The Technical Approach Analytical Phase and pitfalls NordiQC Seminar 2025

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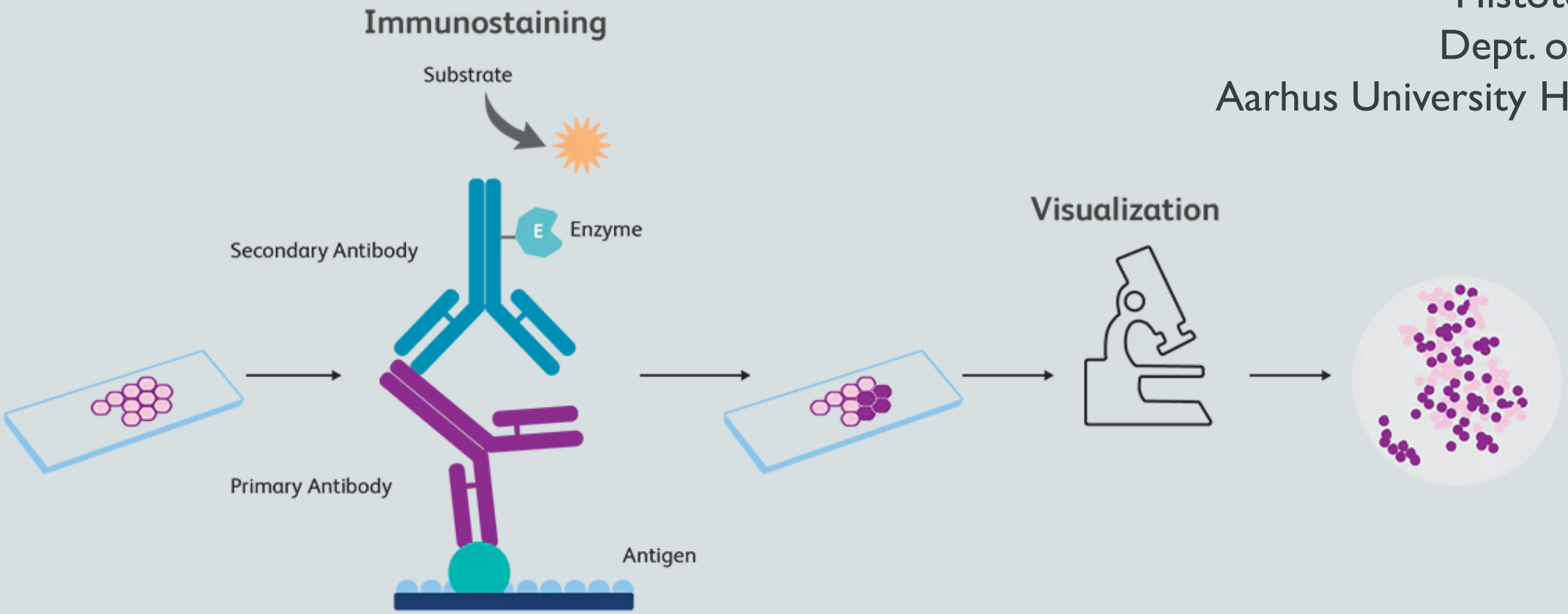
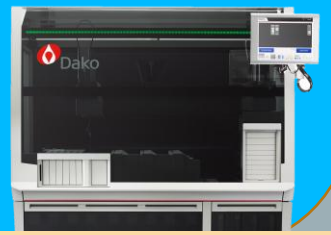


The total test paradigm

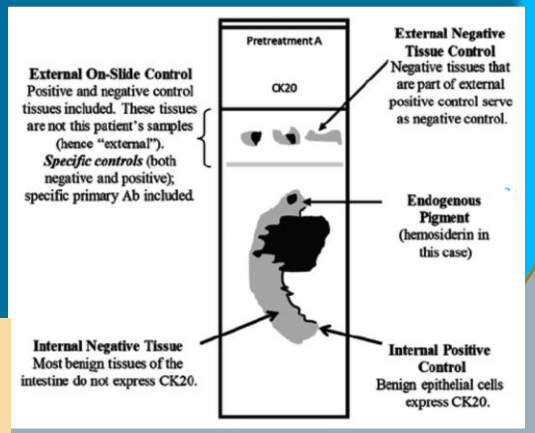
"Immunohistochemistry is technically complex, and no aspect of this complexity can be ignored, from the moment of collecting the specimen to issuance of the final report"
Taylor CR. Arch Pathol Lab Med 2000; 124:945

Preanalytical
- Operating method
- Ischemia
- Fixative and fixation
- Tissue processing
- Paraffin embedding
- Paraffin sectioning
- Slide choice
- Storage

Analytical
- Choice of platform
- Epitope retrieval
- Blocking
- Primary Antibody
- Diluent
- Detection system
- Chromogen
- Counter stain
- Mounting



Interpretation
- Design of controls
- Positive controls
- Negative controls
- Interpretation
- Critical stain indicator



Analytical phase: Begins with dewax of the cut slides and is completed with the coverslipping of the stained slides.

Antibodies

IHC-type 1 markers (Diagnostic)

Often calibrated to produced appropriate level of sensitivity and specificity (positive versus negative)

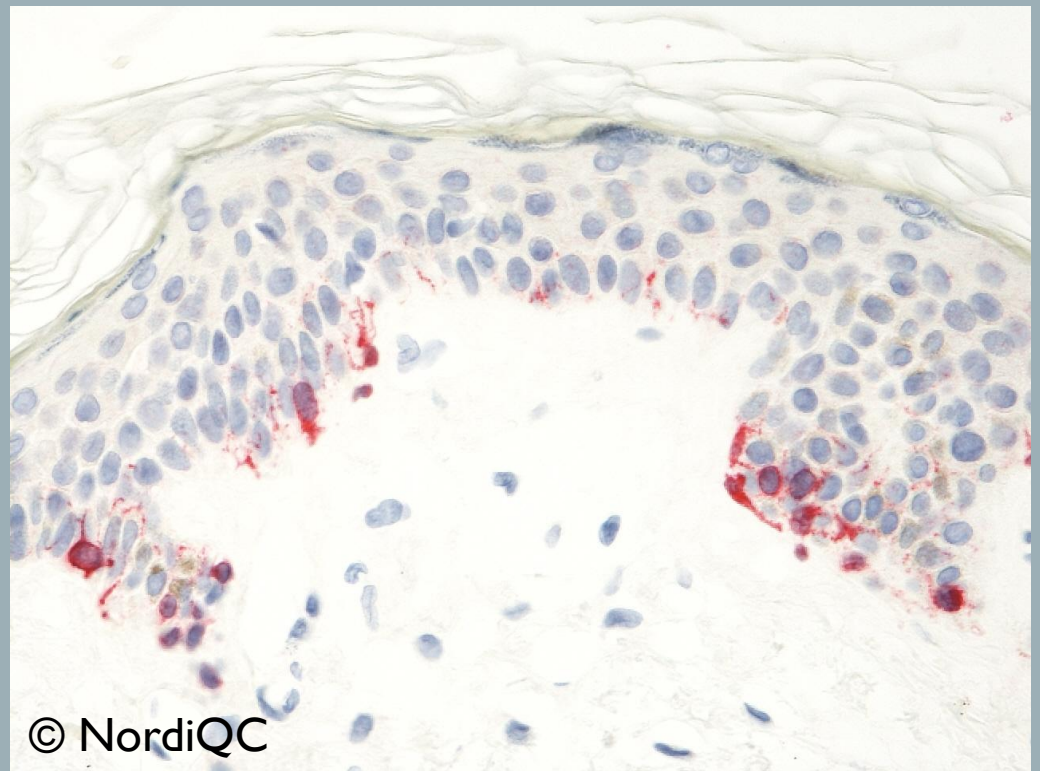
IHC-type 2 markers (Disease screening, predictive treatment & prognosis)

