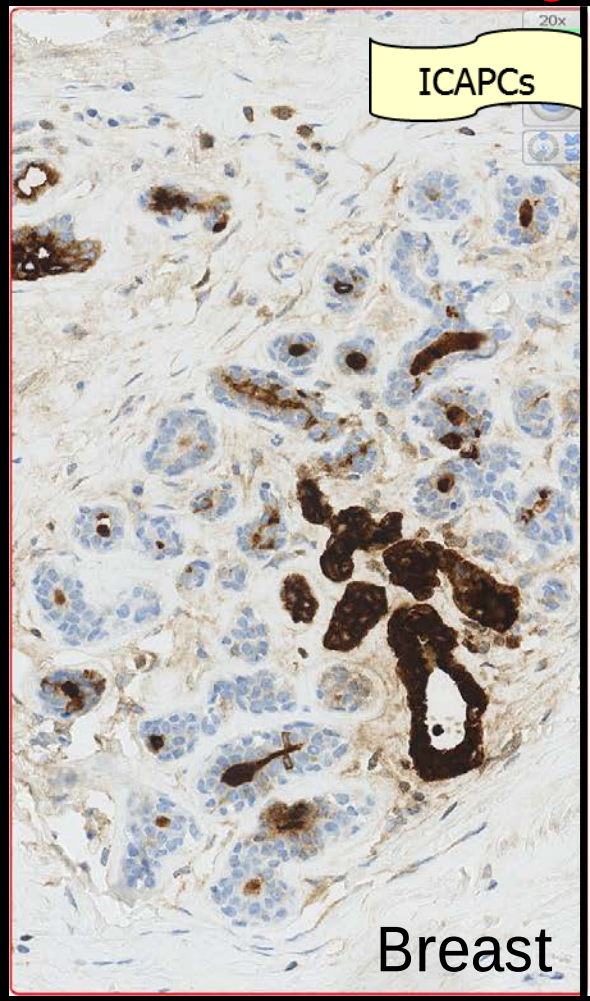
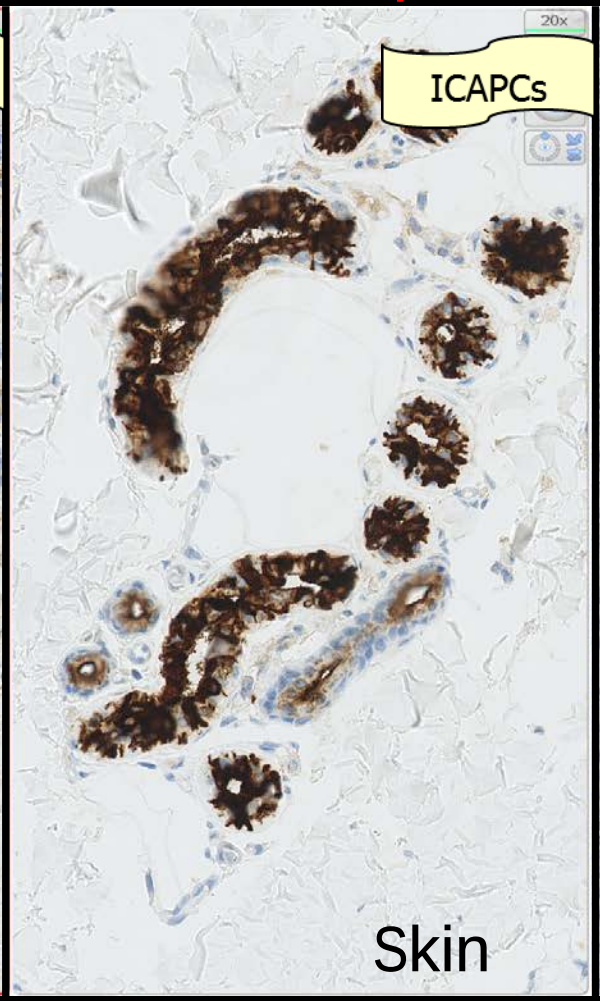
# IHC – Protocols and controls for Breast tumours

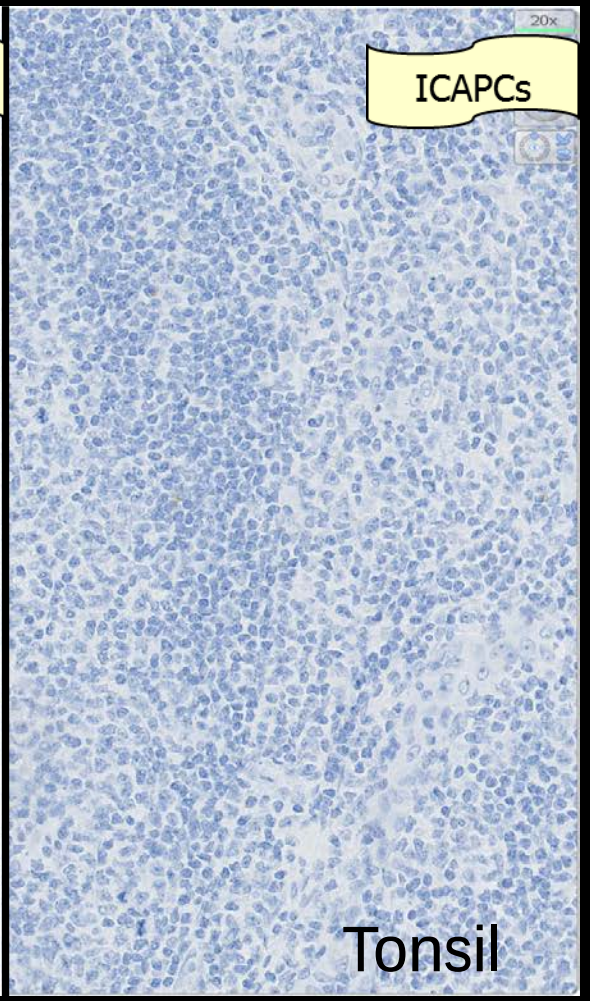
## GCDFP15 / Mammaglobin reaction pattern




A moderate to strong, distinct cytoplasmic staining reaction in scattered ductal epithelial cells and in apocrine metaplastic cells.




A moderate to strong, distinct cytoplasmic staining reaction of the majority of the epithelial cells of the eccrine sweat glands

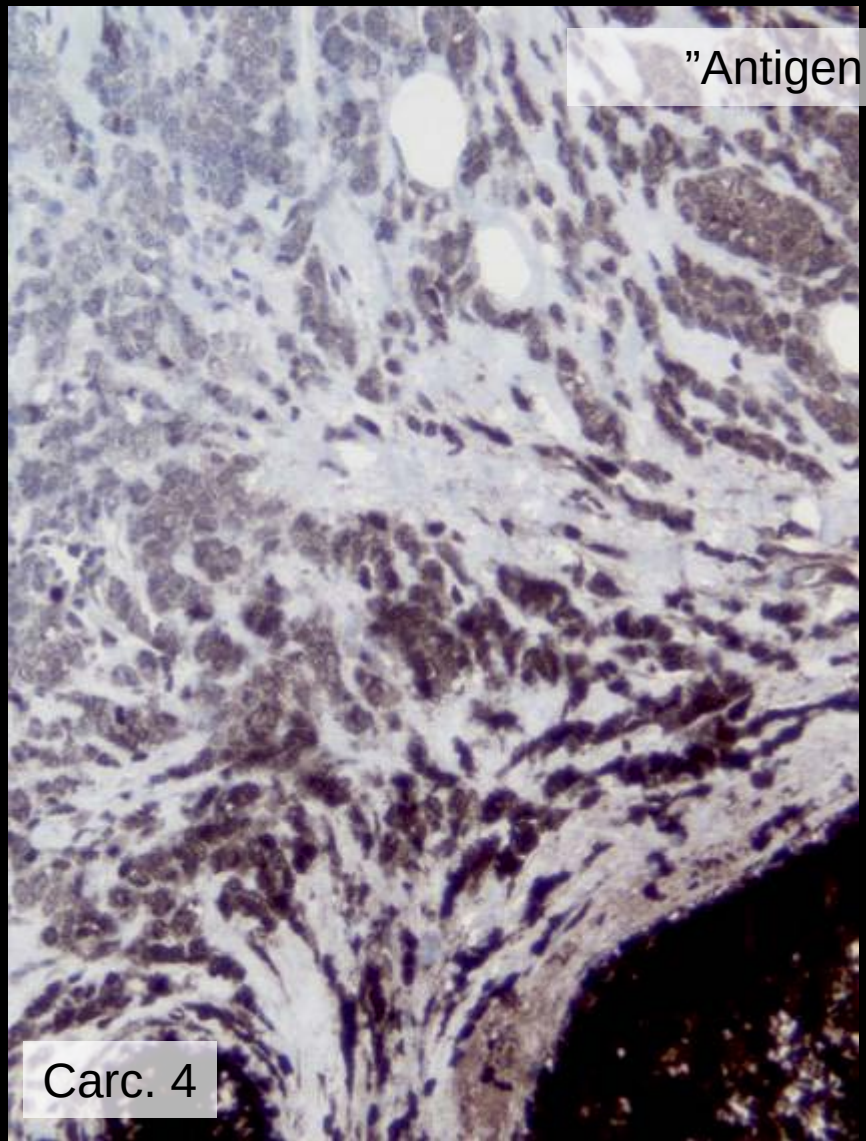



No staining reaction should be seen.

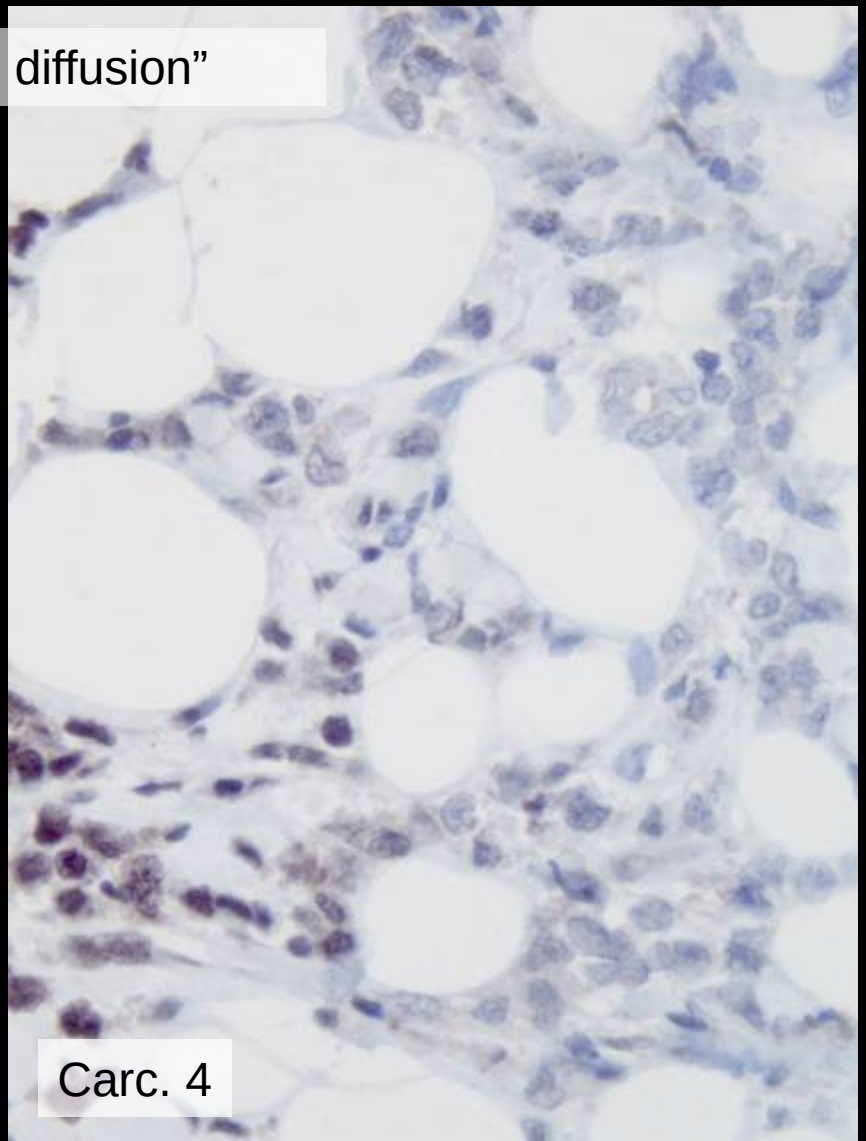
No structure with LE.....

# IHC – Protocols and controls for Breast tumours

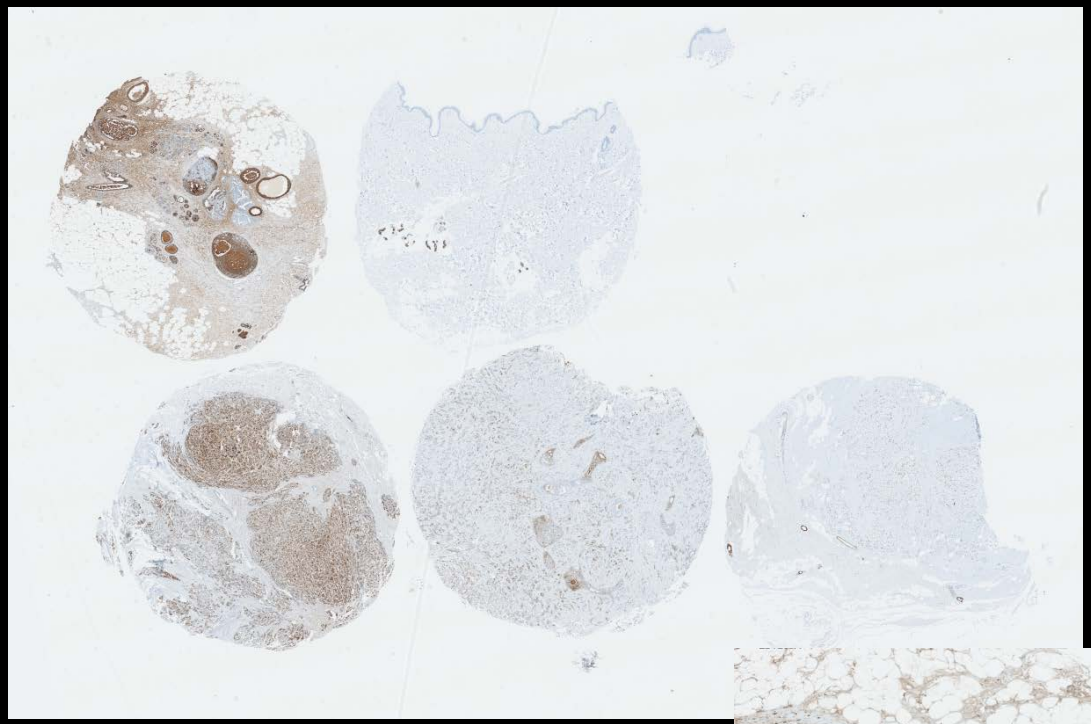
## GCDFP15 / Mammaglobin reaction pattern

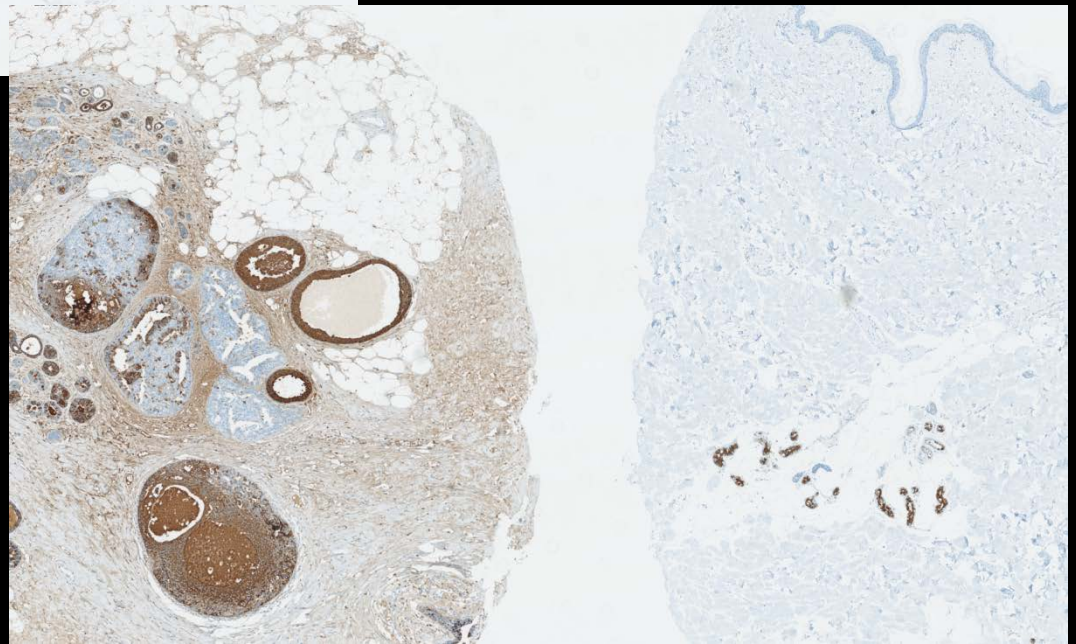



”Antigen diffusion”



# IHC – Protocols and controls for Breast tumours

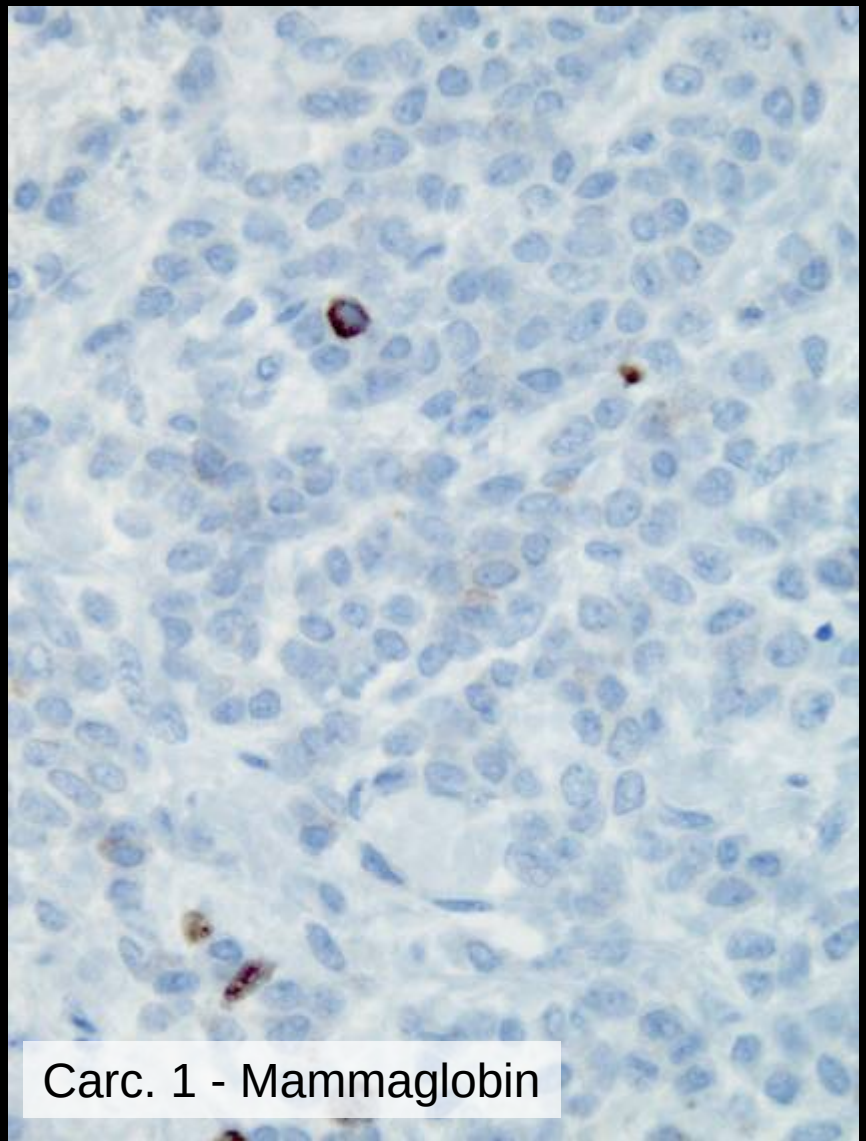




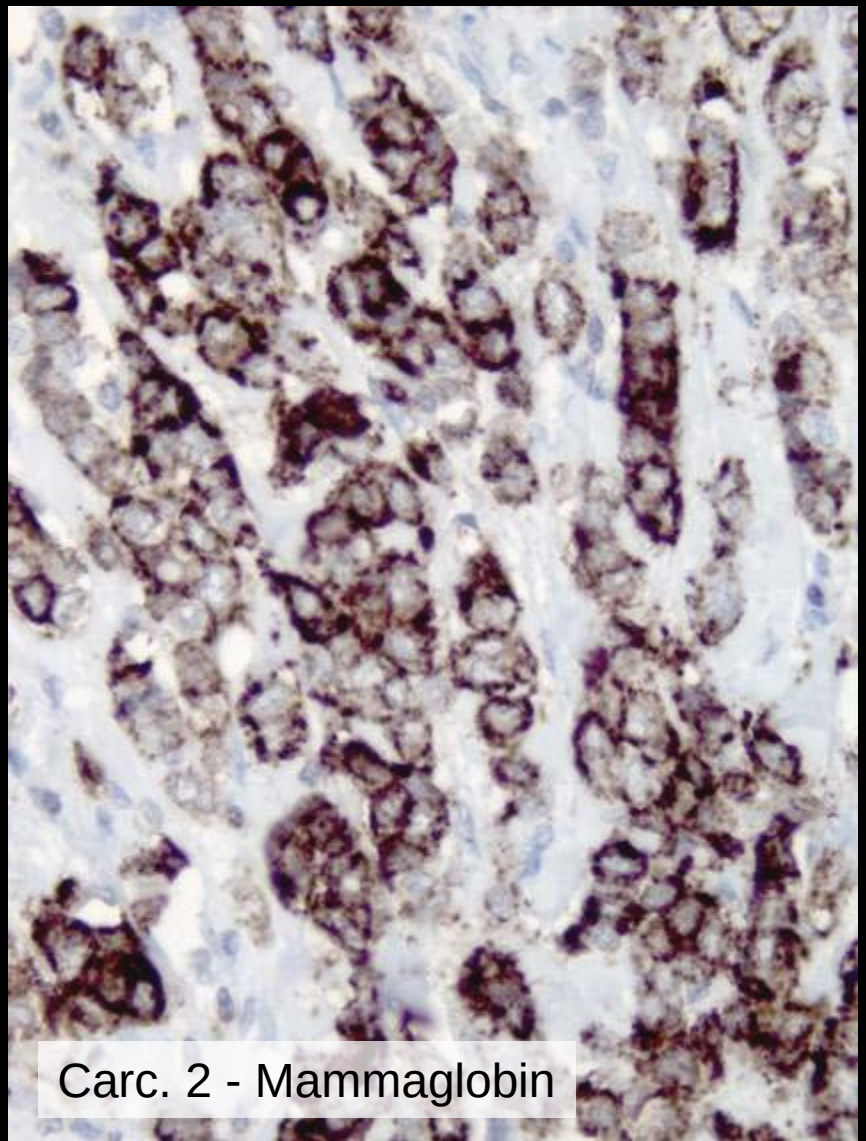
Local diffusion

# IHC – Protocols and controls for Breast tumours

## GCDFP15 / Mammaglobin reaction pattern



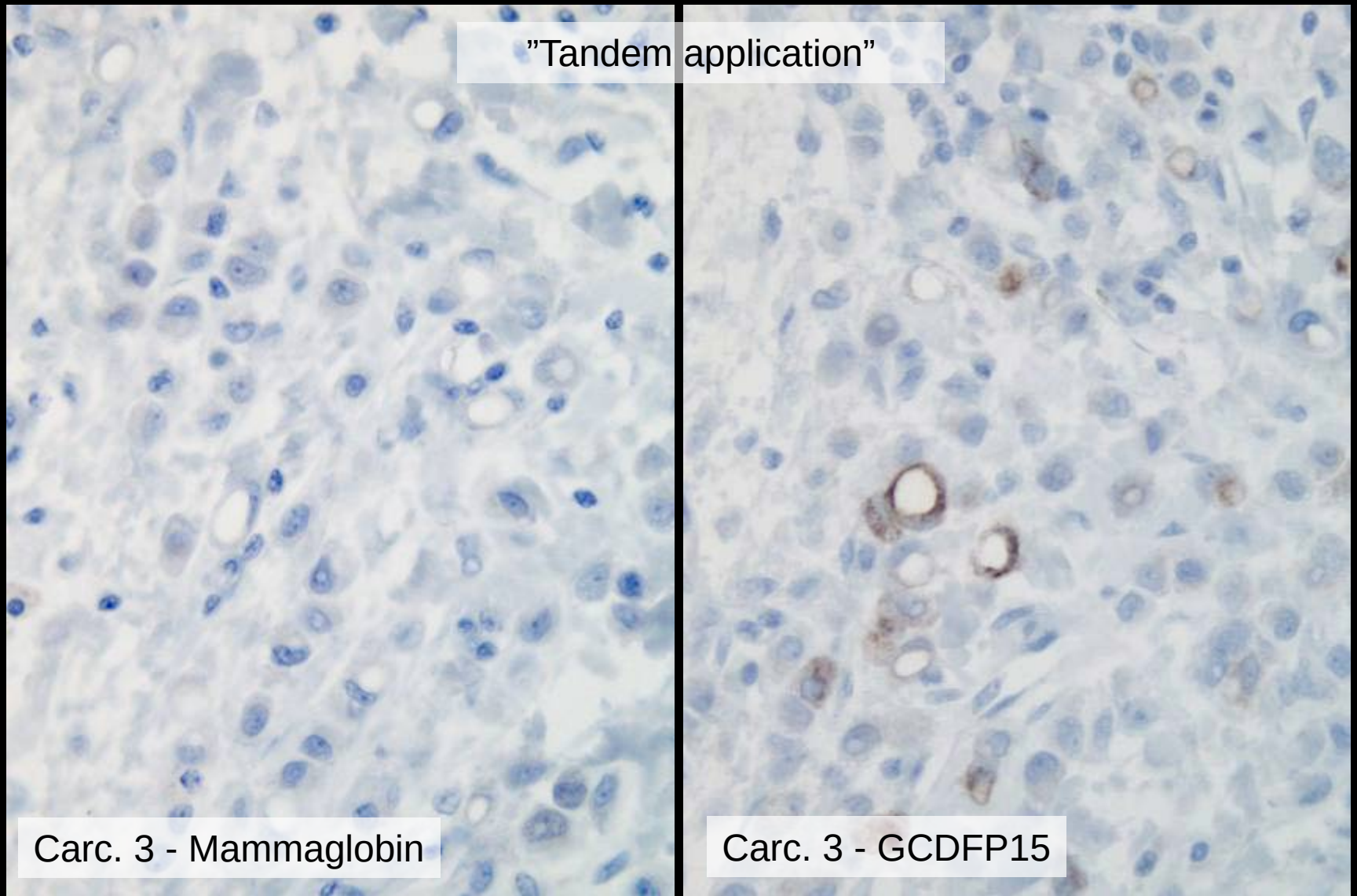
Carc. 1 - Mammaglobin



Carc. 2 - Mammaglobin

# IHC – Protocols and controls for Breast tumours

## GCDFP15 / Mammaglobin reaction pattern



# IHC – Protocols and controls for Breast tumours

**Table 1. Abs and assessment marks for GCD, run 36**

| Concentrated Abs | N | Vendor | Optimal | Good | Borderl. | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **23A3** | 43<br>12<br>10<br>2<br>2<br>1<br>1<br>1 | Leica/Novocastra<br>Thermo/Neomarkers<br>Dako<br>Cell Marque<br>Diagnostic Biosystems<br>Labs Inc.<br>Vector Lab.<br>Abcam | 25 | 33 | 8 | 6 | 81 % | 88 % |
| mAb clone **D6** | 6<br>2<br>1<br>1<br>1 | Covance/Signet<br>ID Labs<br>Biocare<br>Invitrogen<br>Sanbio | 4 | 5 | 1 | 1 | 82 % | 86 % |
| mAb **SPM135** | 1 | Spring Bioscience | 0 | 1 | 0 | 0 | - | - |
| rmAb **EP1582Y** | 2<br>1 | Cell Marque<br>Zytomed systems | 1 | 2 | 0 | 0 | - | - |
| rmAb **EP95** | 1 | Epitomics | 0 | 1 | 0 | 0 | - | - |
| **Ready-To-Use Abs** | | | | | | | | |
| mAb clone **23A3 IS/IR077** | 20 | Dako | 10 | 10 | 0 | 0 | 100 % | 100 % |
| mAb clone **23A3 PA0350** | 1 | Leica/Novocastra | 0 | 1 | 0 | 0 | - | - |
| mAb clone **23A3 257M-17** | 1 | Cell Marque | 0 | 1 | 0 | 0 | - | - |
| mAb clone **23A3 MS-1170** | 1 | Thermo/Neomarkers | 0 | 0 | 1 | 0 | - | - |
| mAb clone **23A3 MAD-001638QD** | 1 | Master Diagnostica | 0 | 0 | 1 | 0 | - | - |
| rmAb clone **EP1582Y Z60-4386** | 18 | Ventana | 10 | 7 | 1 | 0 | 94 % | 94 % |
| rmAb clone **EP1582Y AN481-5M** | 1 | Biogenex | 0 | 1 | 0 | 0 | - | - |
| **Total** | 131 | | 50 | 62 | 12 | 7 | | |
| **Proportion** | | | 38 % | 48 % | 9 % | 5 % | 86 % | |

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

Ins.:

Omission of HIER

and/or

too low conc.

RTU > Conc.

(difficult to calibrate... what is best control...)

# IHC – Protocols and controls for Breast tumours

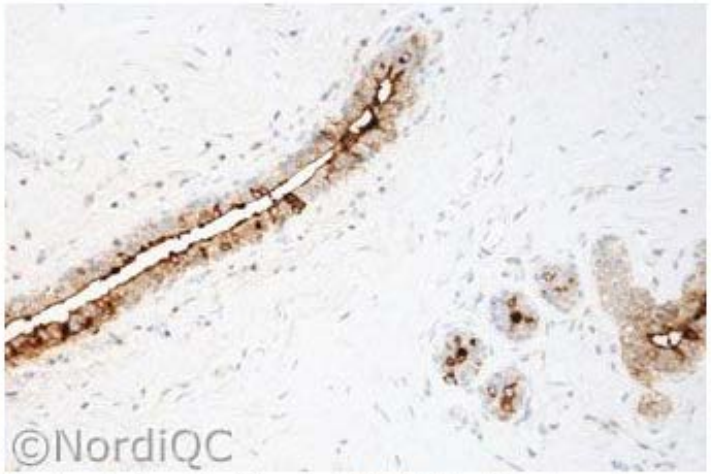



Fig. 1a. Optimal staining for GCDFP-15 of the breast hyperplasia using the mAb clone 23A3 optimally calibrated as a concentrate, HIER in an alkaline buffer and a polymer based detection system. The majority of the ductal epithelia cells show a distinct moderate to strong cytoplasmic staining reaction. Also compare with Fig. 2a – same protocol.

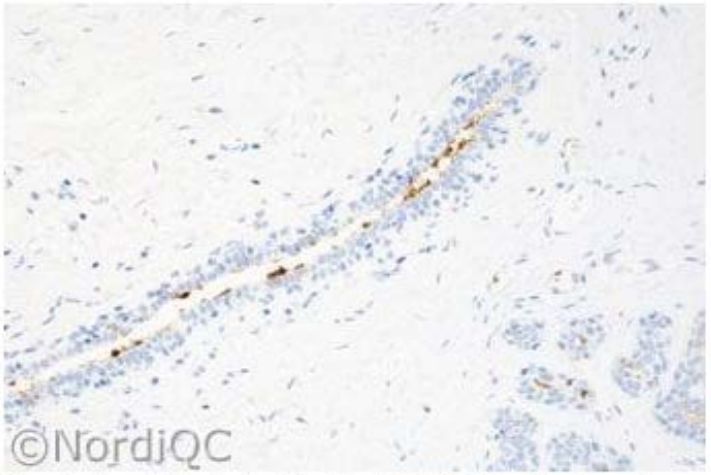



Fig. 1b. Insufficient staining for GCDFP-15 of the breast hyperplasia applying the mAb clone 23A3 as a concentrate using exactly the same protocol settings as used in Fig 1a, except for a 20 fold dilution of the primary antibody. The proportion and the intensity of the cells demonstrated are significantly reduced compared to the result in Fig. 1a. Also compare with Fig. 2b – same protocol.

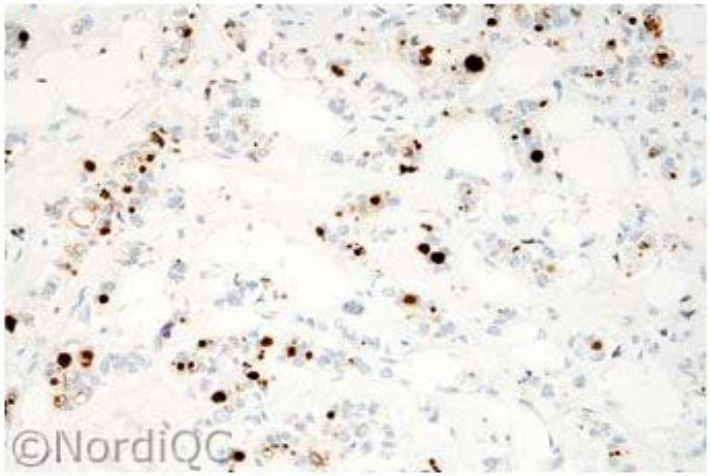



Fig. 2a. Optimal staining for GCDFP-15 of the breast carcinoma no. 5 using same protocol as in Fig. 1a. The majority of the neoplastic cells show a moderate to strong dot-like cytoplasmic staining reaction.

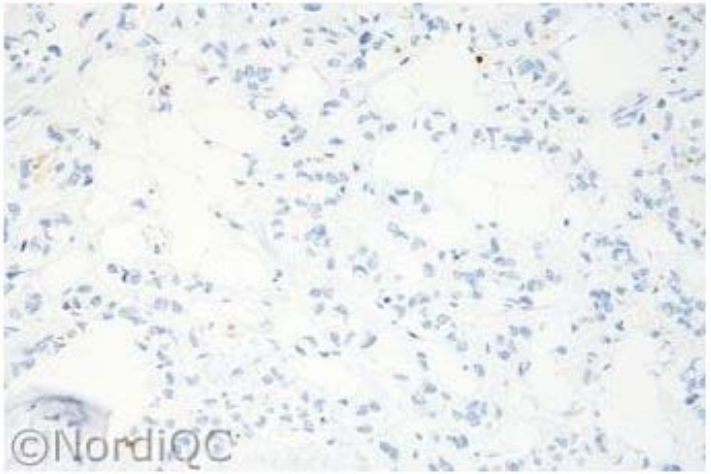


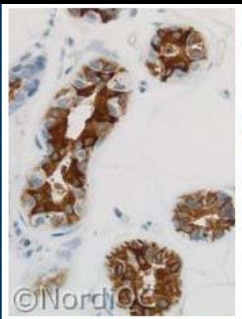

Fig. 2b. Insufficient staining GCDFP-15 of the breast carcinoma no. 5 using same protocol as in Fig. 1b. - same field as in Fig. 2a. Only scattered neoplastic cells show a faint dot-like reaction.

# IHC – Protocols and controls for Breast tumours

**Table 1. Abs and scores for mammaglobin, run 25**

| Concentrated Abs | N | Vendor | Optimal | Good | Borderl. | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **304-1A5** | 14<br>4 | Dako<br>BioLogo | 7 | 8 | 2 | 1 | 83 % | 100 % |
| pAb **53625** | 1 | AnaSpec. Inc. | 0 | 0 | 0 | 1 | - | - |
| **Ready-To-Use Abs** | | | | | | | | |
| mAb clone **304-1A5** | 2 | Dako, IR074 | 2 | 0 | 0 | 0 | - | - |
| rmAb clone **31A5** | 2 | Ventana, 760-4623 | 2 | 0 | 0 | 0 | - | - |
| **Total** | 23 | | 11 | 8 | 2 | 2 | - | - |
| **Proportion** | | | 48 % | 35 % | 9 % | 9 % | 83 % | 100 % |

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



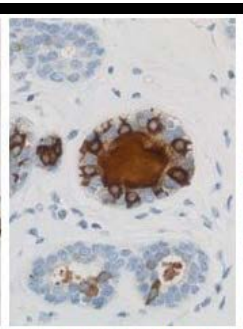
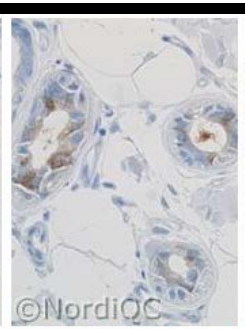



Fig. 1a. Optimal staining for mammaglobin using the mAb clone 304-1A5 optimally calibrated and with HIER.
Left: Skin: The majority of the epithelial cells of the eccrine sweat glands show a distinct cytoplasmic reaction.
Right: Breast: The apocrine metaplastic cells and few ductal epithelial cells show a distinct cytoplasmic reaction.
Also compare with Figs. 2a & 3a – same protocol.



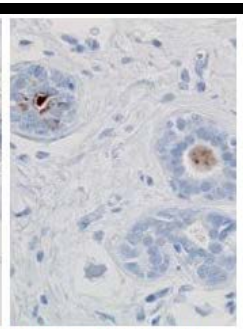



Fig. 1b. Insufficient staining for mammaglobin using the mAb clone 304-1A5 too diluted.
Left: Skin: Only scattered epithelial cells of the eccrine sweat glands show a weak cytoplasmic reaction.
Right: Breast: The epithelial cells are virtually negative and only extracellular mucus is demonstrated. Also compare with Figs. 2b & 3b – same protocol.

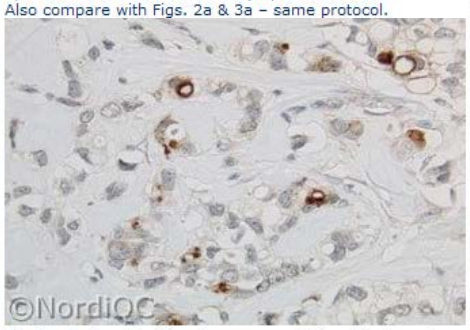



Fig. 3a. Optimal staining for mammaglobin of the breast carcinoma no. 5 using same protocol as in Fig. 1a & 2a. The majority of the neoplastic cells show at least a weak cytoplasmic reaction and focally a dot-like staining.

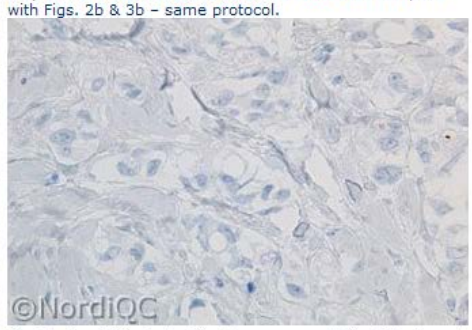



Fig. 3b. Insufficient staining for mammaglobin of the breast carcinoma no. 5 using same protocol as in Fig. 1b & 2b. None or only a dubious reaction is seen in the neoplastic cells.

Ins.:

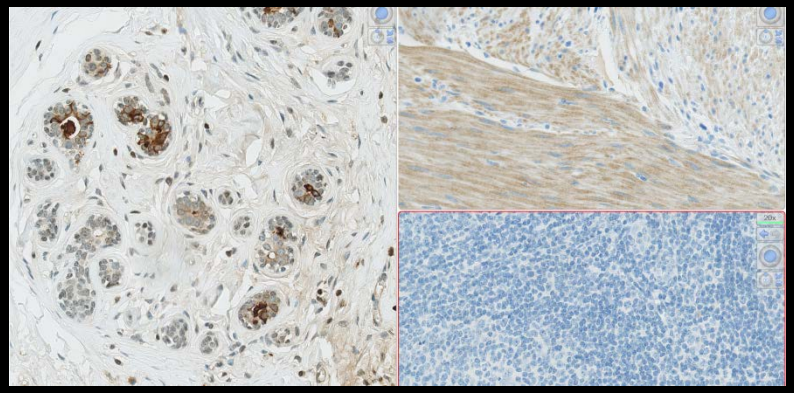
Too low/high conc.

HIER and calibration mandatory for optimal results

# IHC – Protocols and controls for Breast tumours

## Breast panel: GCDFP15 & Mammaglobin
## Basic protocol settings for an optimal staining result (NQC)

| | Retrieval | Titre | Detection | RTU | Detection |
|---|---|---|---|---|---|
| mAb 23A3 | HIER High | 1:10 - 75 | 3-step | Dako | 2- & 3-step |
| mAb D6 | HIER High | 1:4 - 100 | 3-step | - | - |
| rmAb EP1582Y | HIER High | 1:500-1.000 | 3-step | Ventana | 2- & 3-step |
| mAb 304-105 | HIER High | 1:50 - 400 | 2- & 3-step | Dako | 2-step |
| mAb 31A5 | HIER High | - | - | Ventana | 2-step |

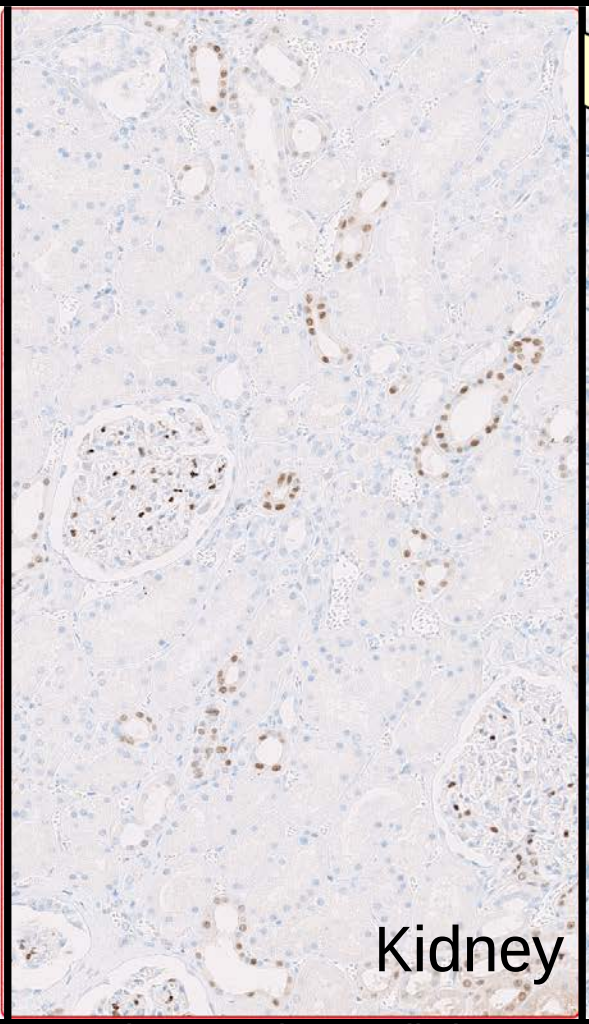


rmAb clone EP1582Y can show positive staining reaction in smooth muscle cells

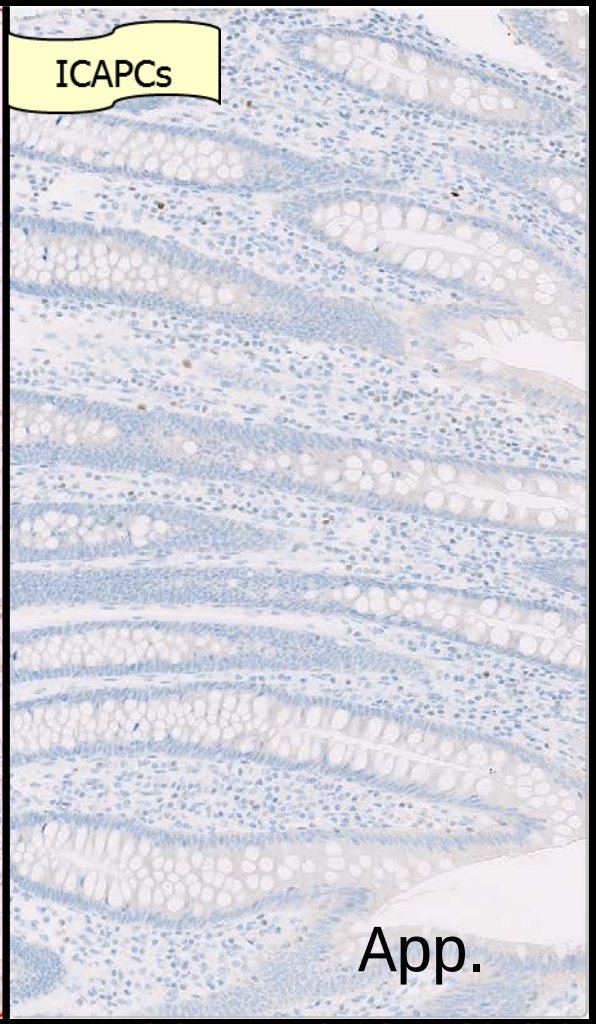
# IHC – Protocols and controls for Breast tumours

## GATA3 reaction pattern


An at least weak nuclear staining reaction of the majority of T-cells in the T-zones in the tonsil.