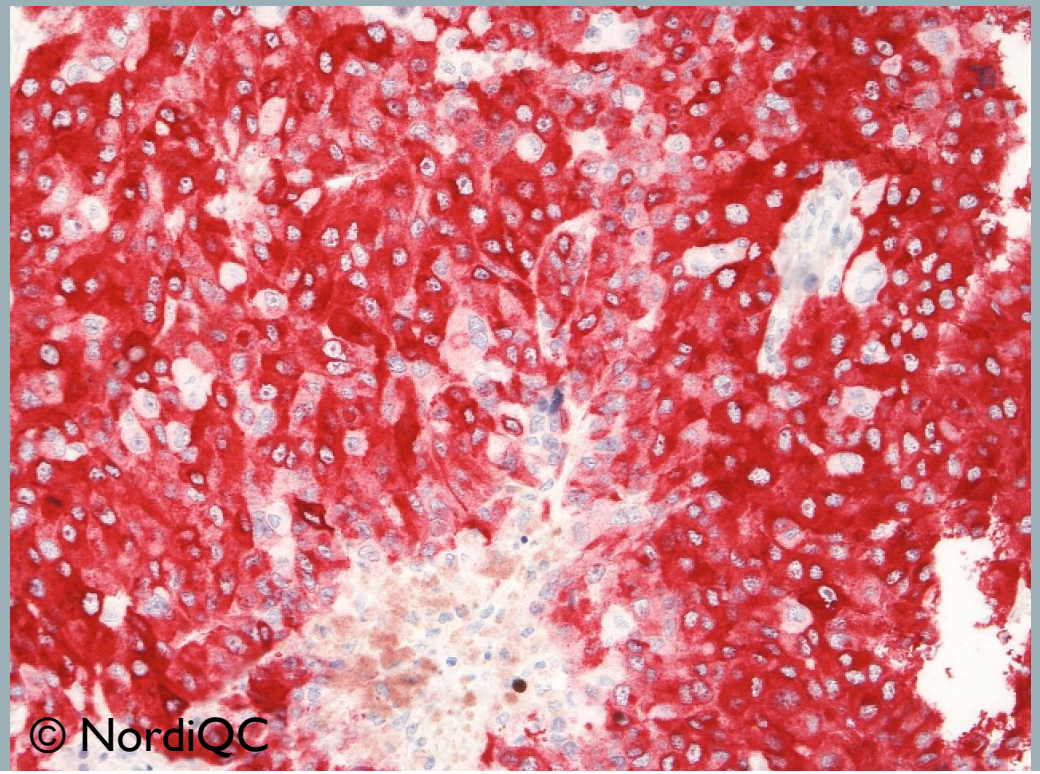
Predictive markers: The assays are calibrated to provide information of which patients will or will not benefit from a specific treatment (HER-2, PD-L1 …….)

Choice of product and clone

- Should we use Ready-to-use (RTU) or a concentrate?
- Is there a RTU on the market and is it intended for my platform?
- Is there an available IVD-R product?
- How do we know if the product available are staining our intended purpose of use?

MELAN A – Fit for purpose





- Mel-a is an antibody that binds to antigens in the melanocytes in the skin.
- Mel-a: positive in 80-100% of malignant melanomas, but also in compound nevi which are benign changes, and in other types of cancer that arise from melanocytes, such as clear cell sarcomas or neurofibromas.
- We describe the great ability to recognize antigens in the melanocytes as an antibody with high sensitivity.

MELAN A

Table 1. **Antibodies and assessment marks for MLA, Run 60**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **A103**	57 19 6 1 1 1 1 1	Dako/Agilent Novocastra/Leica Cell Marque Abcam Biocare Monosan Biogenex Zeta Corporation	21	57	9	0	90%	24%
rmAb clone **EP43**	9 9 1	Nordic Biotite Epitomics Cell Marque	18	1	0	0	100%	95%
mAb clone **BS52**	3	Nordic Biosite	3	0	0	0	-	-
mAb clone **M2-7C10**	1	Zytomed	1	0	0	0	-	-
mAb clone cocktail **HMB45+MC-7310 +M2-9E3+T311**	3	Biocare	2	1	0	0	-	-
mAb clone cocktail **HMB45+A103+T311**	1	Diagnostic Biosystems	0	0	0	1	-	-
mAb clone cocktail **M2-7C10+M2-9E3**	1	Thermo F. Scientific	1	0	0	0	-	-
rmAb clone **EP1442Y**	1	Abcam	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **A103 790-2990**[3]	3	Ventana/Roche	0	0	3	0	-	-
mAb clone **A103 790-2990**[4]	94	Ventana/Roche	6	74	11	3	85%	6%
mAb clone **A103, IR633/IS633**[3]	14	Dako/Agilent	1	13	0	0	100%	7%
mAb clone **A103, IR633/IS633**[4]	56	Dako/Agilent	12	36	7	1	85%	21%
mAb clone **A103, PA0233/PA0044**[3]	7	Leica Biosystems	2	5	0	0	100%	29%
mAb clone **A103, PA0233/PA0044**[4]	10	Leica Biosystems	6	4	0	0	100%	60%

During run 60 MLA assessment 9 different clones was submitted

www. NordiQC.org

Recommended protocols - MLA

Search:

Epitope	Staining Platform	Clone name	Clone format	Version date	View
MLA	Dako Autostainer Link 48 +	A103	RTU	01 Oct 2020	PDF
MLA	Dako Autostainer Link 48 +	Melan-A rabbit monoclonal	Other	06 Aug 2020	PDF
MLA	Dako Autostainer Link 48 +	A103	RTU	14 Aug 2020	PDF
MLA	Dako Autostainer Link 48 +	A103	CONC	25 Aug 2020	PDF
MLA	Dako Omnis	EP43	CONC	06 Feb 2019	PDF
MLA	Dako Omnis	EP43	Other	03 Aug 2020	PDF
MLA	Dako Omnis	A103	RTU	17 Aug 2020	PDF
MLA	Dako Omnis	A103	CONC	20 Aug 2020	PDF