An at least moderate, distinct nuclear staining reaction of virtually all epithelial cells in collecting ducts and podocytes in glomeruli in the kidney.




No staining reaction in epithelial cells should be seen.

# IHC – Protocols and controls for Breast tumours

Table 1. **Antibodies and assessment marks for GATA3, run 44**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **L50-823** | 27<br>48<br>3<br>1 | Biocare<br>Cell Marque<br>Immunologic<br>Zeta | 23 | 31 | 22 | 3 | 68% | 69% |
| mAb clone **HG3-31** | 6 | Santa Cruz | 0 | 0 | 0 | 6 | 0% | - |
| Polyclonal | 1 | Acris | 0 | 0 | 0 | 1 | - | - |
| Ready-To-Use antibodies | | | | | | | | |
| mAb clone **L50-823 760-4897** | 20 | Ventana/Cell Marque | 13 | 7 | 0 | 0 | 100% | 100% |
| mAb clone **L50-823 MAD000632-QD** | 3 | Master Diagnostica | 1 | 1 | 1 | 0 | - | - |
| mAb clone **L50-823 390M-18** | 9 | Cell Marque | 2 | 7 | 0 | 0 | 100% | 100% |
| mAb clone **L50-823 PM405AA** | 3 | BioCare | 1 | 2 | 0 | 0 | - | - |
| mAb clone **L50-823 MAB-0695** | 1 | Maixin | 0 | 1 | 0 | 0 | - | - |
| mAb clone **L50-823 ZM-0498** | 1 | Zeta | 0 | 0 | 1 | 0 | - | - |
| mAb clone **HG3-31** | 1 | Santa Cruz | 0 | 0 | 0 | 1 | - | - |
| Total | 124 | | 40 | 49 | 24 | 11 | - | |
| Proportion | | | 30% | 41% | 20% | 9% | 72% | |

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

Ins.:

Too low conc.

Less succesful Ab

RTU > Conc.

(difficult to calibrate… what is best control…)

# IHC – Protocols and controls for Breast tumours

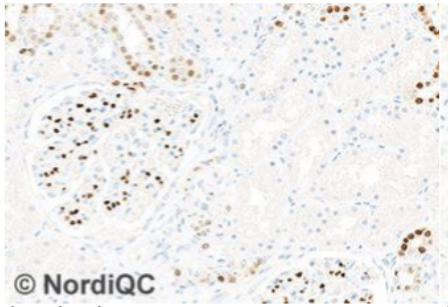



Fig. 1a (x200)
Optimal GATA3 staining of the kidney using the mAb clone L50-823 as a concentrate, optimally calibrated with HIER in an alkaline buffer (CC1 pH 8.5, Ventana) and a 3-step multimer based detection system (UltraView, Ventana). A moderate to strong nuclear staining reaction is seen in the majority of epithelial cells lining the collecting ducts and in podocytes in glomeruli. Same protocol used in Figs. 1a - 4a.

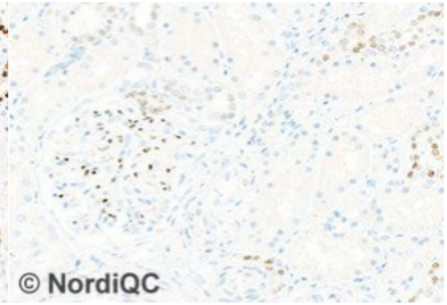



Fig. 1b (x200)
Insufficient GATA3 staining of the kidney using the mAb clone L50-823. The protocol provided an overall reduced sensitivity primarily caused by a too low concentration of the primary Ab. The intensity and proportion of cells demonstrated is reduced compared to the level expected - same field as in Fig. 1a. Also compare with Figs. 2b - 4b, same protocol.

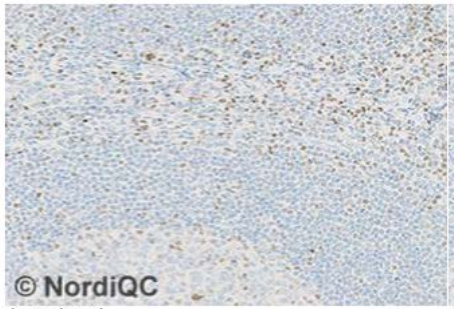



Fig. 2a (x200)
Optimal GATA3 staining of the tonsil using same protocol as in Fig. 1a. T-cells primarily located in the T-zones but also within the germinal center show a weak to moderate, distinct nuclear staining reaction. No background staining

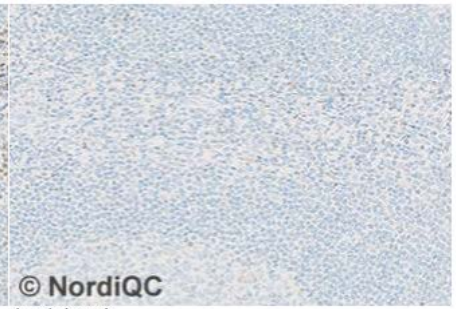



Fig. 2b (x200)
Insufficient GATA3 staining of the tonsil using same protocol as in Fig. 1b – same field as in Fig. 2a. Virtually no staining reaction of T-cells is seen. Also compare with Figs. 3b and 4b, same protocol.

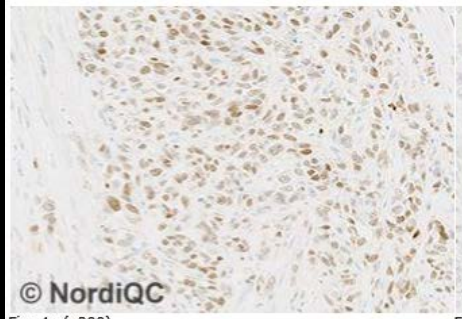



Fig. 4a (x200)
Optimal GATA3 staining of the urothelial carcinoma using same protocol as in Figs. 1a - 3a. The majority of neoplastic cells show a distinct, weak to moderate nuclear staining reaction.

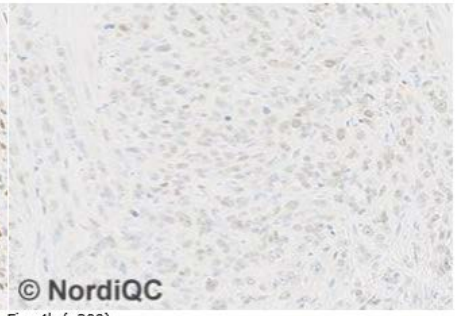



Fig. 4b (x200)
Insufficient GATA3 staining of the urothelial carcinoma using same protocol as in Figs. 1b – 3b – same field as in Fig. 4a. Only a weak and equivocal nuclear staining reaction in dispersed neoplastic cells is observed.

Choice of right tissue controls

Calibrate for the purpose of the assay

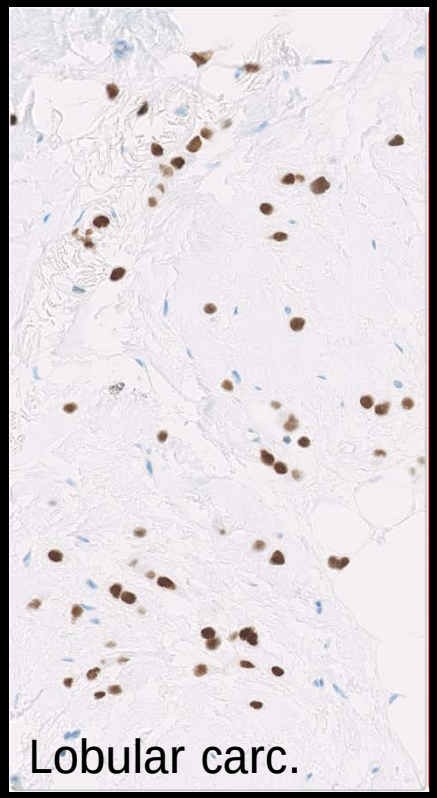
GATA3 as tumour marker

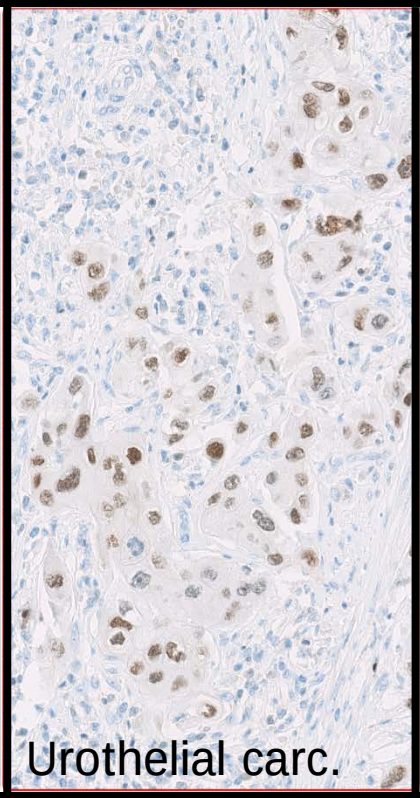
CUP

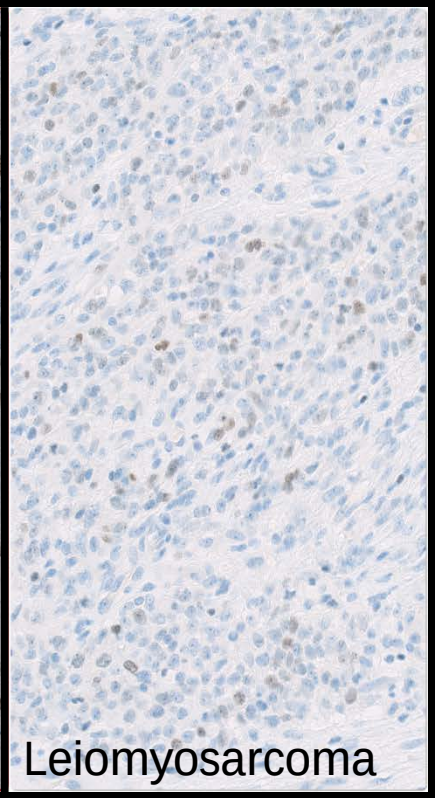
# IHC – Protocols and controls for Breast tumours

## Breast panel: GATA3
## Basic protocol settings for an optimal staining result (NQC)

| | Retrieval | Titre | Detection | RTU | Detection |
|---|---|---|---|---|---|
| mAb L50-8023 | HIER High | 1:70-500 | 2- & 3-step | Ventana | 2- & 3-step |


Lobular carc.


Urothelial carc.


Leiomyosarcoma

GATA3:

Highly sensitive for Breast carcinomas (& Urothelial carcinoma). But also seen in other neoplasias

# IHC - Protocols and controls for Breast tumours

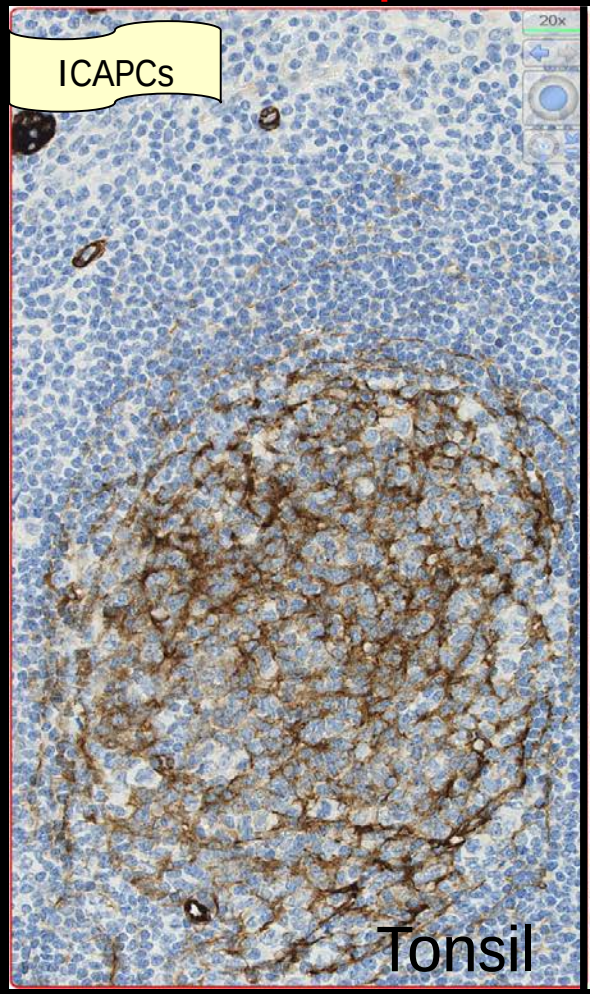
Breast panel:

- GCDFP-15
- Mammaglobin
- Gata 3
- Smooth MHCM
- ASMA
- (p63)
- E-cadherin
- p120
- ER
- PR
- HER-2

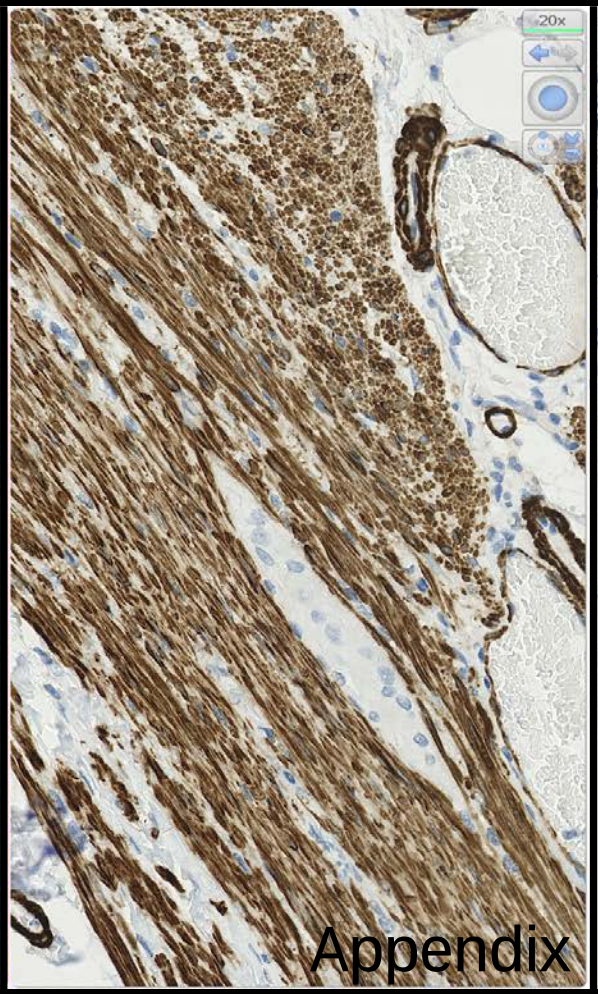
- Is it primary breast ?
- Is it invasive ?
- Is it lobular or ductal ?
- Which teraphy ?

# IHC – Protocols and controls for Breast tumours

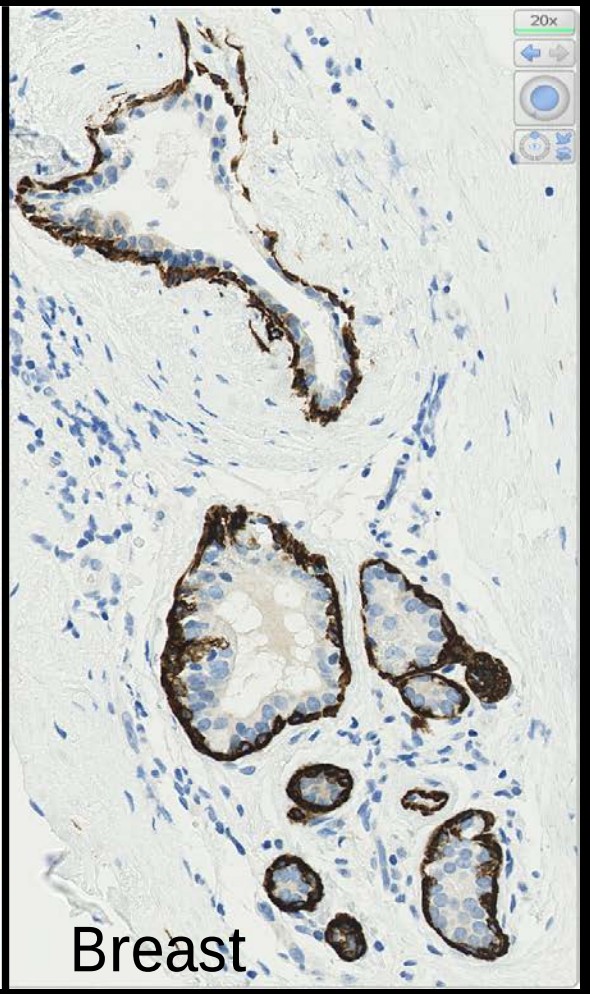
## SMH reaction pattern




A weak to moderate, distinct cytoplasmic staining reaction in the follicular dendritic network of germinal centres. No staining should be seen in epithelial cells.




A moderate to strong, distinct cytoplasmic staining reaction of all smooth muscle cells in muscularis propria and vessels. No staining in epithelium.




A moderate to strong cytoplasmic staining reaction must be seen in myoepithelium. No staining reaction should be seen in luminal epithelial cells.

# IHC – Protocols and controls for Breast tumours

Table 1. **Antibodies and assessment marks for SMH, run 50**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **SMMS-1** | 48<br>5<br>2<br>1<br>1 | Agilent/Dako<br>Cell Marque<br>Thermo/Neomarkers<br>Biocare<br>Zeta Corporation | 24 | 19 | 11 | 3 | 75% | 76% |
| rmAb clone **EP166** | 1 | Cell Marque | 0 | 1 | 0 | 0 | - | - |
| Ready-To-Use antibodies | | | | | | | | |
| mAb clone **S131 PA0493** | 2 | Leica/Novocastra | 2 | 0 | 0 | 0 | - | - |
| mAb clone **SMMS-1 760-2704** | 26 | Roche/Ventana | 13 | 9 | 4 | 0 | 85% | 85% |
| mAb clone **SMMS-1 IR066/IS066** | 17 | Agilent/Dako | 6 | 5 | 3 | 3 | 65% | 100% |
| mAb clones **SMMS-1 pm420aa** | 1 | Biocare | 0 | 1 | 0 | 0 | - | - |
| mAb clone **SMMS-1 PDM175** | 1 | Diagnostic Biosystems | 0 | 1 | 0 | 0 | - | - |
| mAb clone **SMMS-1 MAB-0121** | 1 | Maixin | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **EP166 MAD-000718QB** | 2 | Master Diagnostica | 0 | 2 | 0 | 0 | - | - |
| rmAb clone **EP166 298R-18** | 1 | Cell Marque | 0 | 1 | 0 | 0 | - | - |
| Total | 109 | | 46 | 39 | 18 | 6 | - | |
| Proportion | | | 42% | 36% | 17% | 5% | 67% | |

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

mAb clone SMMS-1

HIER in alk. pH

3-step detection

Insufficient:

LDT;
HIER low pH
Too low conc.
2-step detection

RTU;
Dako – off-label
VMS – on-label

# IHC – Protocols and controls for Breast tumours

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

| RTU systems | Recommended protocol settings* | | Laboratory modified protocol settings** | |
|---|---|---|---|---|
| | Sufficient | Optimal | Sufficient | Optimal |
| Dako AS mAb SMMS-1 **IR/IS066** | 100% (9/9) | 67% (6/9) | 2/3 | 0/3 |
| Leica BOND mAb S131 **PA0493** | 2/2 | 2/2 | - | - |
| VMS Ultra/XT mAb SMMS-1 **760-2704** | 1/3 | 0/3 | 91% (21/23) | 57% (13/23) |

* 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.

The focus of RTU systems……

Dako and Leica; Plug-and-Play

VMS; Play-and-Plug…..