MELAN A

-USED TO DETECT MELANOCYTIC ORIGIN

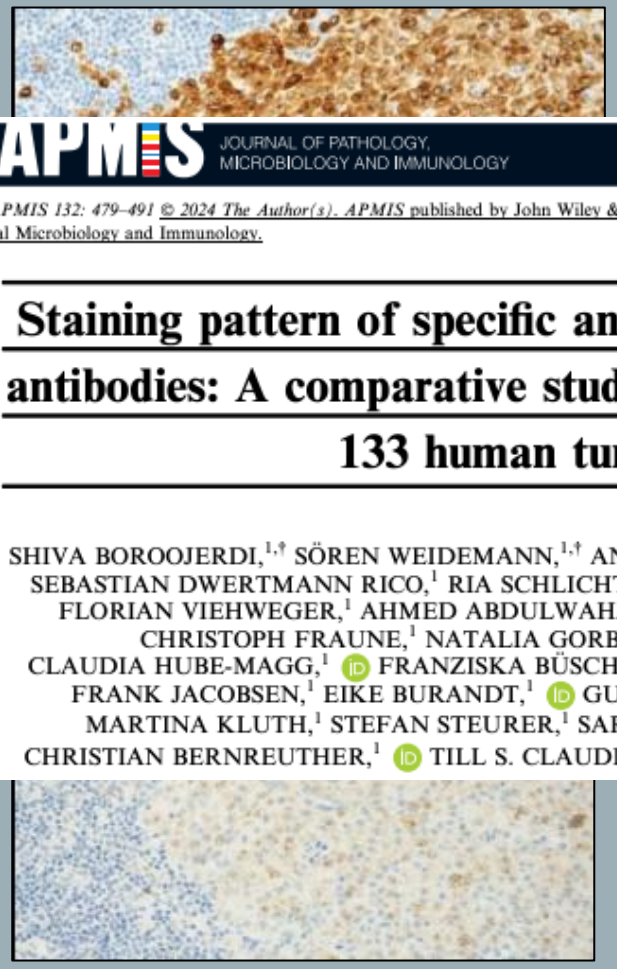
Sentinel node - melanoma

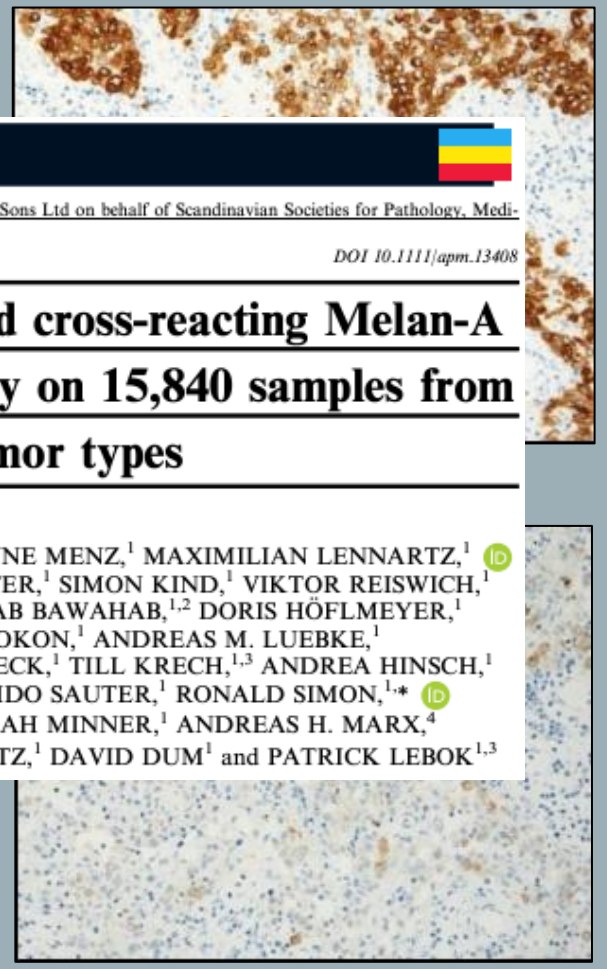
Lymph node - melanoma

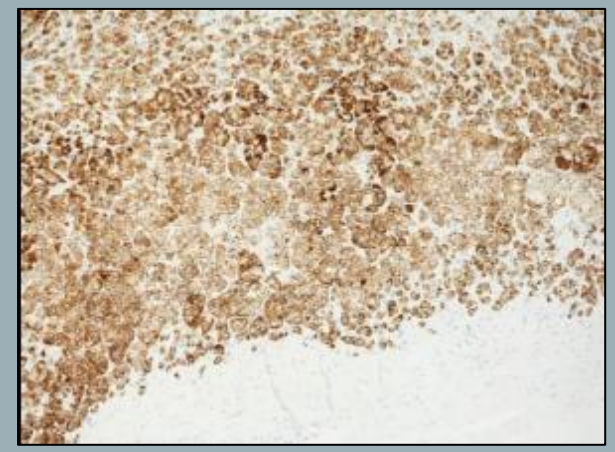
Adrenal gland

MLA, EP43 1:30 Omnis

MLA, A103 1:25 Omnis







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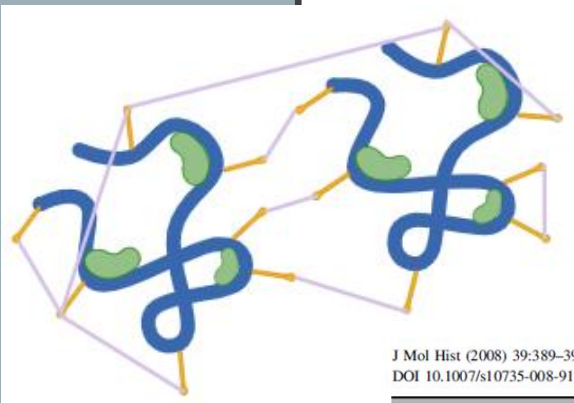
DOI 10.1111/apm.13408

Staining pattern of specific and cross-reacting Melan-A antibodies: A comparative study on 15,840 samples from 133 human tumor types

SHIVA BOROOJERDI,[1,†] SÖREN WEIDEMANN,[1,†] ANNE MENZ,[1] MAXIMILIAN LENNARTZ,[1] SEBASTIAN DWERTMANN RICO,[1] RIA SCHLICHTER,[1] SIMON KIND,[1] VIKTOR REISWICH,[1] FLORIAN VIEHWEGER,[1] AHMED ABDULWAHAB BAWAHAB,[1,2] DORIS HÖFLMEYER,[1] CHRISTOPH FRAUNE,[1] NATALIA GORBOKON,[1] ANDREAS M. LUEBKE,[1] CLAUDIA HUBE-MAGG,[1] FRANZISKA BÜSCHECK,[1] TILL KRECH,[1,3] ANDREA HINSCH,[1] FRANK JACOBSEN,[1] EIKE BURANDT,[1] GUIDO SAUTER,[1] RONALD SIMON,[1,*] MARTINA KLUTH,[1] STEFAN STEURER,[1] SARAH MINNER,[1] ANDREAS H. MARX,[4] CHRISTIAN BERNREUTHER,[1] TILL S. CLAUDITZ,[1] DAVID DUM[1] and PATRICK LEBOK[1,3]

Photo from Michael Bzorek



HIER

– HEAT INDUCED EPITOPE RETRIEVAL

95% of all antibodies uses HIER

J Mol Hist (2008) 39:389–399
DOI 10.1007/s10735-008-9177-y

ORIGINAL PAPER

Hypothesis for the mechanism for heat-induced antigen retrieval occurring on fresh frozen sections without formalin-fixation in immunohistochemistry

Kochi Kakimoto · Susumu Takekoshi ·
Katsuhiro Miyajima · R. Yoshiyuki Osamura

0022-1554/91/$3.30
The Journal of Histochemistry and Cytochemistry

Vol. 39, No. 6, pp. 741–748, 1991
Printed in U.S.A.

Rapid Communication

Antigen Retrieval in Formalin-fixed, Paraffin-embedded Tissues: An Enhancement Method for Immunohistochemical Staining Based on Microwave Oven Heating of Tissue Sections

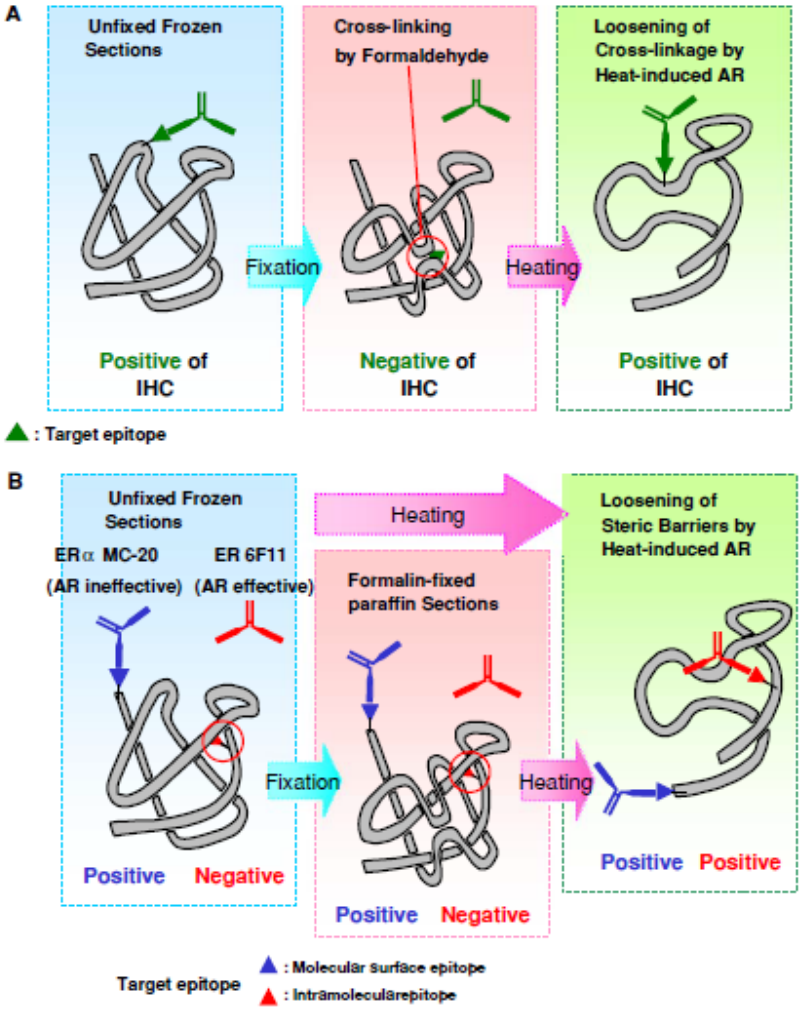
SHAN-RONG SHI, MARC E. KEY,[1] and KRISHAN L. KALRA
BioGenex Laboratories, San Ramon, California 94583.

Received for publication January 15, 1991; accepted March 12, 1991 (IC2212).

We describe a new approach for retrieval of antigens from formalin-fixed, paraffin-embedded tissues and their subse-

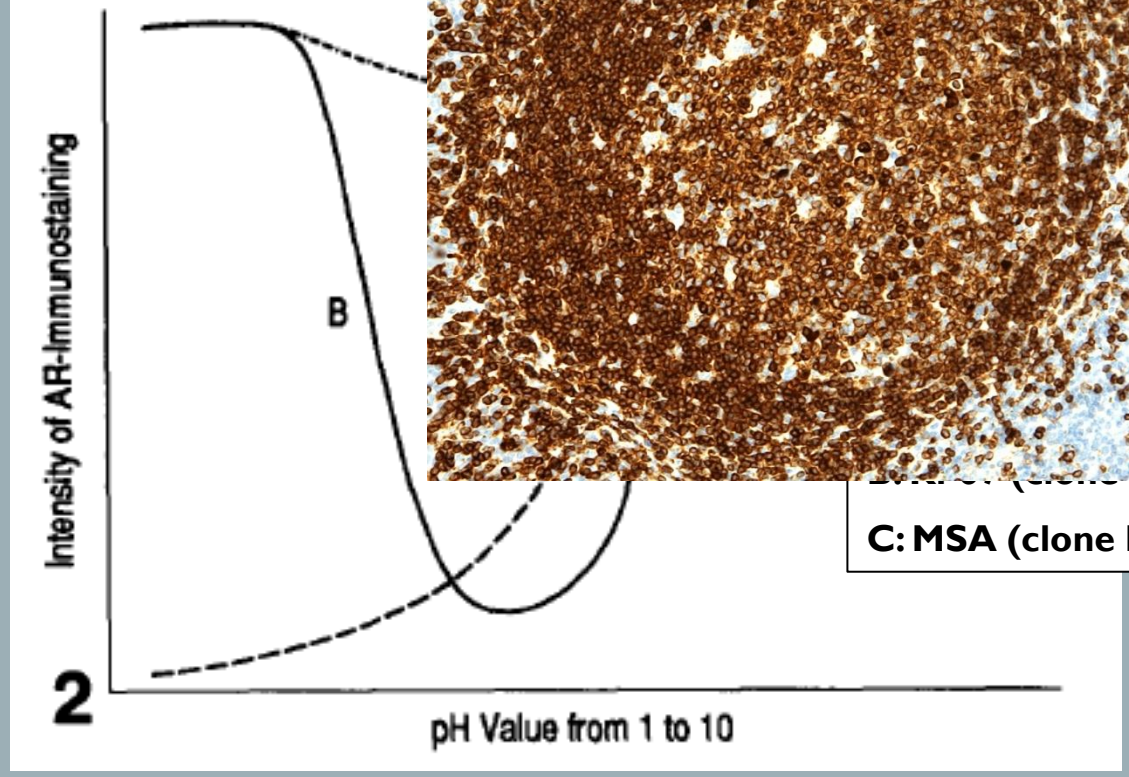
tions after pre-treatment of the slides with this method. These results showed that after antigen retrieval: (a) enzyme pre-

Fig. 7 Conventional hypothesis (**A**). Formaldehyde fixation can alter the three-dimensional structure of the epitope cross-linkages; these can be reversed by high-temperature heating. Our suggested mechanism for AR in IHC (**B**): Antibodies recognizing molecular surface epitopes, such as ERα MC-20, do not show increases in detection levels with or without heating whereas antibodies recognizing intramolecular epitopes, such as ER 6F11, show significantly increased detection levels because the three-dimensional structure is likely to be altered by heat denaturation



HIER

Shi SR et al. *J Histoch*

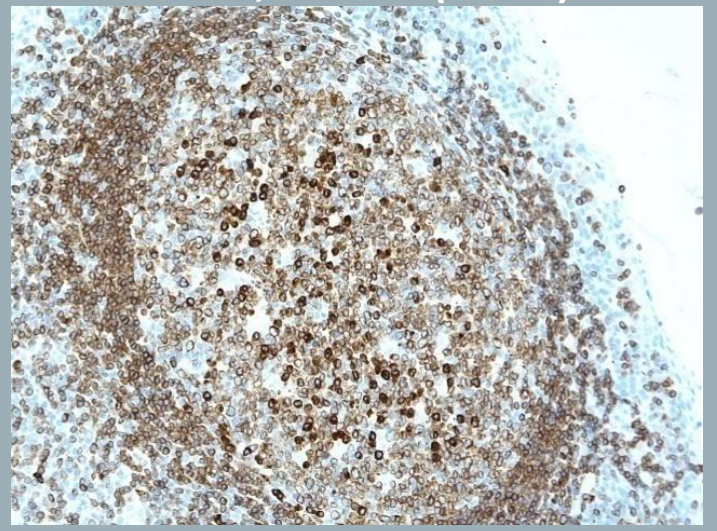


CD79, JCB117 (1:50)