# IHC – Protocols and controls for Breast tumours

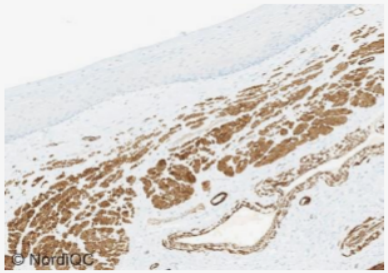



Fig. 1a
Optimal staining for SMH of the esophagus using the mAb clone SMMS-1 within a laboratory developed assay optimally calibrated, using HIER in an alkaline buffer and a 3-step multimer based detection system.
Virtually all smooth muscle cells in vessels and lamina muscularis mucosae show a moderate to strong cytoplasmic staining reaction.
Also compare with Figs. 2a – 5a, same protocol.

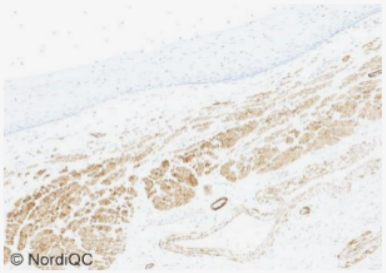



Fig. 1b
SMH staining of the esophagus using an insufficient protocol based on the mAb clone SMMS-1 within a laboratory developed assay providing a too low analytical sensitivity. A too low titre of the primary Antibody and the use of 2-step multimer system, UltraView Ventana, were the main causes for the insufficient result, which especially is seen in Figs. 2b - 5b – same protocol.
In esophagus – same field as Fig. 1a, a moderate staining reaction is seen in virtually all smooth muscle cells.
As described in the assessment report, smooth muscle cells cannot be recommended as positive tissue control for SMH due to the high level of SMH expression.

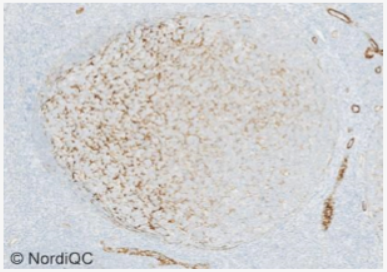



Fig. 2a
Optimal SMH staining of the tonsil using same protocol as in Fig. 1a.
A weak to moderate staining reaction is seen in the follicular dendritic network in the germinal center.
A high signal-to-noise ratio is observed.

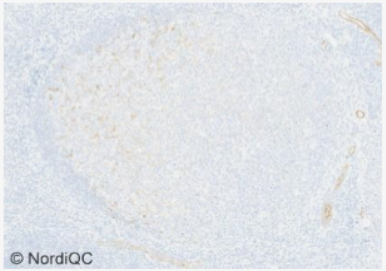



Fig. 2a
Insufficient SMH staining of the tonsil using same protocol as in Fig. 1b.
The follicular dendritic network in the germinal center is virtually negative and only vascular smooth muscle cells are demonstrated.

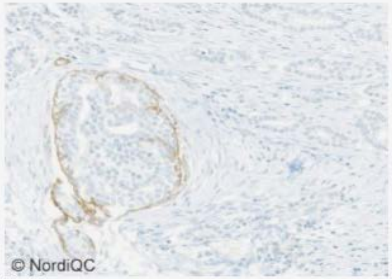



Fig. 5a
Optimal SMH staining of the breast ductal carcinoma using same protocol as in Figs. 1a - 4a.
A moderate, distinct and continuous staining reaction is seen in the myoepithelial cells lining the breast DCIS component, while the invasive components show no staining.

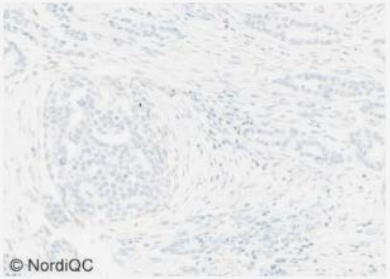



Fig. 5b
Insufficient SMH staining of the breast ductal carcinoma using same protocol as in Figs. 1a - 4a.
No staining is seen in neither the DCIS nor the invasive components and thus not possible to differentiate these two entities.

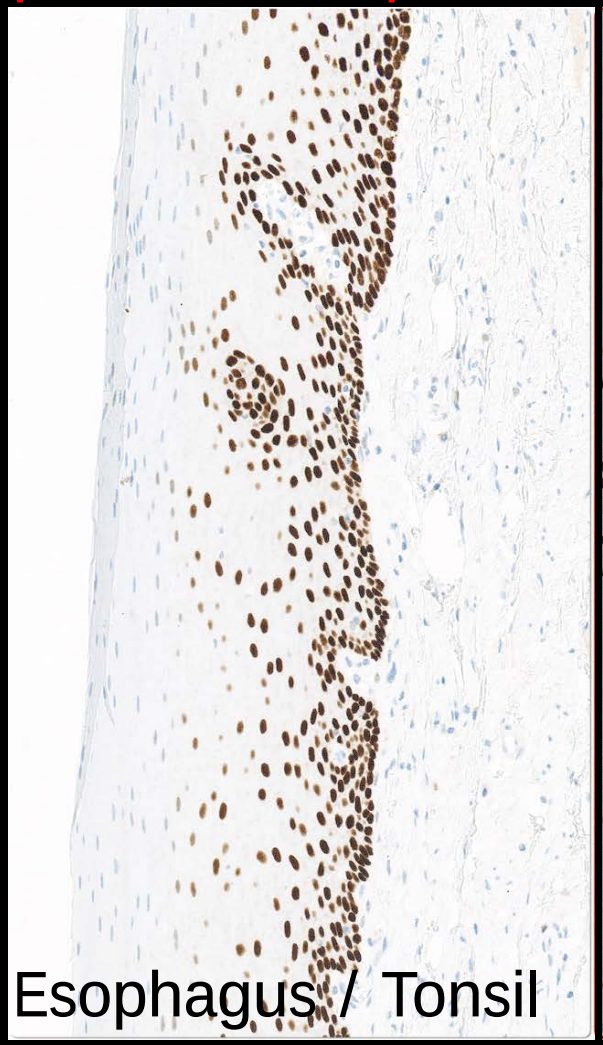
Choice of right tissue controls

Calibrate for the purpose of the assay

SMH as marker to differentiate DCIS vs carcinoma

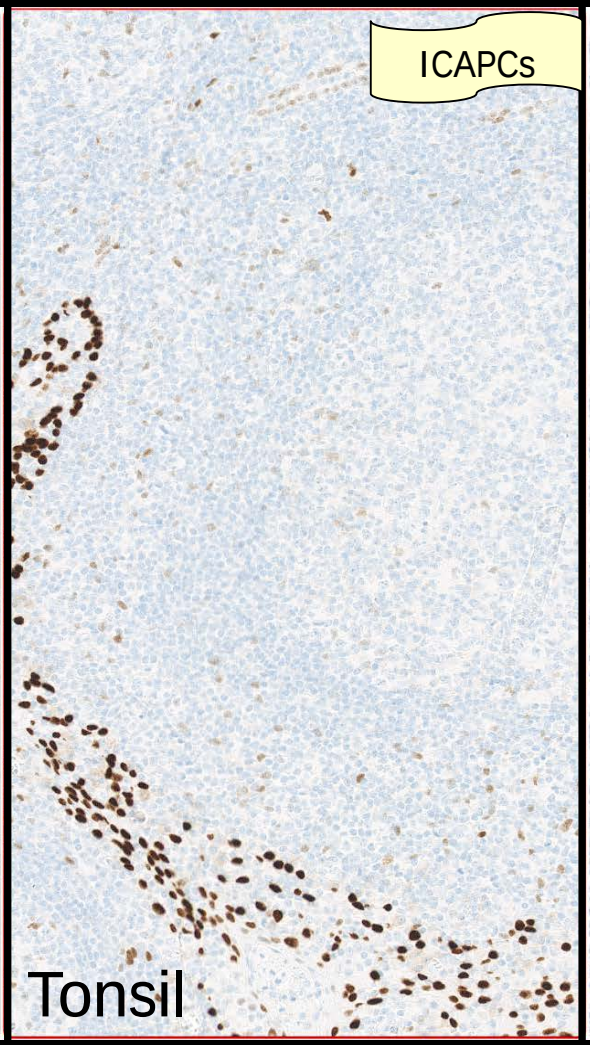
# IHC – Protocols and controls for Breast tumours

## p63 reaction pattern



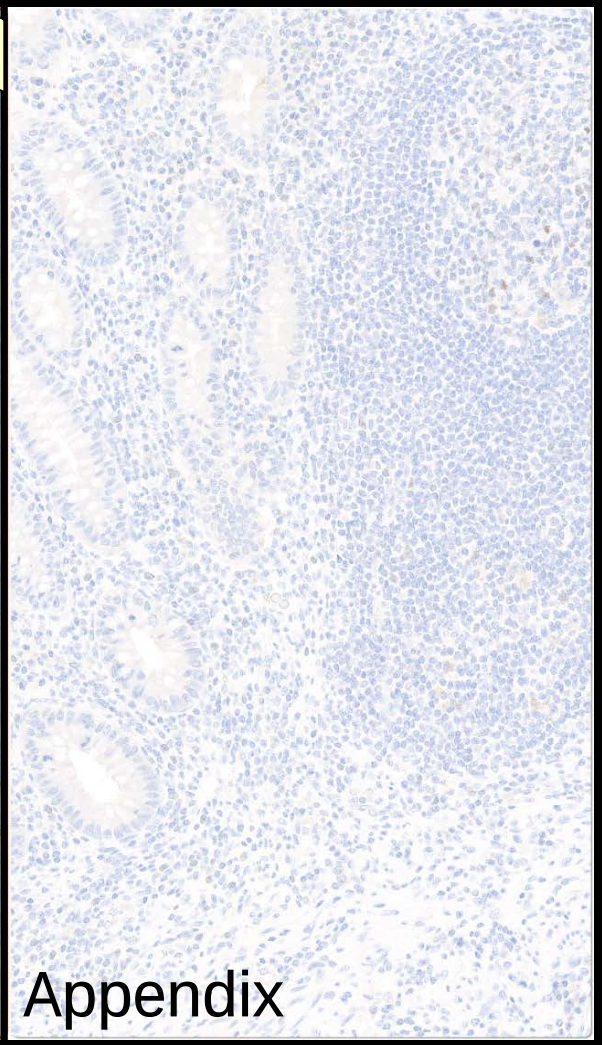
Esophagus / Tonsil

A moderate to strong distinct nuclear staining reaction in the vast majority of squamous epithelial cells.




Tonsil

An at least weak but distinct nuclear staining reaction of scattered lymphocytes and endothelial cells.



Appendix

No staining reaction in columnar epithelial cells – scattered lymphocytes can be expected to be demonstrared.

# IHC – Protocols and controls for Breast tumours

Table 1. **Antibodies and assessment marks for p63, run 48**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **4A4** | 26<br>4<br>3<br>3<br>2<br>2<br>1<br>1<br>1<br>1<br>1 | BioCare Medical<br>ImmunoLogic<br>Dako<br>Zeta Corporation<br>Thermo Scientific<br>Zytomed Systems<br>BioGenex<br>Diagnostic BioSystems<br>Klinipath<br>Minarini<br>Nordic Biosite<br>Santa Cruz | 13 | 20 | 11 | 2 | 72% | 76% |
| mAb clone **DAK-p63** | 47 | Dako | 20 | 21 | 6 | 0 | 87% | 91% |
| mAb clone **7JUL** | 12 | Leica/Novocastra | 0 | 1 | 3 | 8 | 8% | - |
| mAb clone **SFI-6** | 2 | DCS Immunoline | 0 | 0 | 2 | 0 | - | - |
| rmAb clone **BSR6** | 1 | Nordic Biosite | 0 | 0 | 1 | 0 | - | - |
| rmAb clone **DBR16.1** | 1 | Diagnostic Biosystems | 1 | 0 | 0 | 0 | | |
| rmAb clone **EPR5701** | 1 | Epitomics | 0 | 0 | 1 | 0 | - | - |
| Unknown Ab | 1 | Unknown | 1 | 0 | 0 | 0 | - | - |
| Ready-To-Use antibodies | | | | | | | | |
| mAb clone **4A4 790-4509** | 102 | Ventana | 59 | 36 | 5 | 2 | 93% | 95% |
| mAb clone **DAK-p63 IR662** | 46 | Dako | 21 | 23 | 2 | 0 | 96% | 94% |
| mAb clone **4A4 PM163** | 3 | BioCare | 1 | 1 | 1 | 0 | - | - |
| mAb clone **7JUL PA0103** | 5 | Leica/Novocastra | 0 | 0 | 3 | 2 | - | - |
| mAb clone **4A4 AM418** | 2 | BioGenex | 0 | 1 | 0 | 1 | - | - |
| mAb clone **4A4 ARB-56695** | 1 | Nordic Biosite | 1 | 0 | 0 | 0 | - | - |
| mAb clone **MX013 MAB-0694** | 1 | Maixin | 0 | 1 | 0 | 0 | - | - |
| mAb clone **4A4 MAD-000479QD** | 3 | Master Diagnostica SL | 3 | 0 | 0 | 0 | - | - |
| Total | 274 | | 120 | 104 | 35 | 15 | - | |
| Proportion | | | 44 % | 38 % | 13 % | 5 % | 82 % | |

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

Clone:
4A4
DAK-p63

7JUL – no-go...

HIER settings
high pH – time

Detection kit
3-step

RTU superior

# IHC – Protocols and controls for Breast tumours

p63 / RUN 41 2014

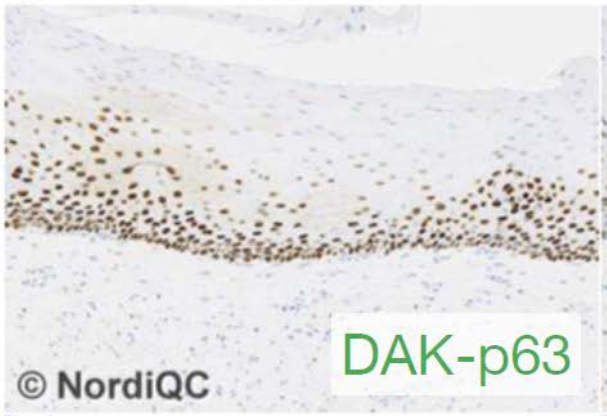



Fig. 1a
Optimal p63 staining of the esophagus using the mAb clone DAK-p63 (Dako RTU) with HIER in an alkaline buffer (TRS pH 9.0, Dako) and performed on the Dako Autostainer. A strong nuclear staining reaction is seen in the majority of the squamous epithelial cells in the esophagus. No background staining is seen. Same protocol used in Figs. 1a - 4a.

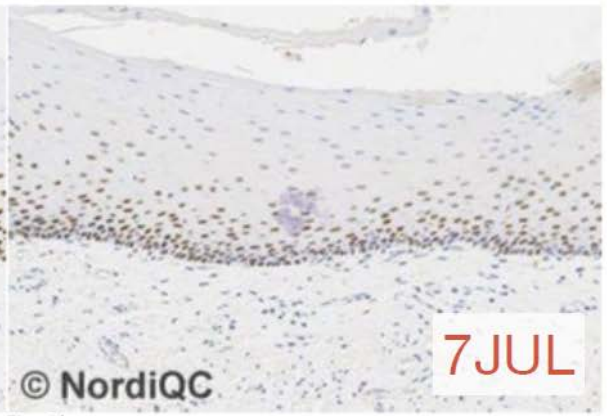



Fig. 1b
Insufficient p63 staining of the esophagus using the mAb clone 7JUL (Leica/Novocastra, 1:100) with HIER in an alkaline buffer (BERS2, Bond) and performed on the Bond III, Leica. A moderate nuclear staining reaction is seen in the majority of the squamous epithelial cells in the esophagus. Compare with Fig. 1a – same field. Also compare with Figs. 2b, 3b and 4b – same protocol.

Primary antibody with a too low sensitivity.

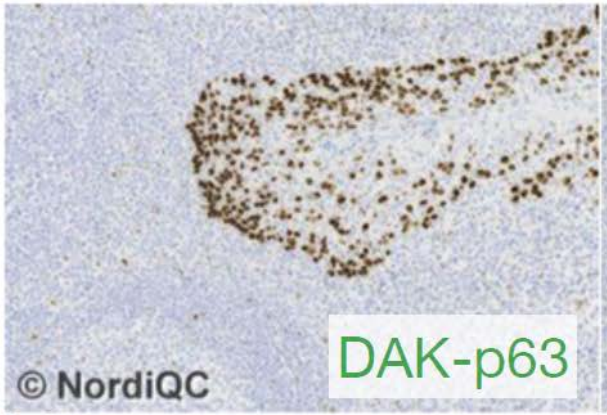



Fig. 2a
Optimal p63 staining of the tonsil using the same protocol as in Fig. 1a. A moderate to strong, distinct nuclear staining is seen in virtually all the squamous epithelial cells in the tonsil. In addition to the epithelial staining a weak but distinct nuclear reaction is present in scattered lymphocytes in the tonsil.

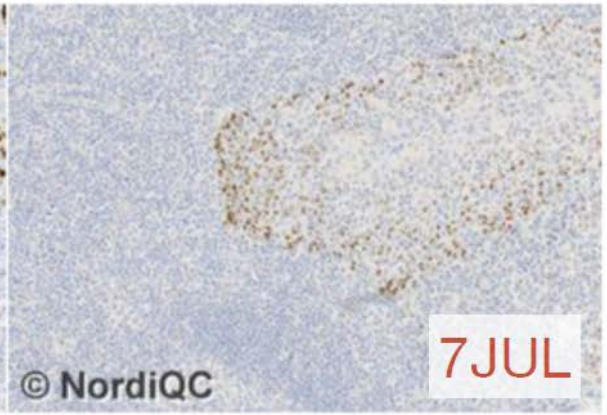



Fig. 2b
Insufficient p63 staining of the tonsil using the same protocol as in Fig. 1b. A weak to moderate, distinct nuclear staining is seen in the majority of the squamous epithelial cells in the tonsil. But in the insufficient protocol no staining is seen in lymphocytes. Compare with Fig. 2a. - same field.

# IHC – Protocols and controls for Breast tumours

p63 / RUN 41 2014

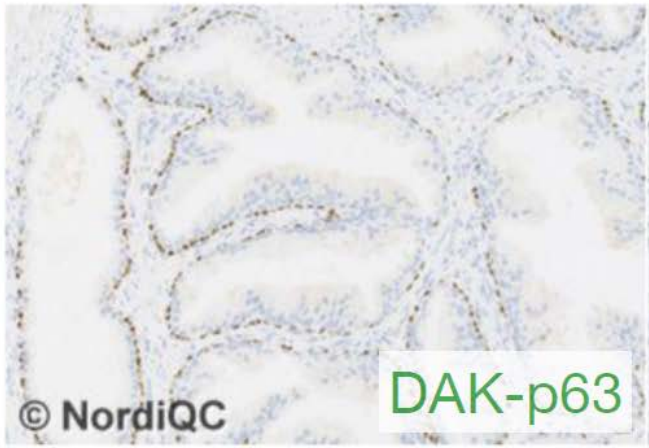



Fig. 3a
Optimal p63 staining in the prostate hyperplasia using the same protocol as in Figs. 1a & 2a. Virtually all the basal cells show a moderate to strong distinct nuclear staining reaction. No background staining is seen.

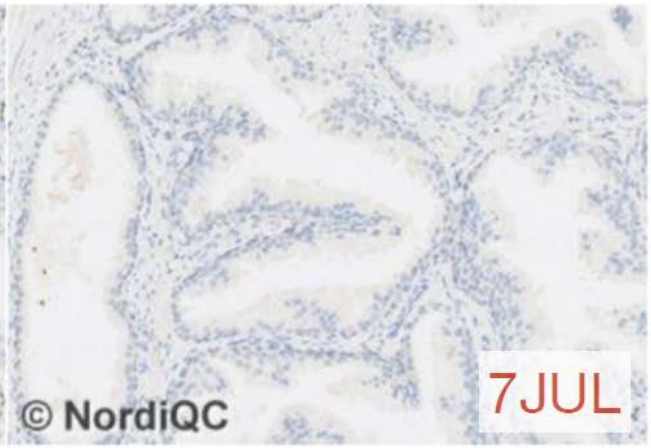



Fig. 3b
Insufficient p63 staining in the prostate hyperplasia using the same protocol as in Figs. 1b & 2b. Virtually all basal cells in the prostate hyperplasia are negative. Compare with Fig. 3a – same field.

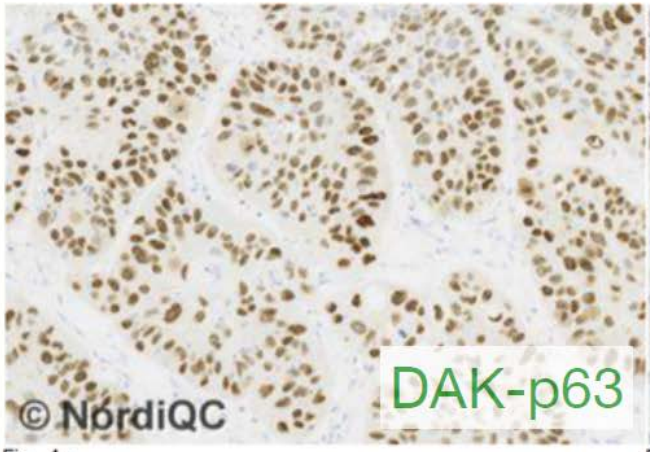



Fig. 4a
Optimal p63 staining of the lung squamous cell carcinoma using the same protocol as in Figs. 1a, 2a & 3a. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. No background staining is seen.