C: MSA (clone HMB45)

HIER in TRS pH 6.1
(20 min at 97°C)

CD79, JCB117 (1:300)



HIER in TRS pH 9
(20 min at 97°C)

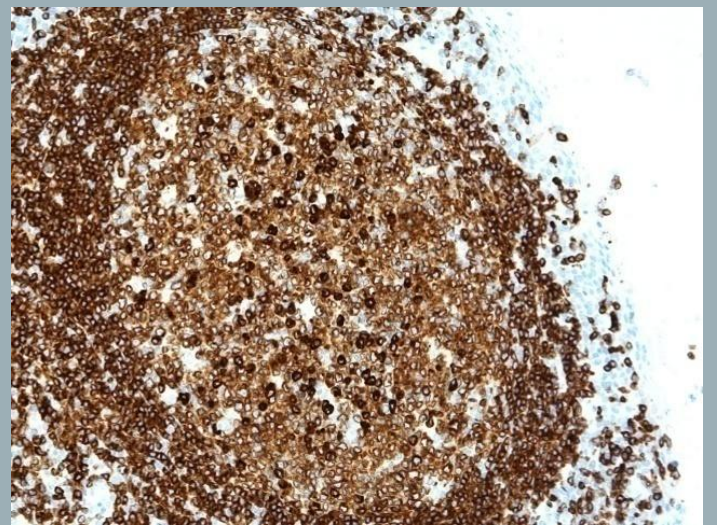


Foto fra Michael Bzorek

Alternatives for HIER

- Proteolytic pretreatment

Enzyme	Typical conc.	Activation Temperature	Typical Incubation time	Cleavage nature
Proteinase K	0.1%, pH 8.0	25-37 °C	5-10 min.	Broad - all amino acids
Trypsin	0.1-0.25%, pH 7.6	37 °C	10 min.	Arginin / Lysin
Pepsin	0.2-0.4%, pH 2.0	37 °C	5-20min.	Broad, favors peptider with aromatic amino-groups
Protease XXIV	0.05-0.1%, pH 7.6	37 °C	5-10 min.	Broad - all amino acids
Protease XIV	0.05-0.1%,pH 7.6	25-37 °C	10-30min.	Broad, favors peptidse with aromatic recidues

Courtesy of Michael Bzorek

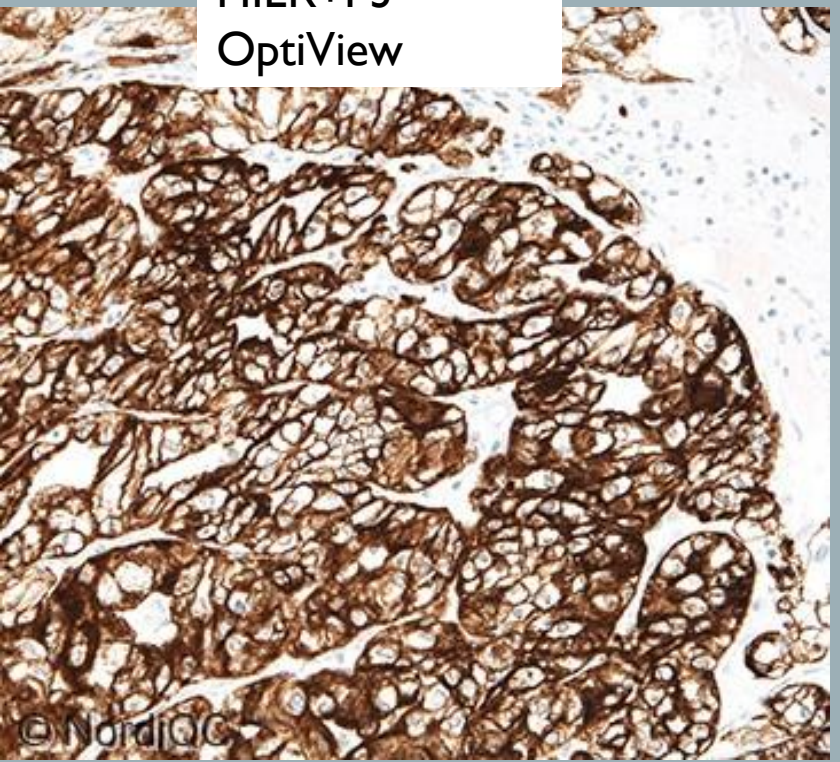
Alternatives for HIER
- Proteolytic pretreatment

Table 4. **Pass rates for antibody cocktails combined with epitope retrieval methods in nine NordiQC runs**

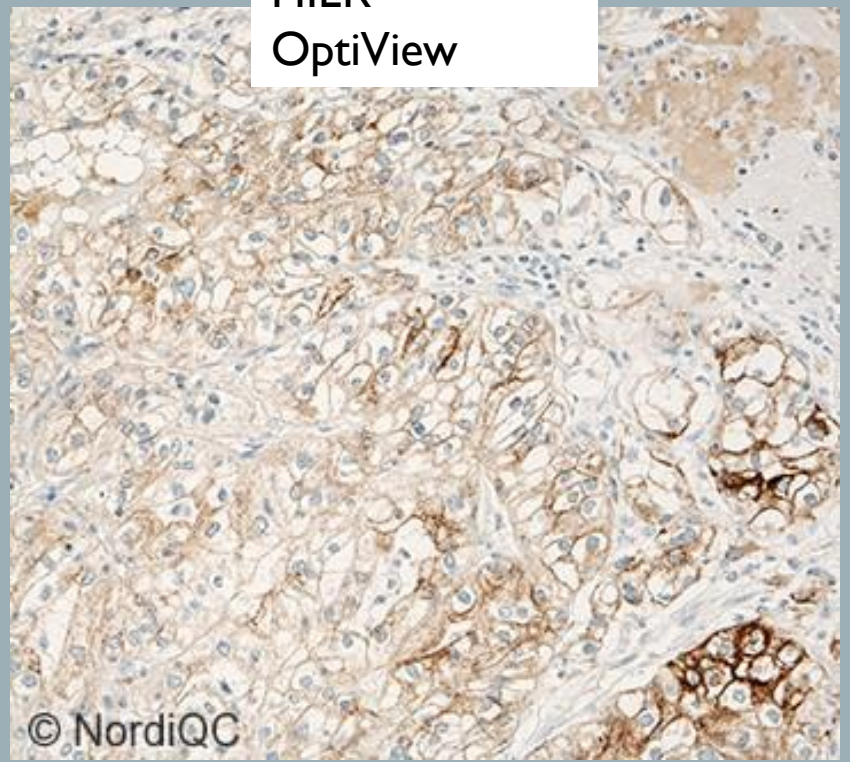
Pass rate for compiled data from run 15, 20, 24, 30, 36, 41, 47, 54 & 58								
	Total		HIER		Proteolysis		HIER + proteolysis	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb AE1/AE3	1145	836 (73%)	1075	826 (77%)	49	6 (12%)	9	3 (33%)
mAb AE1/AE3/5D3	48	42 (88%)	47	42 (89%)	1	0	0	0
mAb AE1/AE3/PCK26	361	219 (61%)	48	22 (46%)	48	3 (6%)	258	192 (74%)
mAb MNF116	111	31 (28%)	53	9 (17%)	48	22 (46%)	9	2 (22%)

Ventana RTU AE1/AE3/PCK26

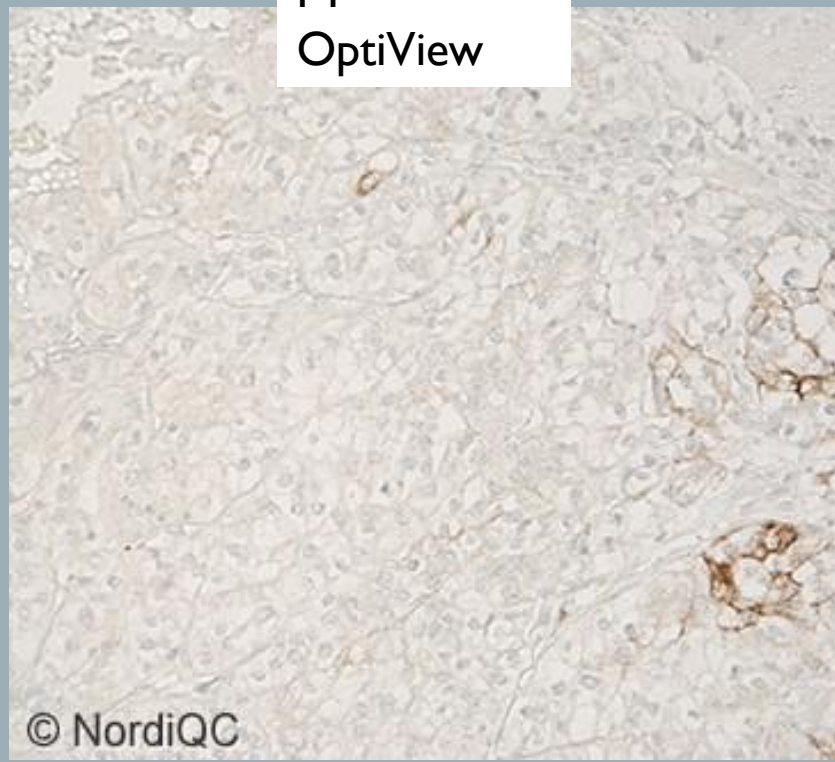
HIER+P3
OptiView



HIER
OptiView



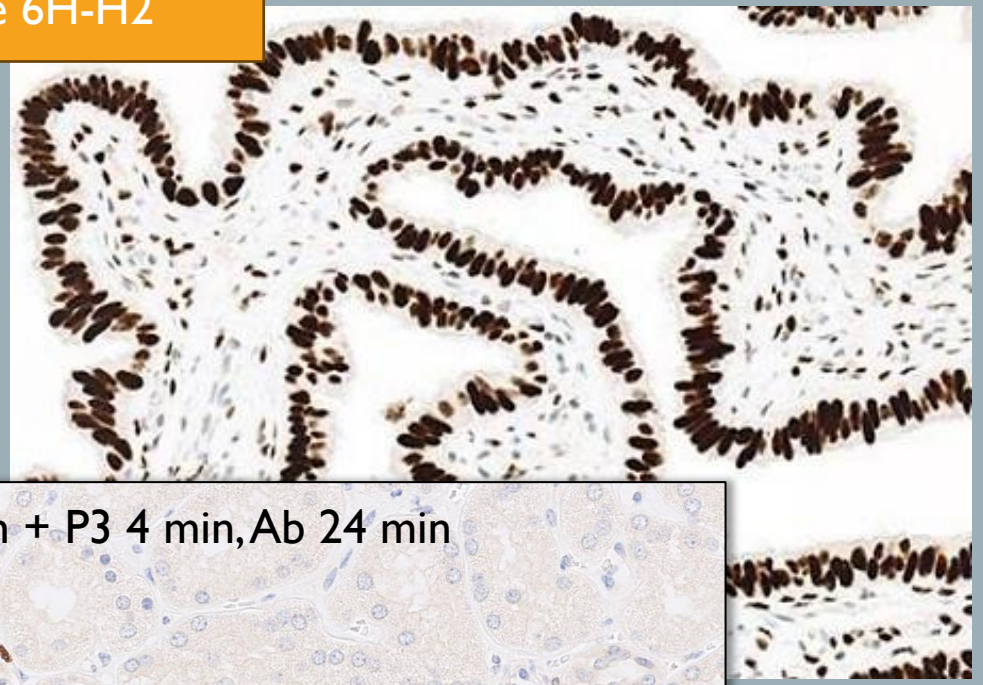
P1
OptiView

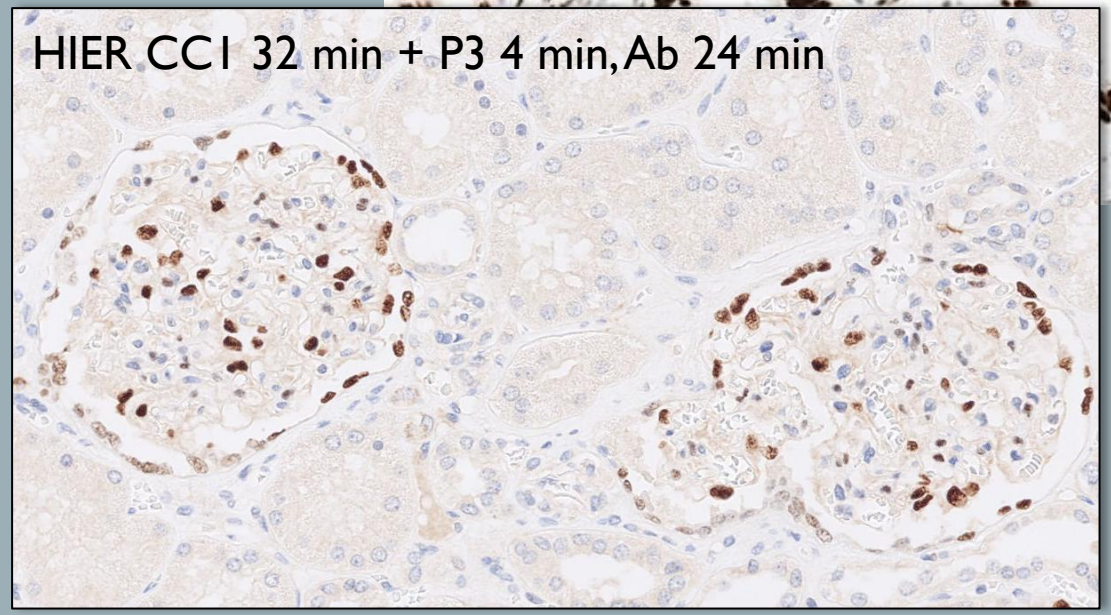


Alternatives for HIER

- Proteolytic pretreatment

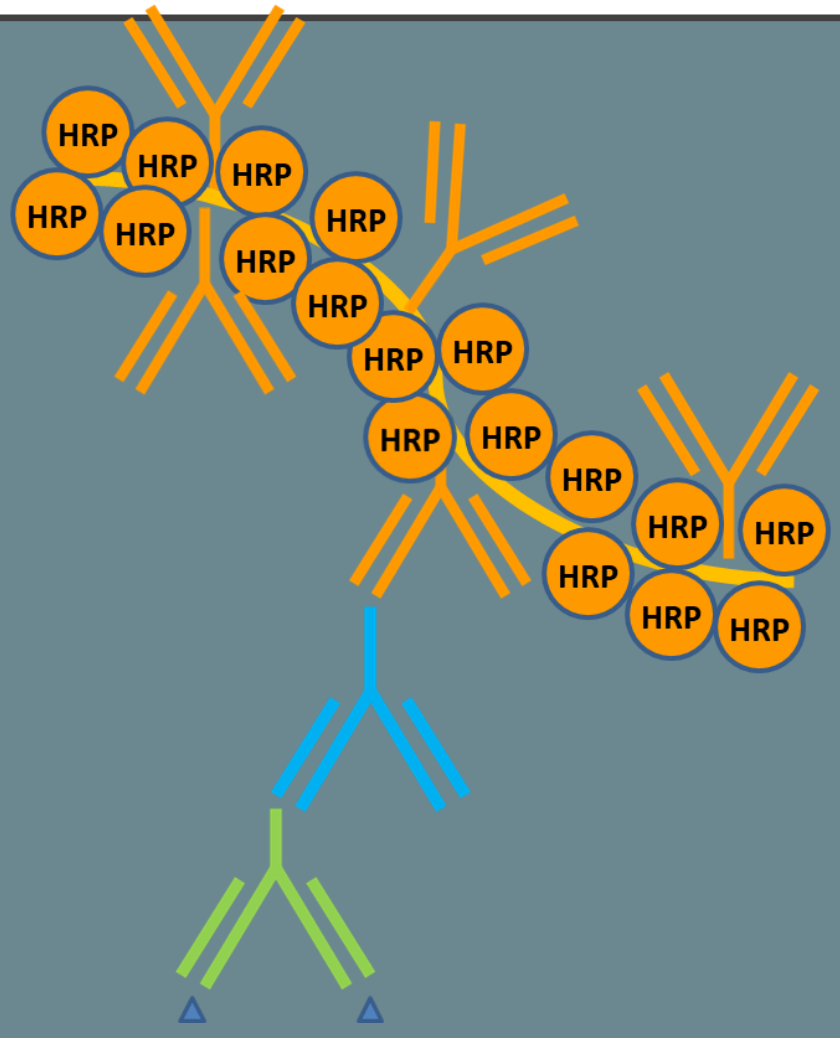
WT1 – clone 6H-H2



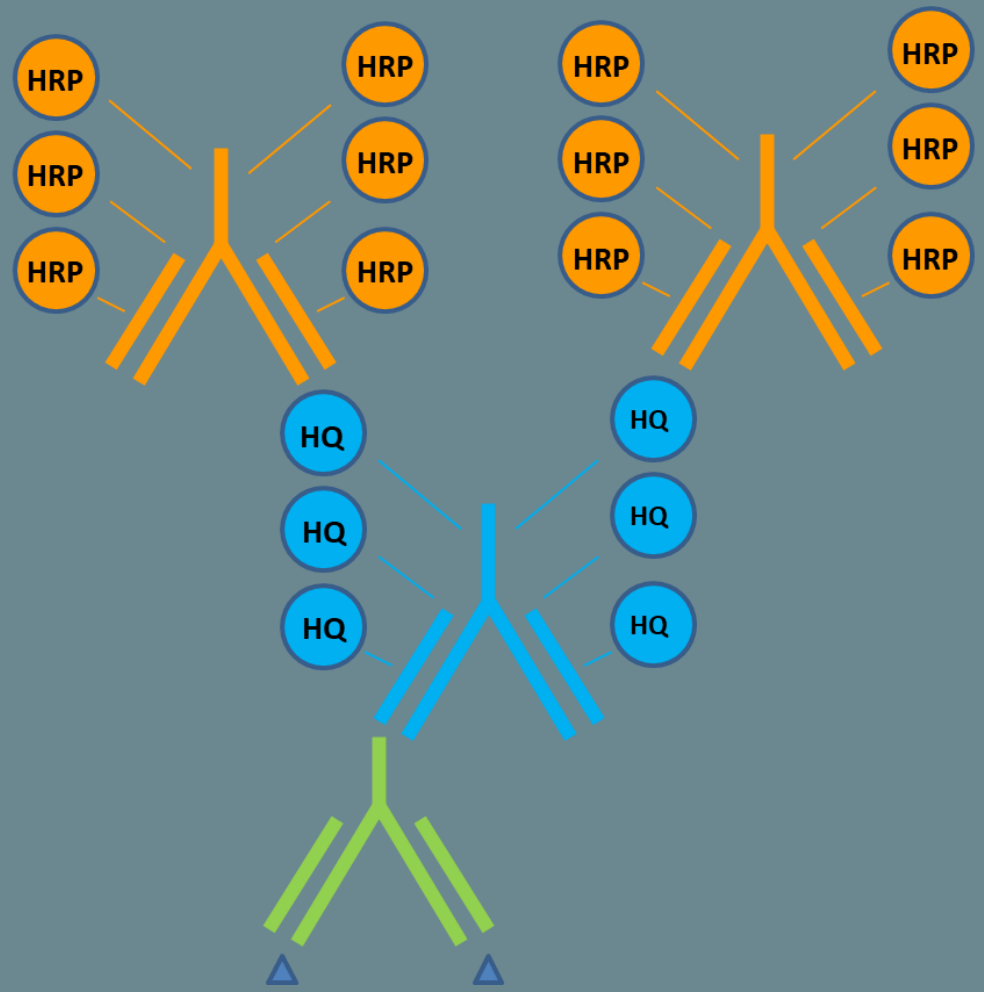


Recommended staining protocol with OptiView	
Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, 32 minutes
Enzyme (Protease)	Not required
Pre-primary peroxidase inhibition	Selected
Antibody (Primary)	BenchMark ULTRA or ULTRA PLUS instrument: 32 minutes, 36°C BenchMark XT instrument: 32 minutes, 37°C BenchMark GX instrument: 32 minutes, 37°C
OptiView HQ Linker	8 minutes
Optiview HRP Multimer	8 minutes
Amplification	Not selected
Counterstain	Hematoxylin II, 8 minutes
Post Counterstain	Bluing, 4 minutes