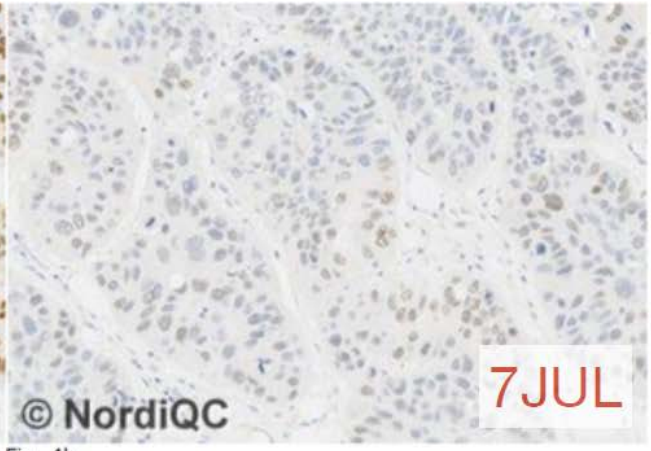



Fig. 4b
Insufficient P63 staining of the lung squamous cell carcinoma using the same protocol as in Figs. 1b, 2b & 3b. Only faint nuclear staining is seen and only in a minor fraction of the neoplastic cells. Compare with Fig. 4a – same field.

Primary antibody with a too low sensitivity.

# IHC - Protocols and controls for Breast tumours

p63 / RUN 41 2014

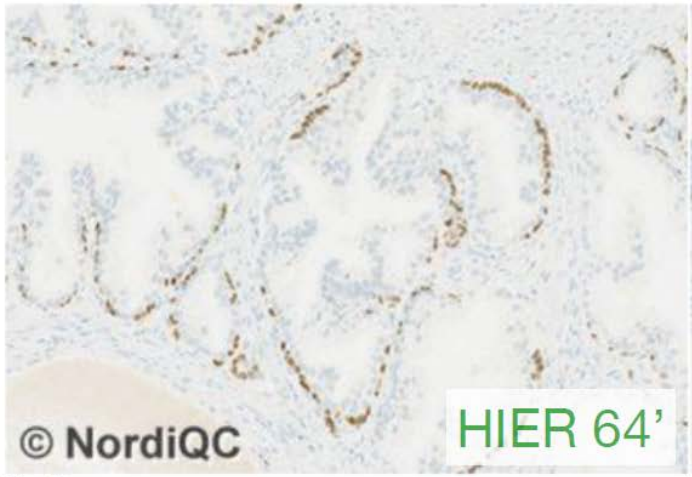



Fig. 5a
Optimal p63 staining of the prostate hyperplasia using the mAb clone 4A4 (Ventana, RTU) with HIER in CC1 (Ventana) for 64 min. Moderate to strong nuclear reaction is seen in virtually all basal cells. Efficient HIER pretreatment is essential to optimal P63 staining. Compare with Fig. 5b.

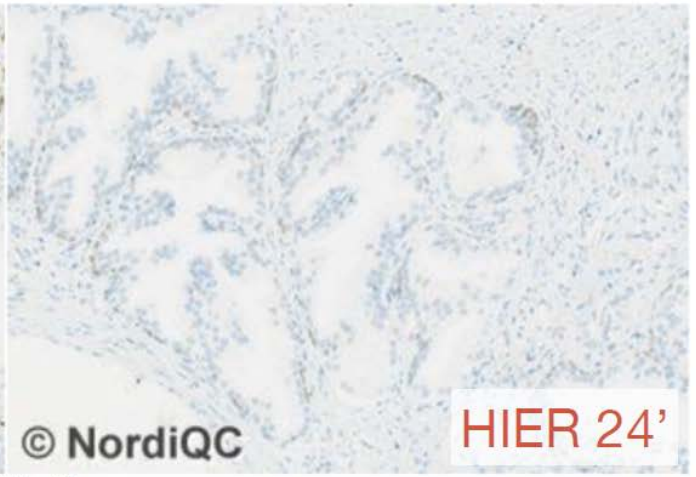
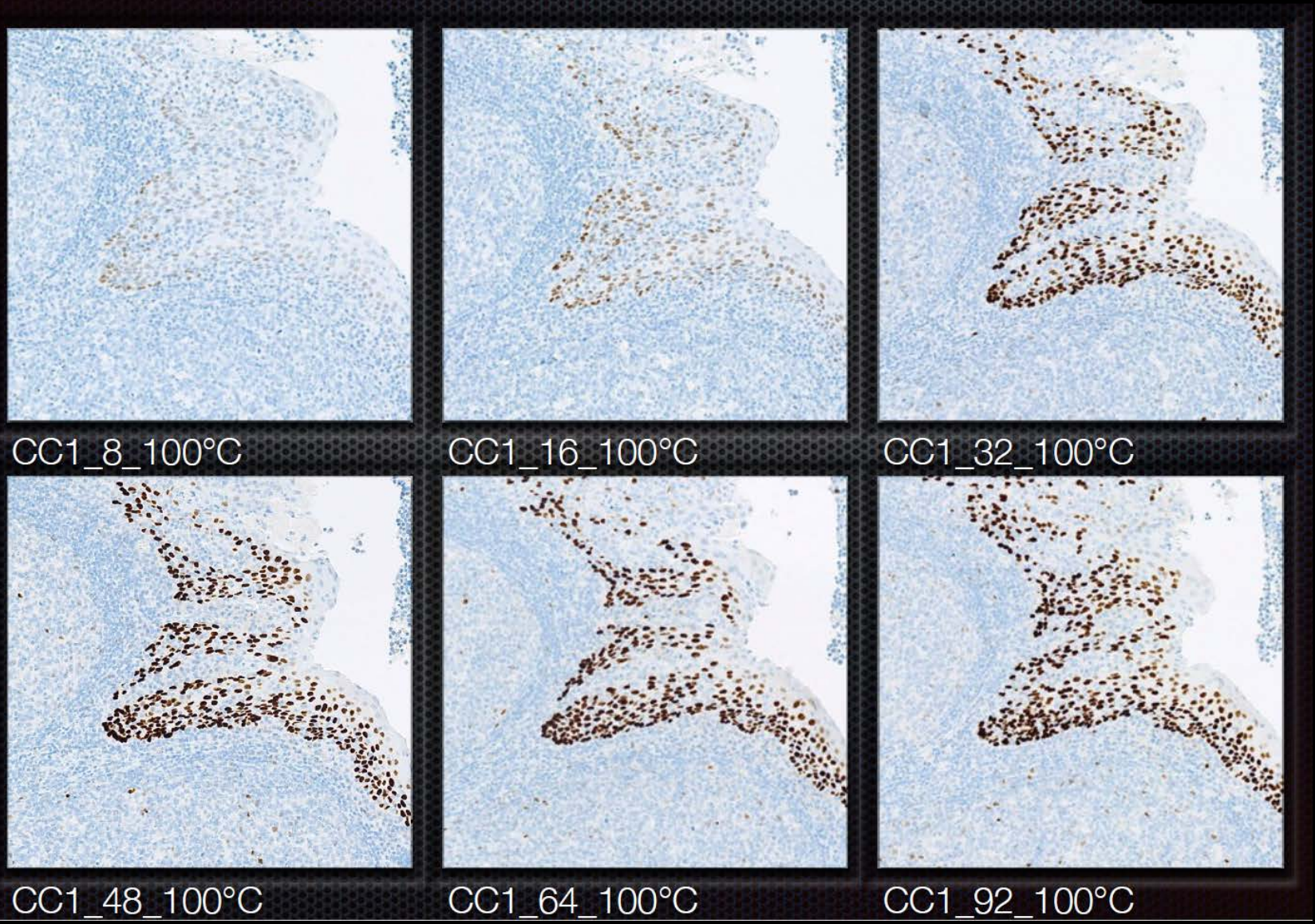



Fig. 5b
Insufficient p63 staining in the prostate hyperplasia using the mAb clone 4A4 (Ventana, RTU) in the same protocol as in Fig. 5a, except for the reduction in HIER pretreatment to 24 min compared to the 64 min i Fig 5a. Consequenctly a dramatic reduction in staining intensity is seen making the identification of the basal cell difficult. Compare with Fig. 5a – same field.

Insufficient HIER.

# IHC - Protocols and controls for Breast tumours

## p63, 4A4 - OptiView (3-step) - Various HIER time



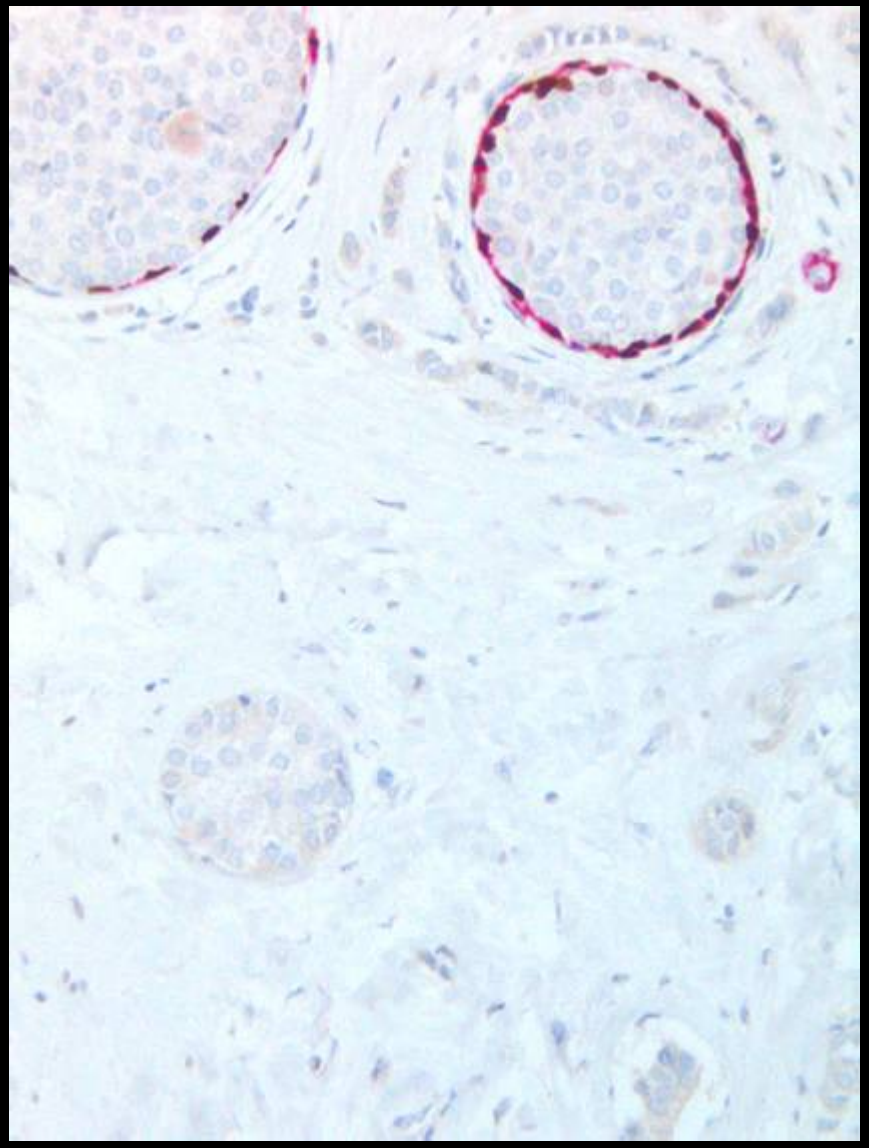
# IHC – Protocols and controls for Breast tumours


p63 + SMH;
single colour or dual colour

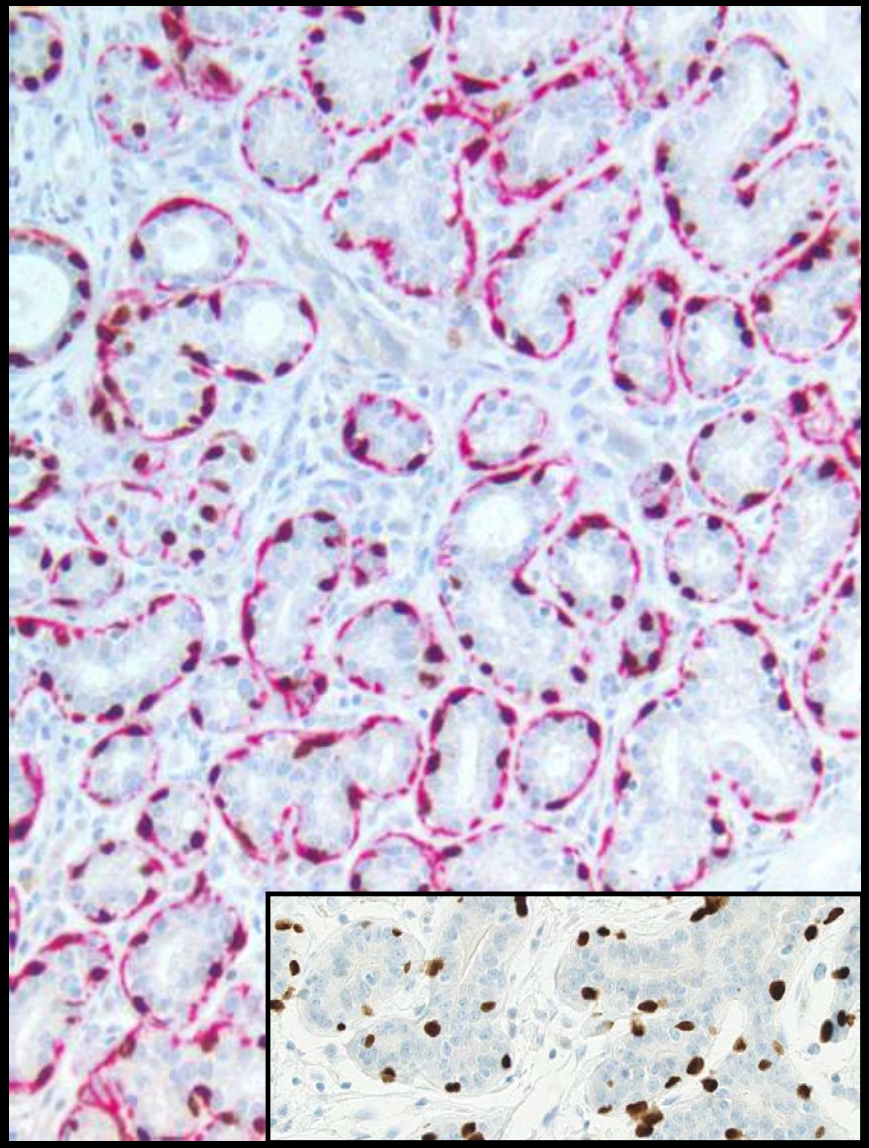
(simultanously or sequentially)

.....

# IHC – Protocols and controls for Breast tumours



p63 / SMH



p63 / SMH

# IHC – Protocols and controls for Breast tumours

Breast panel: SMH

Basic protocol settings for an optimal staining result (NQC)

| | Retrieval | Titre | Detection | RTU | Detection |
|---|---|---|---|---|---|
| mAb SMMS1 | HIER High | 1:200-1.500 | 3-step | Ventana<br>Dako (AS) | 3-step<br>2-step |
| mAb S131 | HIER High | - | - | Leica | 3-step |

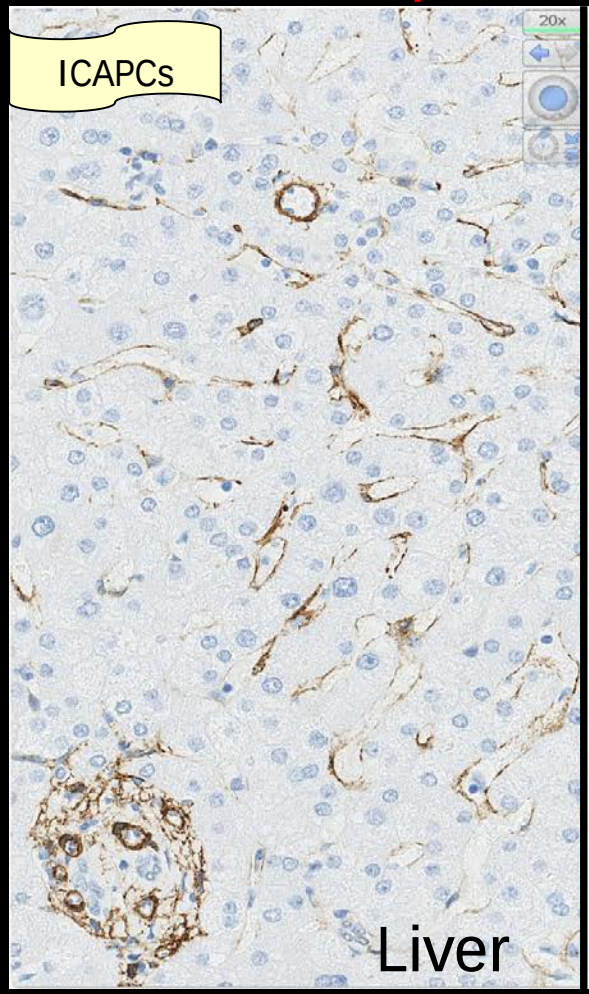
p63

Basic protocol settings for an optimal staining result (NQC)

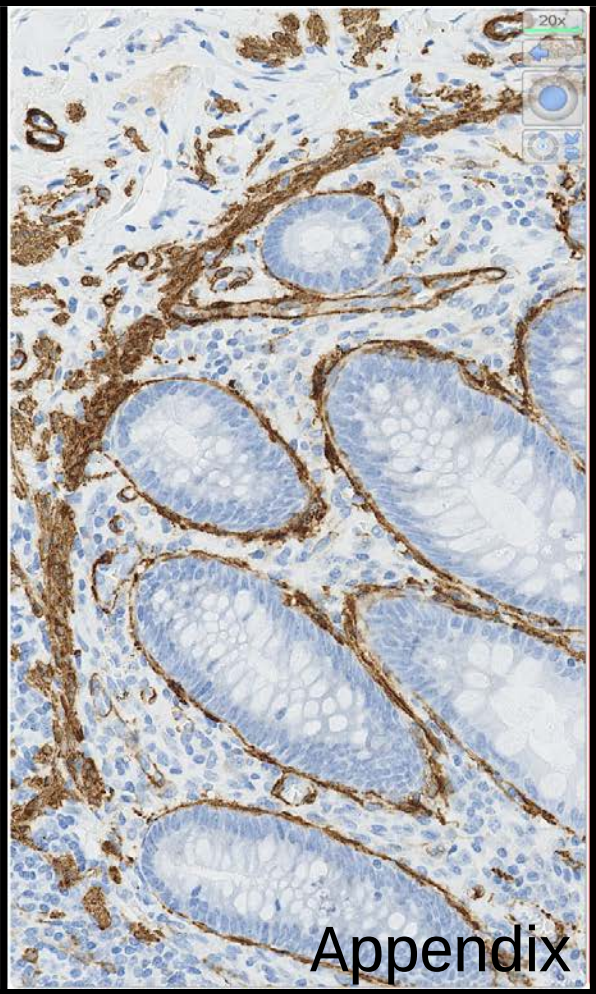
| | Retrieval | Titre | Detection | RTU | Detection |
|---|---|---|---|---|---|
| mAb 4A4 | HIER High | 1:50-600 | 3-step | Ventana | 3-step |
| mAb DAK-p63 | HIER High | 1:50-300 | 3-step | Dako | 3-step |

# IHC – Protocols and controls for Breast tumours

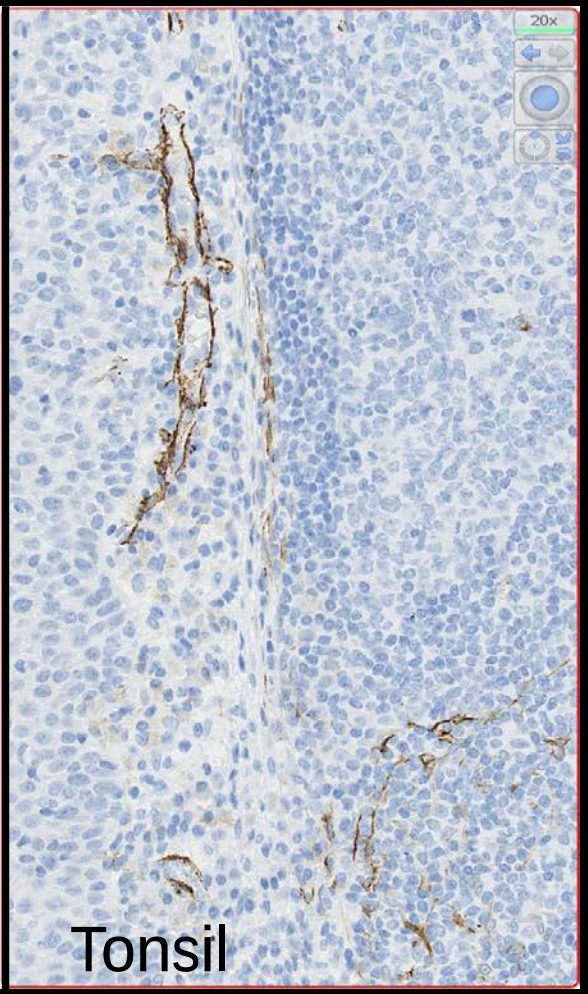
## ASMA reaction pattern




A moderate to strong, distinct cytoplasmic staining of the majority of the perisinusoidal cells in the liver. No staining should be seen in hepatocytes.




A strong, distinct cytoplasmic staining of all the smooth muscle cells in the muscularis propria, lamina muscularis mucosae and myofibroblasts lining the crypts.




A moderate to strong cytoplasmic staining must be seen in smooth muscle cells – e.g. vessels
No staining should be seen in lymphocytes and epithelial cells.

# IHC – Protocols and controls for Breast tumours

Table 1. **Antibodies and assessment marks for ASMA, run 44**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **1A4** | 98<br>6<br>5<br>1<br>1<br>1<br>1<br>1<br>1 | Dako<br>Thermo/NeoMarkers<br>Sigma Aldrich<br>AbD Serotec<br>Biocare<br>BioGenex<br>Genemed<br>Immunologic<br>Spring Bioscience<br>Zytomed | 34 | 49 | 23 | 10 | 72% | 85% |
| mAb clone **asm-1** | 10 | Leica/Novocastra | 2 | 4 | 4 | 0 | 60% | 100% |
| mAb clone **BS66** | 1 | Nordic Biosite | 0 | 1 | 0 | 0 | - | - |
| rmAb clone **E184** | 1 | Biocare | 0 | 0 | 0 | 1 | - | - |
| rmAb clone **EP188** | 1 | Epitomics | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **SP171** | 1 | Spring Bioscience | 0 | 0 | 1 | 0 | - | - |
| Unknown | 2 | Unknown | 1 | 0 | 1 | 0 | - | - |
| Ready-To-Use antibodies | | | | | | | | |
| mAb clone **1A4 IR/IS611** | 44 | Dako | 23 | 13 | 7 | 1 | 82% | 91% |
| mAb clone **1A4 760-2833** | 44 | Ventana/Cell Marque | 0 | 6 | 29 | 9 | 14% | - |
| mAb clone **1A4 202M-9x** | 3 | Cell Marque | 0 | 0 | 2 | 1 | - | - |
| mAb clone **1A4 MAD-001195QD** | 3 | Master Diagnostica | 0 | 0 | 3 | 0 | - | - |
| mAb **1A4 PM001** | 1 | Biocare | 0 | 0 | 1 | 0 | - | - |
| mAb clone **1A4 AM128-5M** | 1 | BioGenex | 0 | 1 | 0 | 0 | - | - |
| mAb clone **1A4 Kit-0006** | 1 | Maixin | 0 | 0 | 1 | 0 | - | - |
| mAb clone **asm-1 PA0943** | 5 | Leica/Novocastra | 1 | 3 | 1 | 0 | 80% | 100% |
| Total | 234 | | 62 | 77 | 73 | 22 | - | |
| Proportion | | | 27% | 33% | 31% | 9% | 60% | |

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

Low pass rate

1A4
Platform dependant..

# IHC – Protocols and controls for Breast tumours

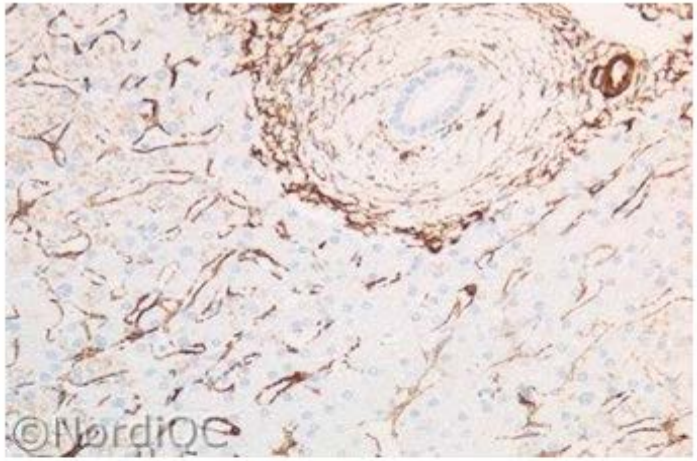



Fig. 1a. Optimal ASMA staining of the liver using the mAb clone 1A4 with HIER. The smooth muscle cells in the portal vessels as well as the perisinusoidal smooth muscle cells show a distinct staining. The liver cells are negative (a weak granular staining is due to lipofuscin).

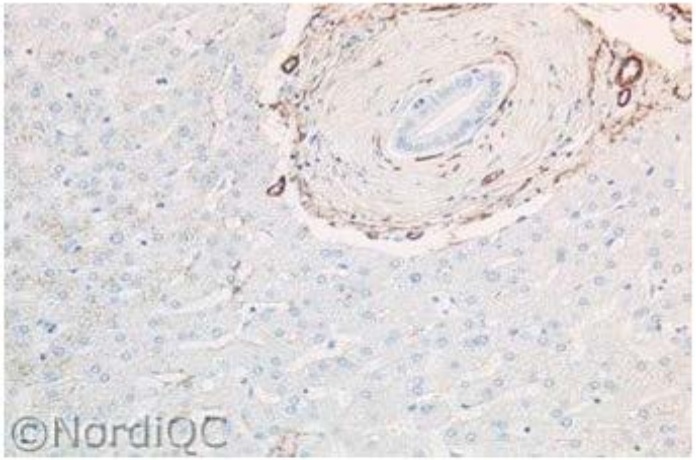



Fig. 1b. Insufficient ASMA staining of the liver using the mAb clone 1A4 in a protocol omitting HIER – same field as in Fig. 1a. The smooth muscle cells in the portal vessels are demonstrated, while the perisinusoidal smooth muscle cells are virtually negative. Also compare with Figs. 2b & 3 b same protocol.

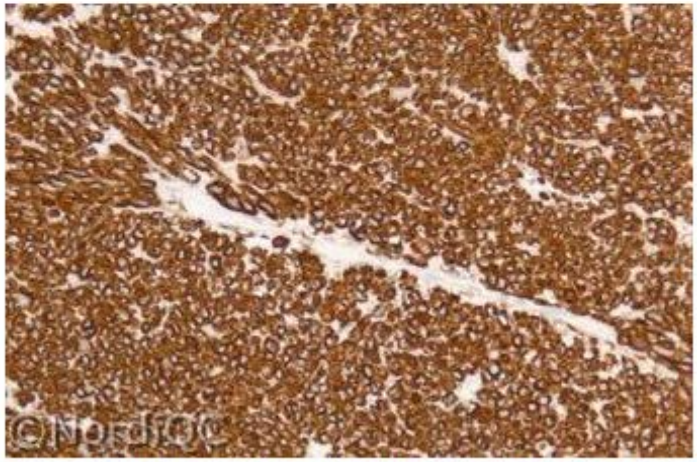



Fig. 2a. Optimal ASMA staining of the leiomyosarcoma tissue no. 3 in the multitissue block using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct reaction with no background reaction.

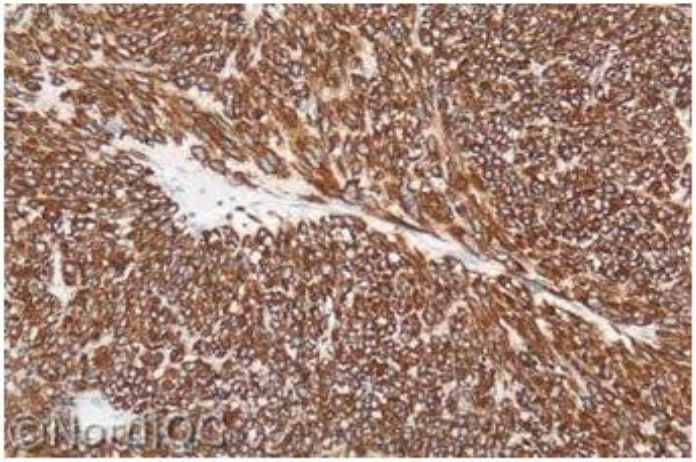



Fig. 2b. ASMA staining of the leiomyosarcoma tissue no. 3 in the multitissue block using same insufficient protocol as in Fig. 1b. Virtually all the neoplastic cells show a strong and distinct reaction with no background reaction – same field as in Fig. 2a. However, also compare with Fig. 3b – same protocol.

# IHC – Protocols and controls for Breast tumours

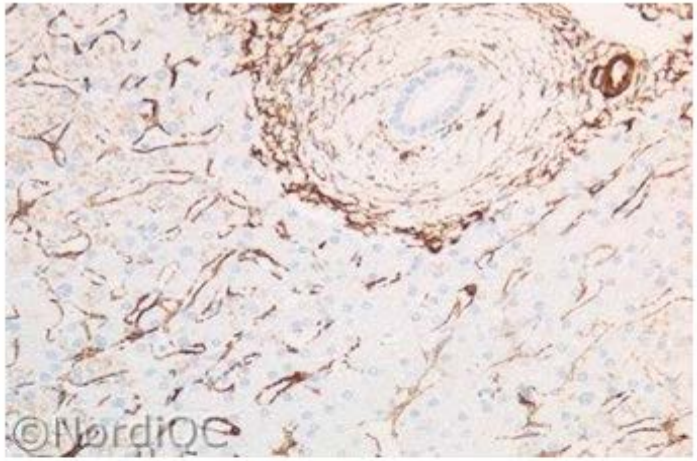



Fig. 1a. Optimal ASMA staining of the liver using the mAb clone 1A4 with HIER. The smooth muscle cells in the portal vessels as well as the perisinusoidal smooth muscle cells show a distinct staining. The liver cells are negative (a weak granular staining is due to lipofuscin).

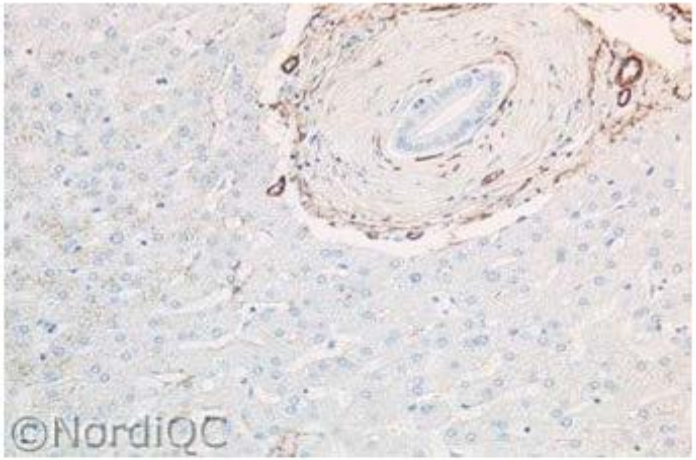



Fig. 1b. Insufficient ASMA staining of the liver using the mAb clone 1A4 in a protocol omitting HIER – same field as in Fig. 1a. The smooth muscle cells in the portal vessels are demonstrated, while the perisinusoidal smooth muscle cells are virtually negative. Also compare with Figs. 2b & 3 b same protocol.

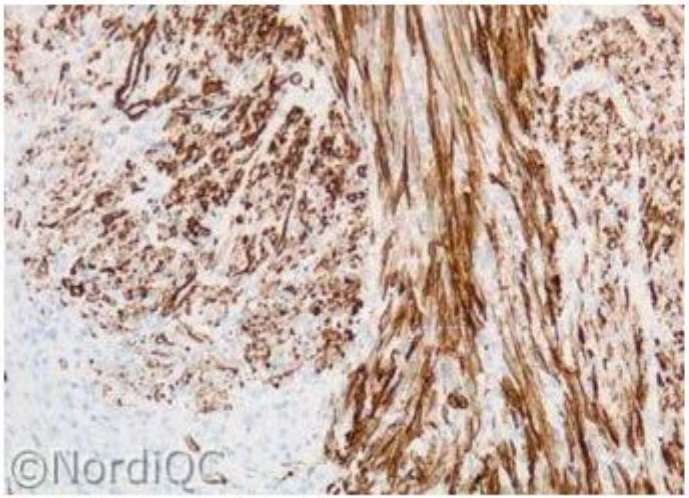



Fig. 3a. Optimal ASMA staining of the leiomyosarcoma tissue no. 4 in the multitissue block using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate to strong and distinct reaction with no background reaction.

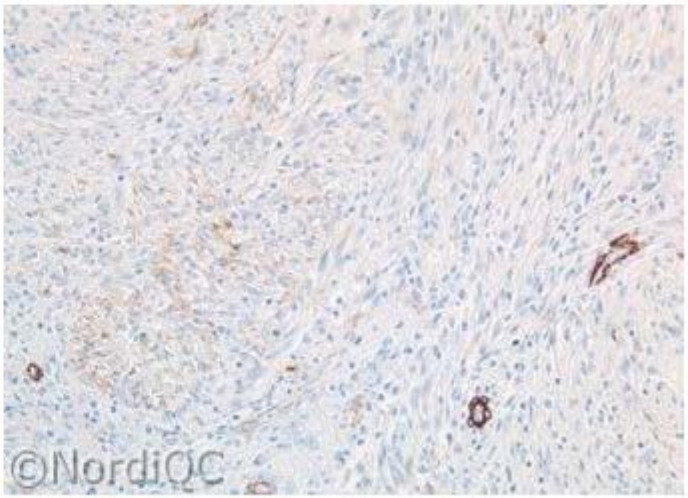



Fig. 3b. Insufficient ASMA staining of the leiomyosarcoma tissue no. 4 in the multitissue block using same protocol as in Figs. 1b & 2b. Only scattered neoplastic cells show a weak reaction – same field as in Fig. 3a.

# IHC – Protocols and controls for Breast tumours

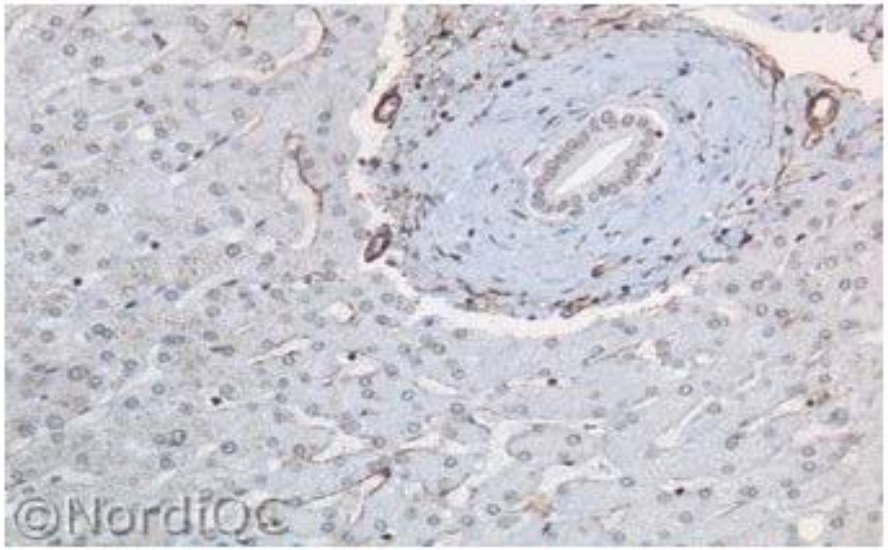



Fig. 4a. Insufficient ASMA staining of the liver using the mAb clone 1A4 with HIER in Cell Conditioning 1 (CC1) on the BenchMark XT, Ventana.
Scattered perisinusoidal smooth muscle cells are demonstrated, but the liver cells and the epithelial cells of the bile duct show a false positive nuclear reaction. This pattern was frequently seen when the mAb clone 1A4 was applied with HIER in CC1 and stained on the BenchMark XT, Ventana. Compare with Fig. 1a.

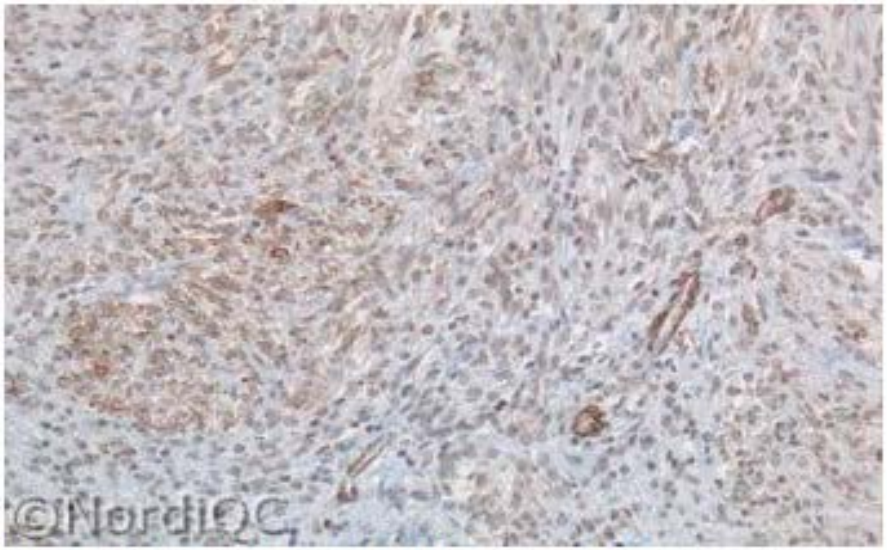


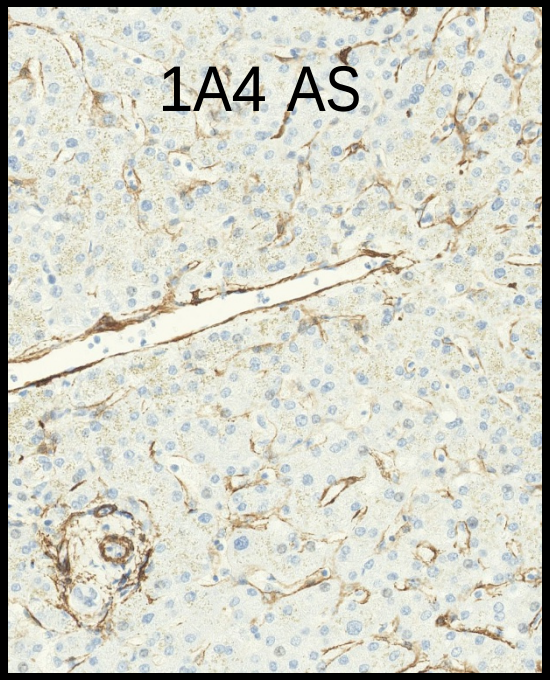

Fig. 4b. Left: Insufficient ASMA staining of the leiomyosarcoma tissue no. 4 in the multi block using same protocol as in Fig. 4a. The neoplastic cells show a false positive positive nuclear reaction, while the specific cytoplasmic reaction is virtually absent. Compare with Fig. 3a - same field.

Table 3. **Proportion of optimal results for ASMA for the most commonly used antibody 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 **1A4** | 14/25** (56%) | 0/2 | 1/29 (3%) | 0/2 | 5/7 (71%) | 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)

# IHC – Protocols and controls for Breast tumours






## Protocol for ASMA depending on IHC stainer

| mAb clone 1A4 (Dako*) | Dako AS48 | Leica Bond III | VMS Dako Ultra Omnis |
|---|---|---|---|
| Titre | 1:100-500* / RTU | 1:200-500* |  |
| Retrieval | HIER TRS High | HIER ER 2 | |
| Detection | 2- or 3-step | 3-step | |

# IHC – Protocols and controls for Breast tumours

**Performance history**
This was the fourth NordiQC assessment of ASMA and as shown in table 2 the pass rates have been constant at a relatively low level throughout all runs.

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

| | Run 10 2004 | Run 21 2007 | Run 27 2009 | Run 44 2015 |
|---|---|---|---|---|
| Participants, n= | 71 | 106 | 124 | 234 |
| Sufficient results | 62% | 63% | 64% | 60% |

**Conclusion**
The mAb clones **1A4**, **asm-1** and rmAb clone **EP188** could all be used to obtain an optimal staining result. Using the two most widely used ASMA antibodies (clone 1A4 and asm-1) HIER and careful calibration of the titre of the primary antibody were the main prerequisites for optimal results.
The performance of clone 1A4 seems to be influenced by the stainer platform as a significantly reduced proportion of sufficient results was observed when used on the Ventana BenchMark platform compared to Dako Autostainer and Leica BOND platforms.

# IHC – Protocols and controls for Breast tumours

Breast panel: ASMA – Ventana BenchMark and Dako Omnis
Protocol settings for an optimal staining result (NQC internal data)

| | Retrieval | Titre | Detection | RTU | Detection |
|---|---|---|---|---|---|
| rmAb EP188 | P2 4M + CC1M | 1:200 | 3-step OP + AMP | - | - |
| mAb BS66 | HIER High | 1:1000-1500 | 3-step | - | - |

The mAb BS66 is the secret from Miraculix……

ASMA should be in good shape…

# IHC - Protocols and controls for Breast tumours

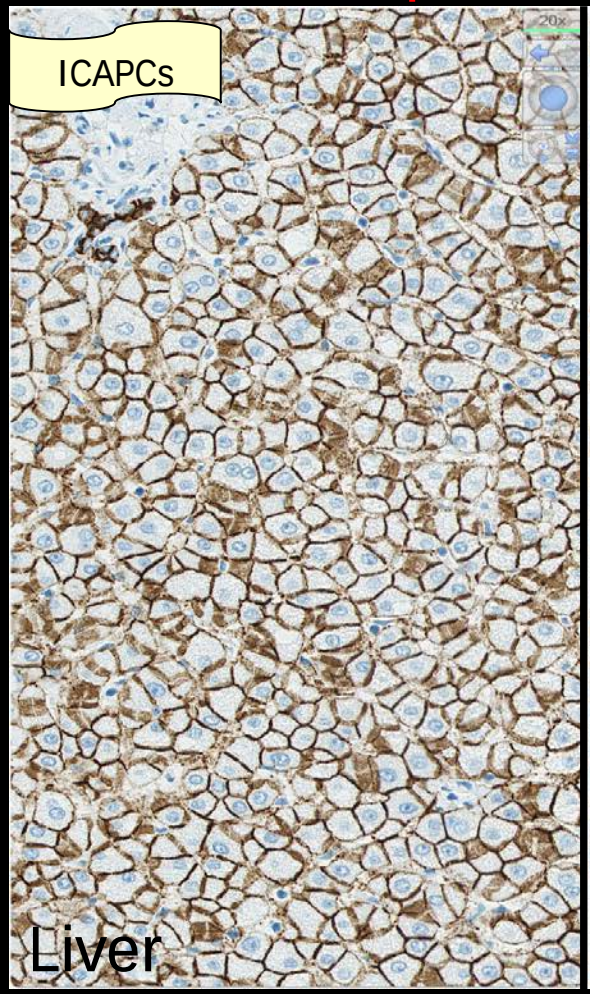
Breast panel:

- GCDFP-15
- Mammaglobin
- Gata 3
- Smooth MHCM
- ASMA
- (p63)
- E-cadherin
- p120
- ER
- PR
- HER-2

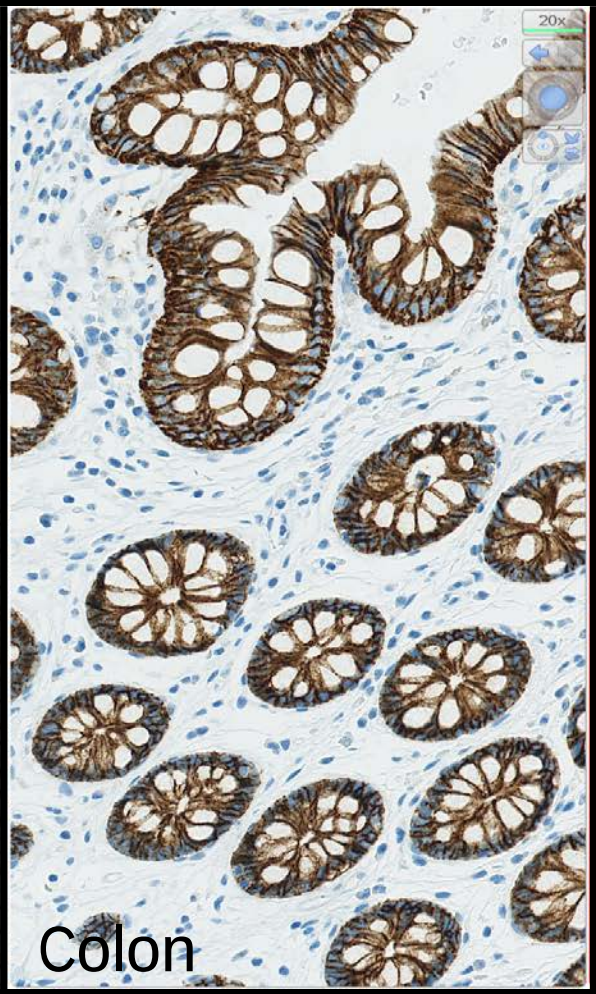
- Is it primary breast ?
- Is it invasive ?
- Is it lobular or ductal ?
- Which teraphy ?

# IHC – Protocols and controls for Breast tumours

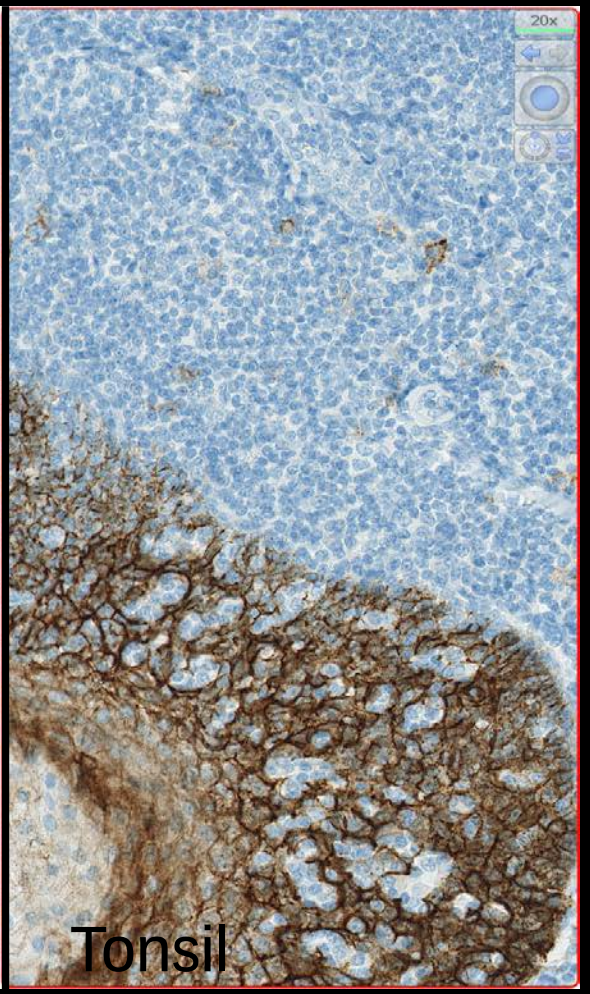
## ECAD reaction pattern




An at least weak to moderate membranous staining reaction of virtually all the hepatocytes.




A moderate to strong, distinct membranous staining reaction of virtually all the columnar epithelial cells in the colon / appendix.




A moderate to strong, distinct membranous staining reaction of virtually all squamous epithelial cells. No staining reaction of the vast majority of lymphocytes.

# IHC – Protocols and controls for Breast tumours

Table 1. **Antobodies and assessment marks for ECAD run 39**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **36** | 1 | BD Biosciences | 0 | 0 | 1 | 0 | - | - |
| mAb clone **36B5** | 16<br>1 | Leica/Novocastra<br>Vector | 2 | 13 | 2 | 0 | 88 % | 100 % |
| mAb clone **4A2C7** | 5 | Invitrogen/Zymed | 0 | 3 | 2 | 0 | 60 % | - |
| mAb clone **BSH38** | 1 | Nordic Biosite | 0 | 1 | 0 | 0 | - | - |
| mAb clone **ECH-6** | 1 | Zytomed | 1 | 0 | 0 | 0 | - | - |
| mAb clone **HECD-1** | 9<br>2<br>1<br>1 | Invitrogen/Zymed<br>Immunologic<br>Abcam<br>Biocare | 7 | 6 | 0 | 0 | 100 % | 100 % |
| mAb clone **NCH-38** | 90<br>5 | Dako<br>Thermo/NeoMarkers | 59 | 30 | 6 | 0 | 94 % | 94 % |
| mAb clone **SPM471** | 1 | Thermo/NeoMarkers | 0 | 1 | 0 | 0 | - | - |
| rmAb clone **EP6** | 2 | Epitomics | 0 | 2 | 0 | 0 | - | - |
| rmAb clone **EP700Y** | 6<br>1<br>1<br>1<br>1 | Cell Marque<br>Biocare<br>Bio SB<br>Thermo/NeoMarkers<br>Zytomed | 0 | 7 | 1 | 2 | 70 % | - |
| Unknown | 1 | Unknown | 1 | 0 | 0 | 0 | - | - |
| Ready-To-Use Antibodies | | | | | | | | |
| mAb clone **36 790-4497** | 51 | Ventana | 6 | 12 | 32 | 1 | 35 % | 38 % |
| mAb clone **36B5 PA0387** | 10 | Leica | 0 | 10 | 0 | 0 | 100 % | - |
| mAb clone **NCH-38 IR/IS059** | 44 | Dako | 40 | 4 | 0 | 0 | 100 % | 100 % |
| mAb clone **NCH-38 GA059** | 1 | Dako | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **EP700Y 760-4440** | 16 | Ventana/Cell Marque | 0 | 15 | 1 | 0 | 94 % | - |
| rmAb clone **EP700Y 246R-1x** | 1 | Cell Marque | 0 | 1 | 0 | 0 | - | - |
| rmAb clone **EP700Y MAD-000051QD** | 1 | Master Diagnostica | 0 | 1 | 0 | 0 | - | - |
| Total | 271 | | 117 | 106 | 45 | 3 | - | |
| Proportion | | | 43 % | 39 % | 17 % | 1 % | 82 % | |

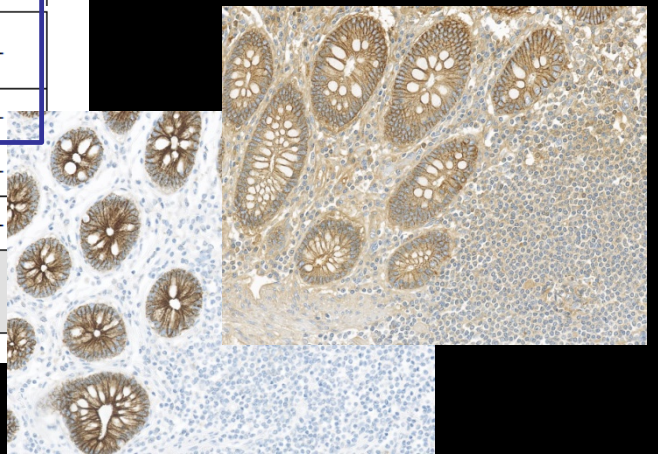
1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.

mAb clones
HECD-1 & NCH-38 most successful

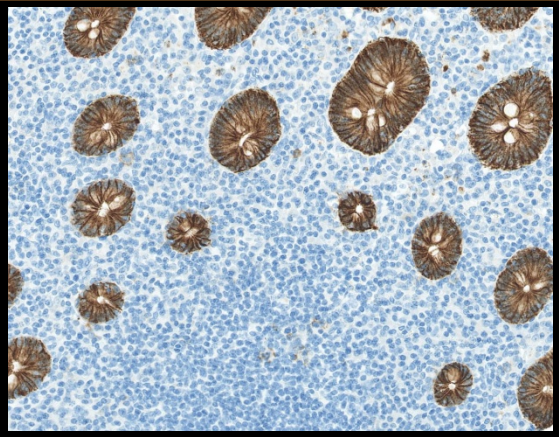
HIER
2 or 3-step mul/pol.

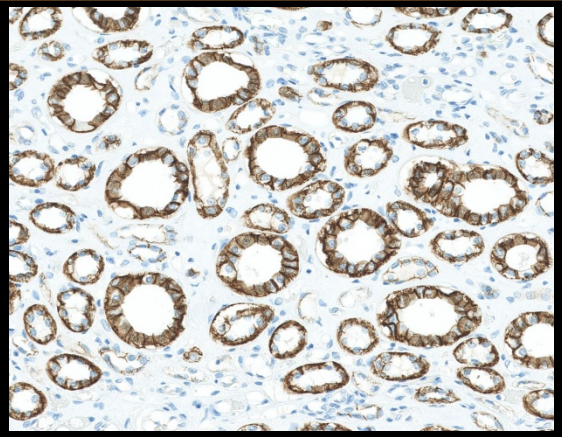
mAb clone 36 aberrant nuclear staining reaction

rmAb clone EP700y inferior signal-to-noise



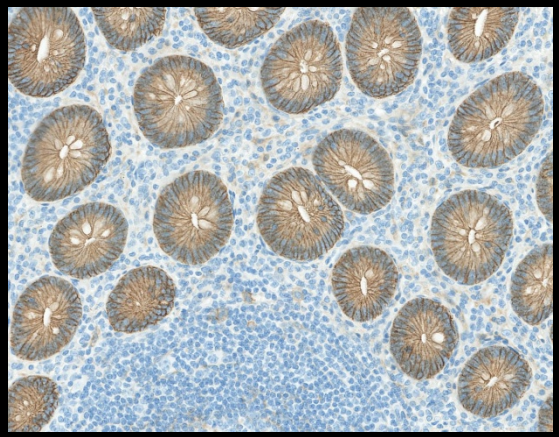
# IHC – Protocols and controls for Breast tumours

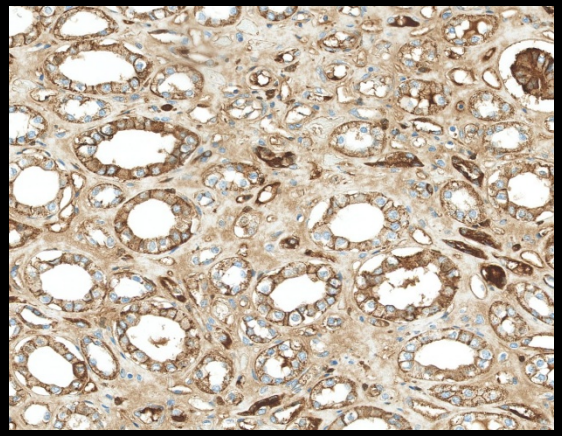




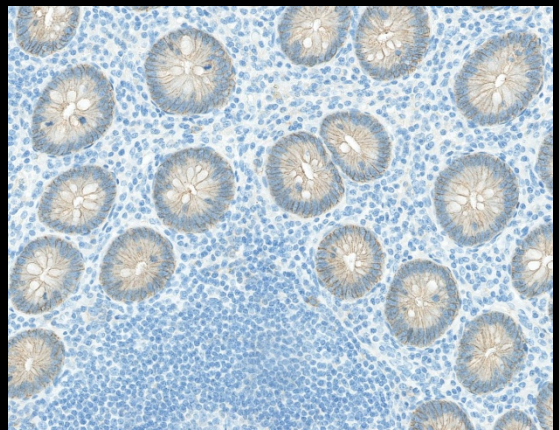
NCH-38 vs EP700Y

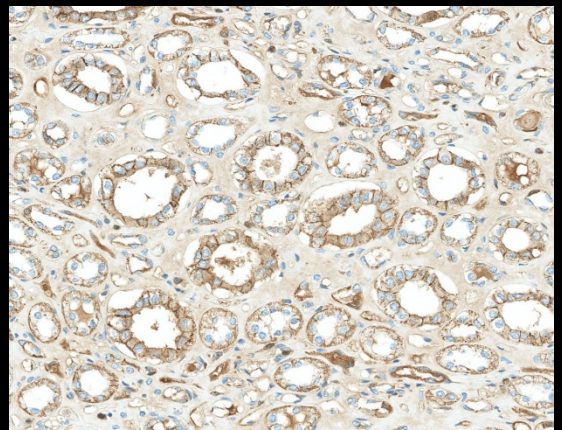
Colon - Kidney
NCH-38





Colon - Kidney
EP700Y Titre A



Colon – Kidney
EP700Y Titre B

# IHC – Protocols and controls for Breast tumours

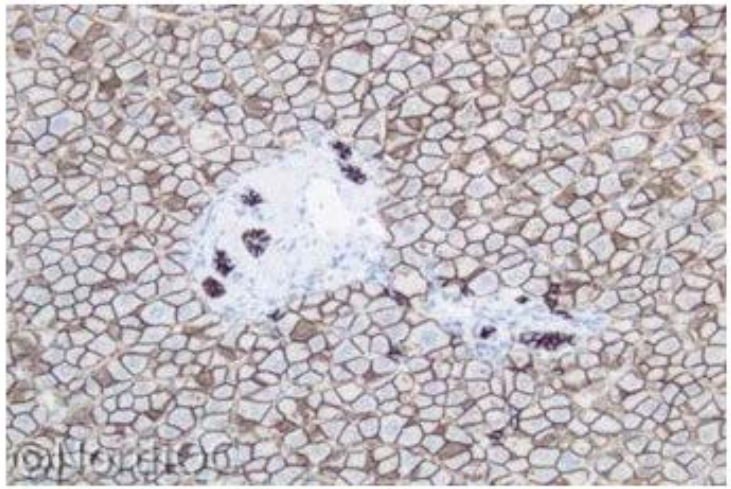



Fig. 1a. Optimal staining for ECAD of the liver using the mAb clone NCH-38 with HIER. Virtually all the hepatocytes show a moderate distinct membranous reaction, while the epithelial cells of the bile ducts show a strong staining.

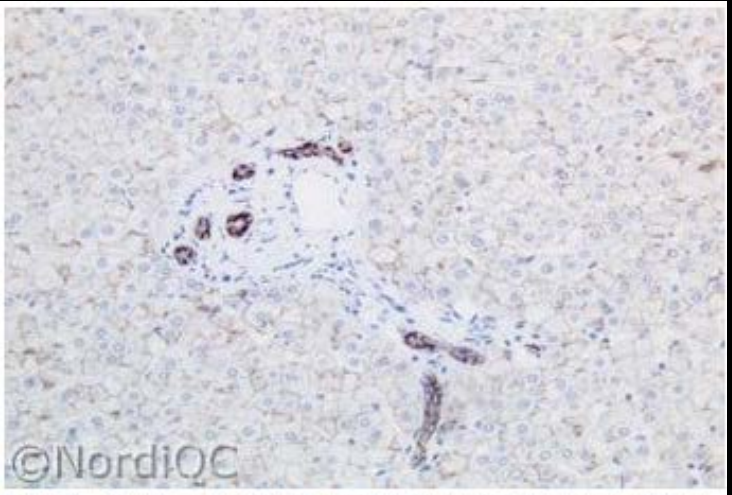



Fig. 1b. Staining for ECAD of the liver using an insufficient protocol based on the same mAb clone NCH-38 as in Fig. 1a, but in a too low concentration. The hepatocytes only show a weak disrupted membranous reaction – same field as in Fig. 1a.

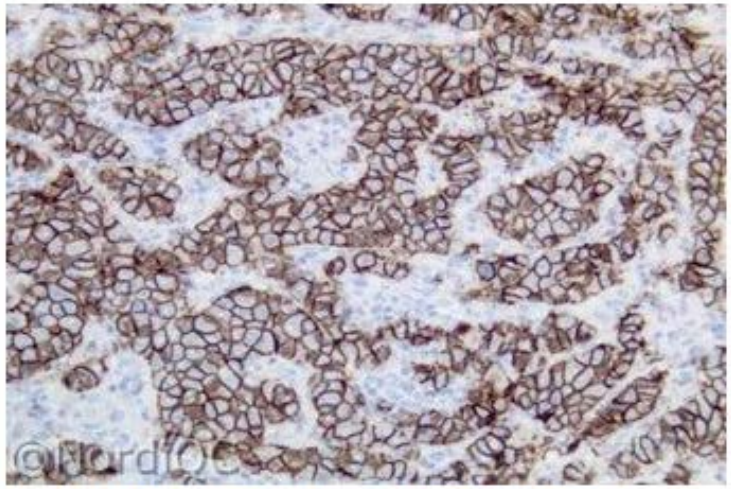



Fig. 2a. Optimal ECAD staining of the ductal breast carcinoma using same protocol as in Fig. 1a. The majority of the neoplastic cells show a strong distinct membranous reaction with no background reaction.

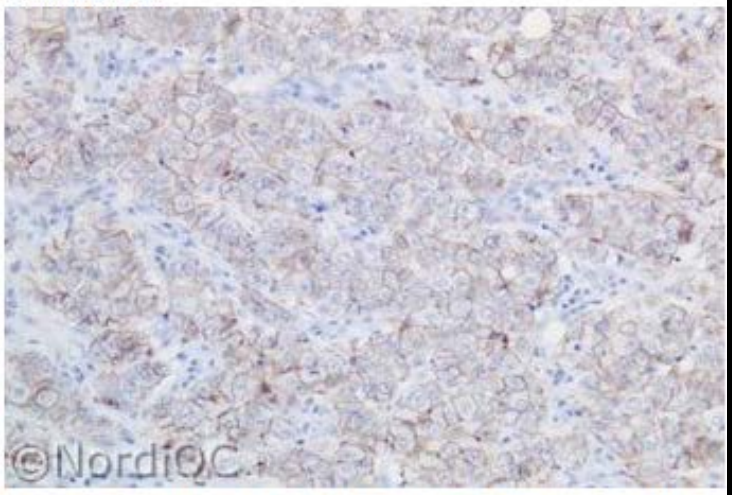


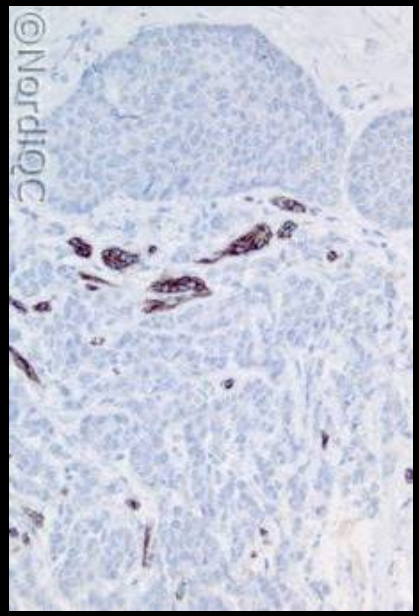

Fig. 2b. Staining for ECAD of the ductal breast carcinoma using same insufficient protocol as in Fig. 1b. The neoplastic cells only show a weak diffuse membranous reaction – also compare with Fig. 3b – same protocol.

**E-Cadherin**

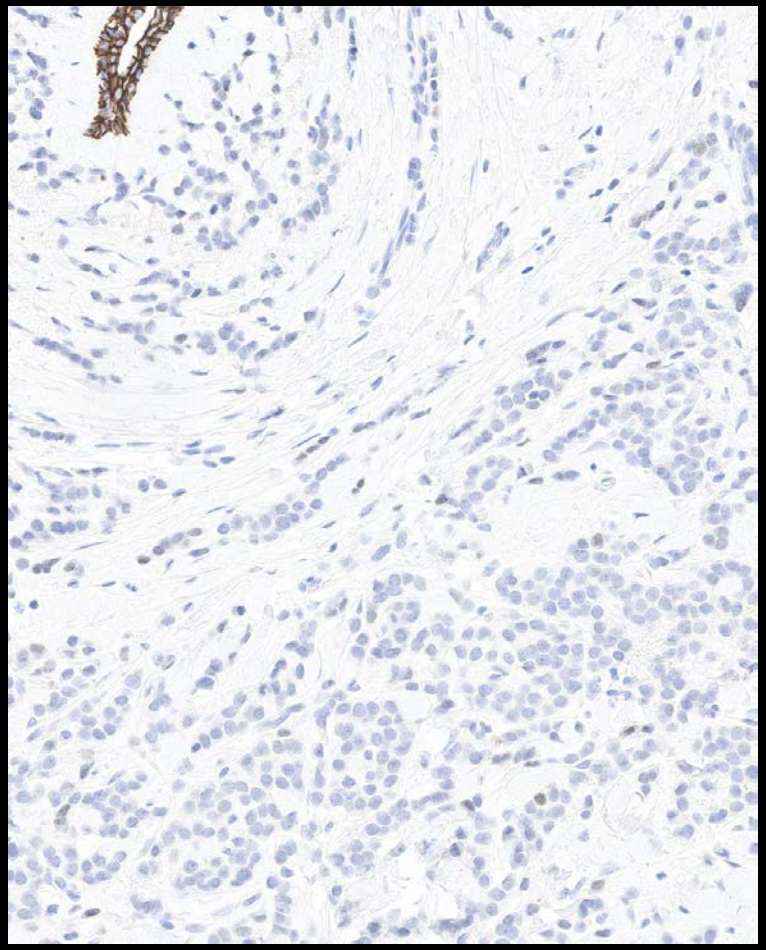
**Liver**

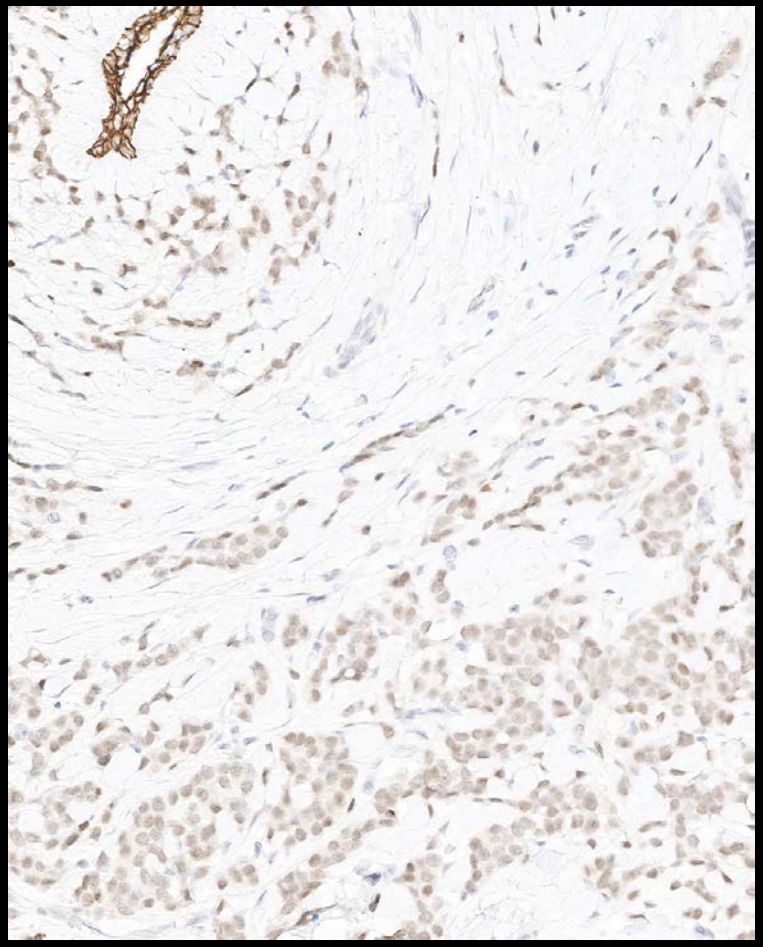
CSQI:

Hepatocytes



# IHC – Protocols and controls for Breast tumours





Lobular breast carcinoma

mAb clone HECD-1 or NCH-38

mAb clone 36

Technical ? Biology ?

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*Histopathology* 2008, **52**, 325–330. DOI: 10.1111/j.1365-2559.2007.02949.x

## Membrane loss and aberrant nuclear localization of E-cadherin are consistent features of solid pseudopapillary tumour of the pancreas. An immunohistochemical study using two antibodies recognizing different domains of the E-cadherin molecule

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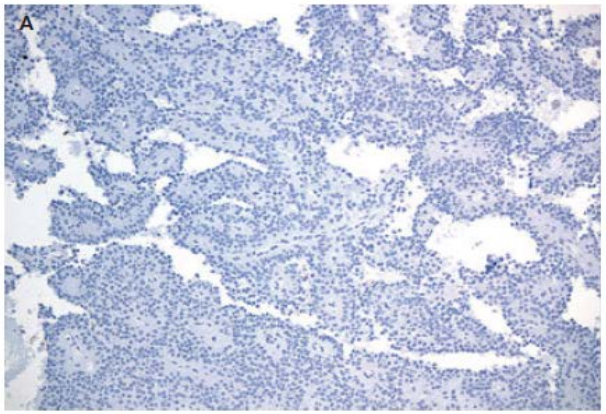
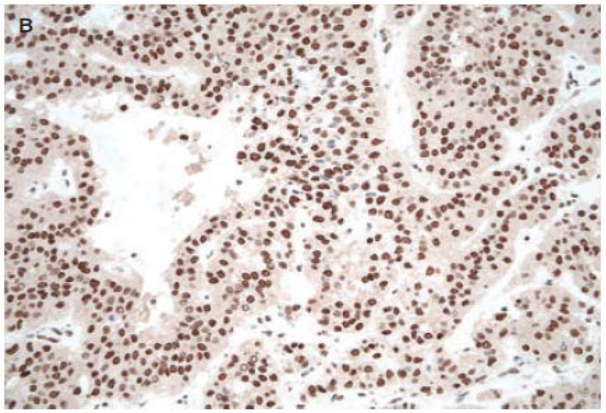
**Table 1.** Clinicopathological features and immunohistochemistry

| Case | Gender | Age (years) | Size (mm) | Site | β-Catenin | E-cadherin clone 36 | E-cadherin clone 36B |
|---|---|---|---|---|---|---|---|
| 1 | F | 32 | 52 | Head | Nuc/cyto | Nuclear | Negative |
| 2 | F | 40 | 68 | Tail | Nuc/cyto | Nuclear | Negative |
| 3 | F | 45 | 55 | Body/tail | Nuc/cyto | Nuclear | Negative |
| 4 | F | 39 | 35 | Head | Nuc/cyto | Nuclear | Negative |
| 5 | F | 24 | 45 | Head | Nuc/cyto | Nuclear | Negative |
| 6 | F | 31 | 88 | Tail | Nuc/cyto | Nuclear | Negative |
| 7 | F | 43 | 40 | Tail | Nuc/cyto | Nuclear | Negative |
| 8 | M | 45 | 35 | Tail | Nuc/cyto | Nuclear | Negative |
| 9 | F | 39 | 32 | Tail | Nuc/cyto | Nuclear | Negative |
| 10 | F | 20 | 31 | Body/tail | Nuc/cyto | Nuclear | Negative |
| 11 | F | 43 | 130 | Tail | Nuc/cyto | Nuclear | Negative |
| 12 | F | 39 | 63 | Tail | Nuc/cyto | Nuclear | Negative |
| 13 | F | 11 | 80 | Body/tail | Nuc/cyto | Nuclear | Negative |
| 14 | F | 61 | 90 | Tail | Nuc/cyto | Nuclear | Negative |
| 15 | M | 62 | 170 | Body/tail | Nuc/cyto | Nuclear | Negative |
| 16 | F | 40 | 45 | Tail | Nuc/cyto | Nuclear | Negative |
| 17 | F | 52 | 20 | Body/tail | Nuc/cyto | Nuclear | Negative |
| 18 | F | 19 | 53 | Body/tail | Nuc/cyto | Nuclear | Negative |
| 19 | F | 36 | 70 | Tail | Nuc/cyto | Nuclear | Negative |
| 20 | F | 13 | 50 | Head | Nuc/cyto | Nuclear | Negative |




mAb 36B5




mAb 36

Clone 36 reacts with cytoplasmic component

Nuclear localization might occur due to B-Cat mutation (has to be confirmed and no data on breast tumours).

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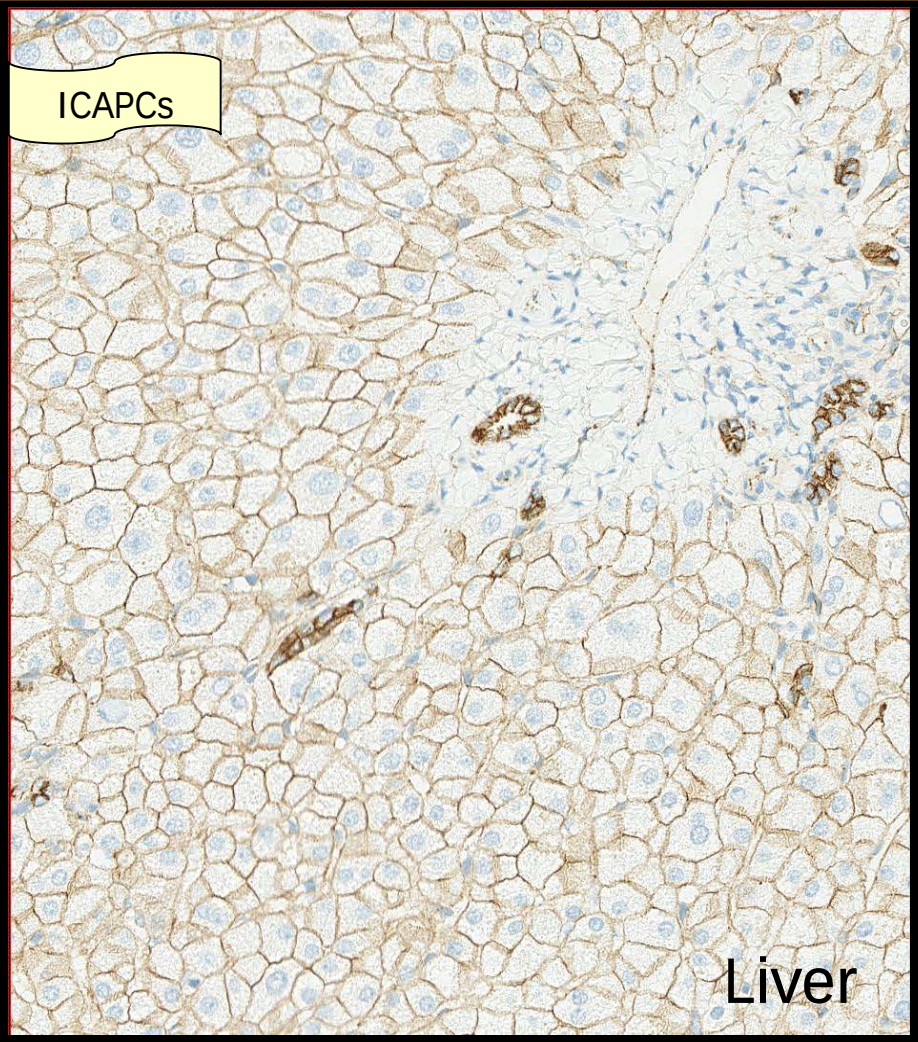


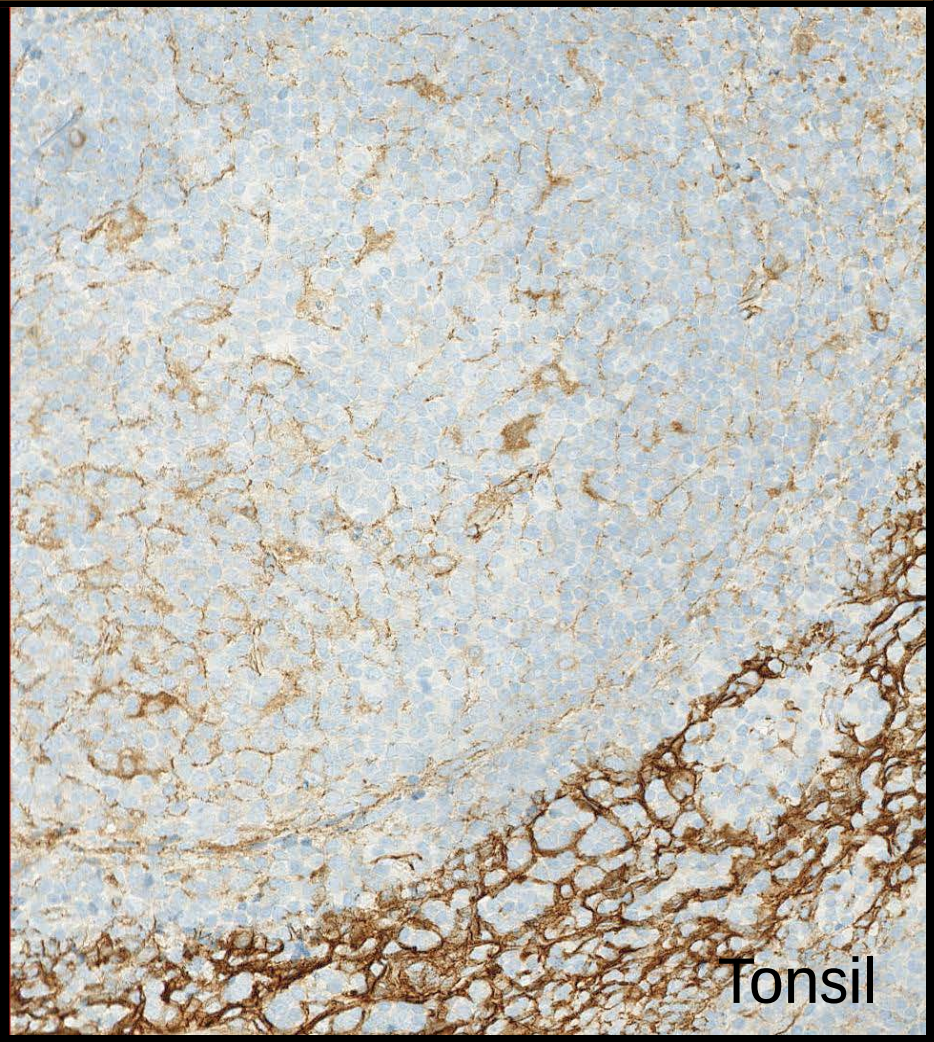


## p120 Catenin

An at least weak to moderate membranous staining reaction of virtually all the hepatocytes.
A moderate to strong pre-dominantly membranous staining reaction must be seen all epithelial cells of bile ducts.

An at least weak to moderate membranous staining reaction of germinal centre macrophages and the follicular dendritic network.

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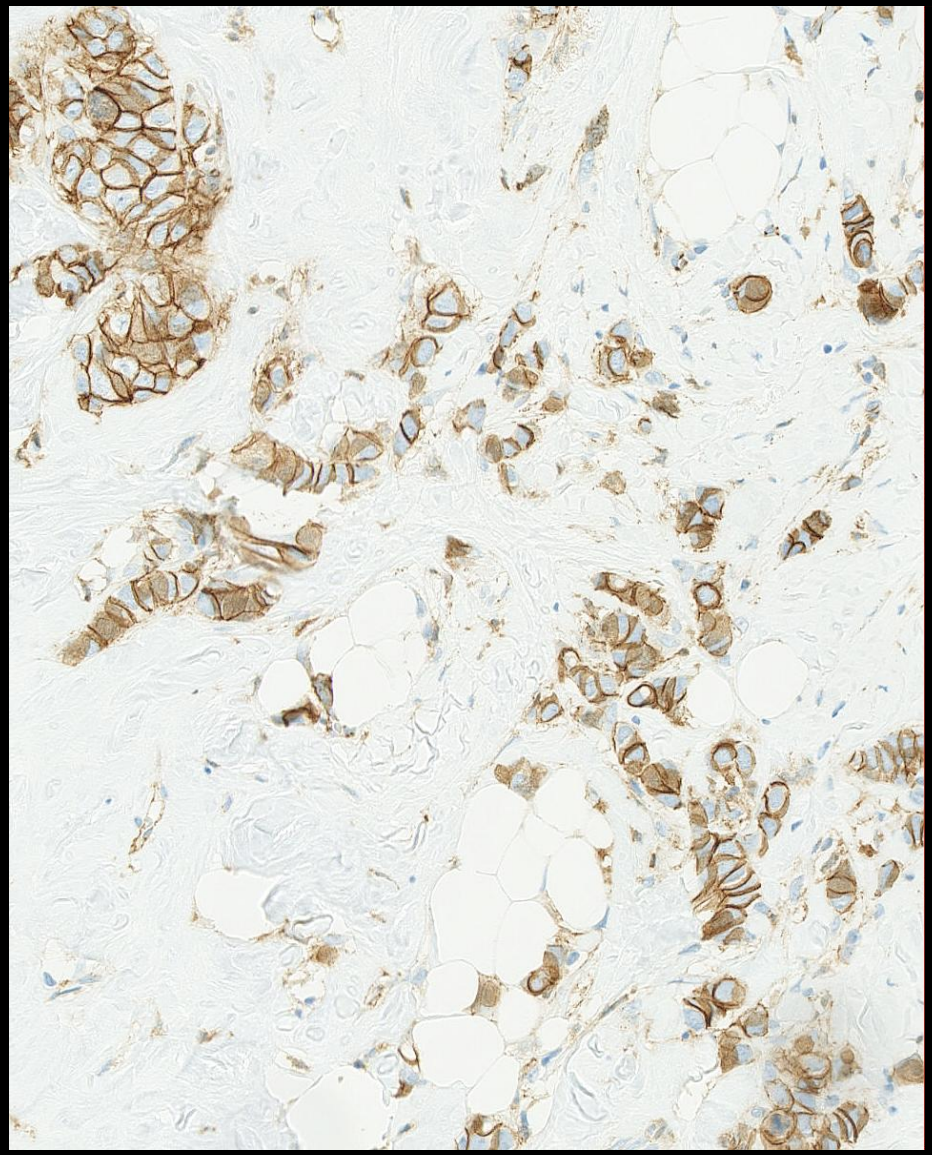

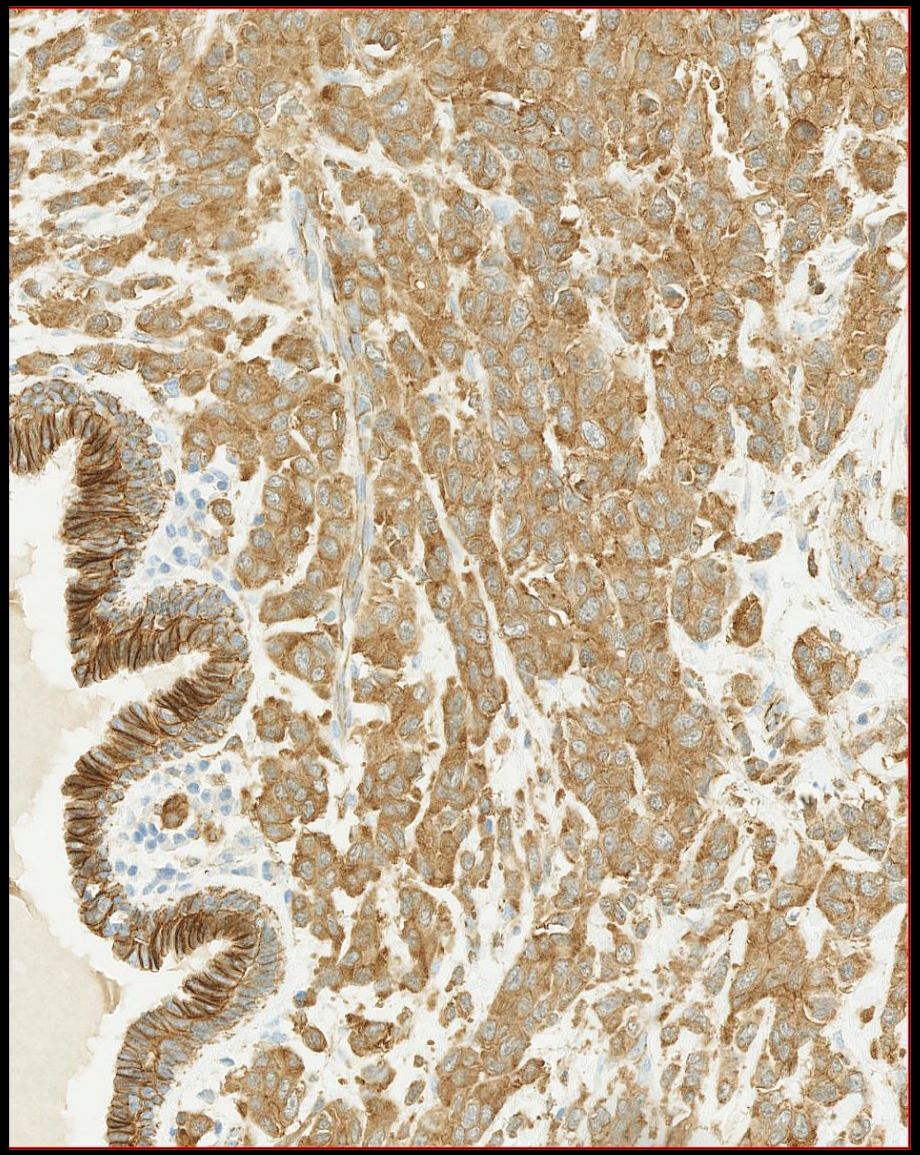

p120 Catenin



Ductal carc.

Lobular carc.

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Breast panel: E-Cadherin (& *p120 NordiQC internal data*)
Basic protocol settings for an optimal staining result (NQC)

| E-CAD | Retrieval | Titre | Detection | RTU | Detection |
|---|---|---|---|---|---|
| mAb NCH-38 | HIER High | 1:25-100 | 2- & 3-step | Dako | 2- & 3-step |
| mAb HECD-1 | HIER High | 1:200–1.000 | 2- & 3-step | - | - |
| mAb 36B5 | HIER High | 1:50 | 2- & 3-step | - | - |
| mAb ECH-6 | HIER High | 1:100 | 2-step | - | - |
| *mAb 36* | *HIER High* | *-* | *-* | *Ventana* | *2-step** |

* *Short incubation time 8-16 min. and 2-step multimer*

| p120 | Retrieval | Titre | Detection | RTU | Detection |
|---|---|---|---|---|---|
| *mAb MRQ-5* | *HIER high* | *1:25-100* | *2- & 3-step* | - | - |

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