Detectionssystems



Polymer detection systems – Envision flex (Omnis), Bond Refine

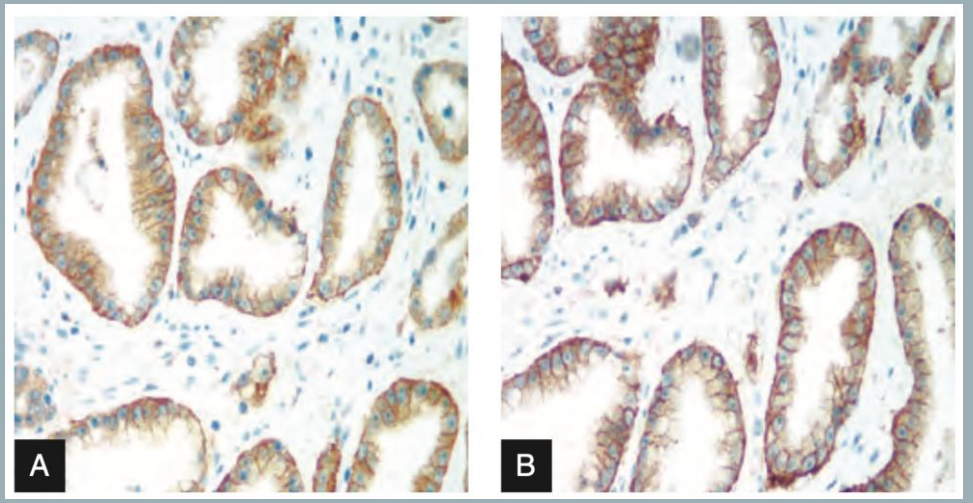


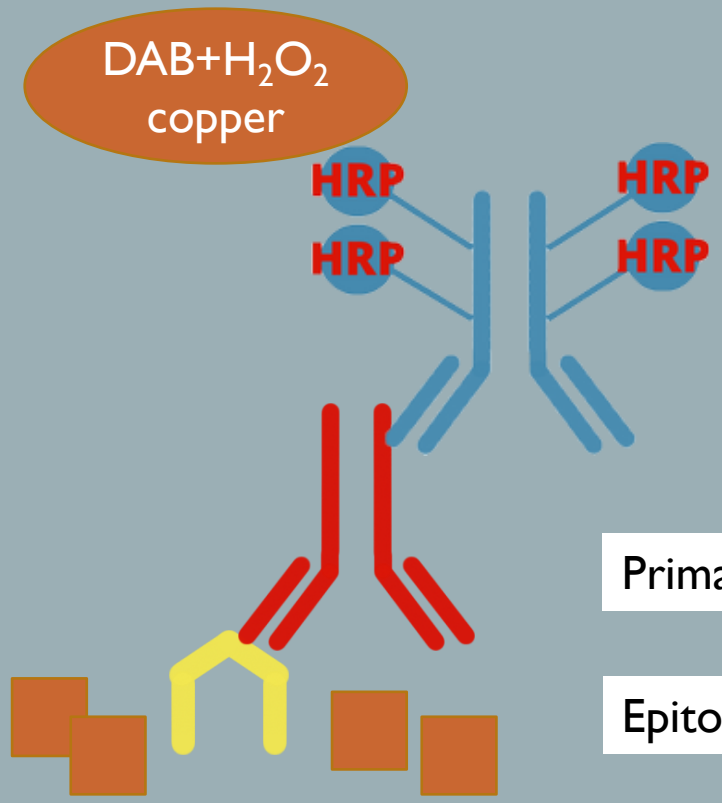
Multimer detection systems – OptiView, UltraView (Ventana)

UltraView – 2-layer system

Without copper

With copper



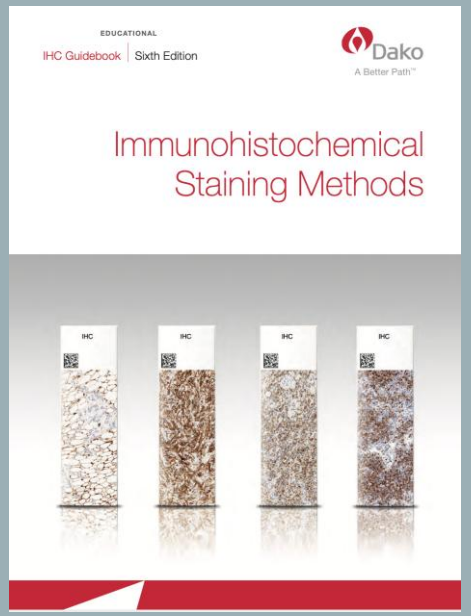


HRP multimer

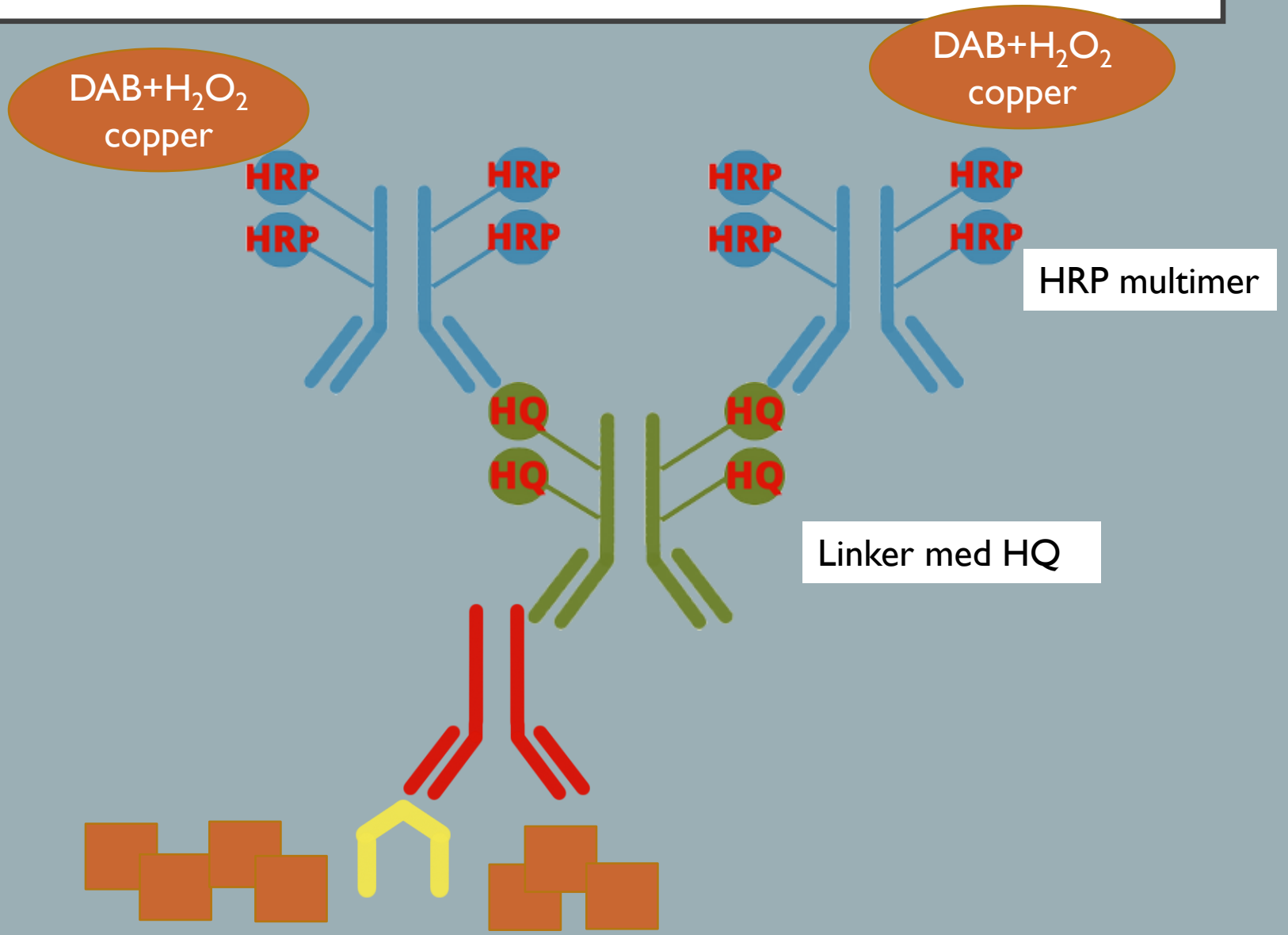
Primary Ab

Epitope

UltraView Universal HRP Multimer is a cocktail of HRP labeled antibodies (goat anti-mouse IgG, goat anti-mouse IgM, and goat anti-rabbit)



Optiview – 3 layer system



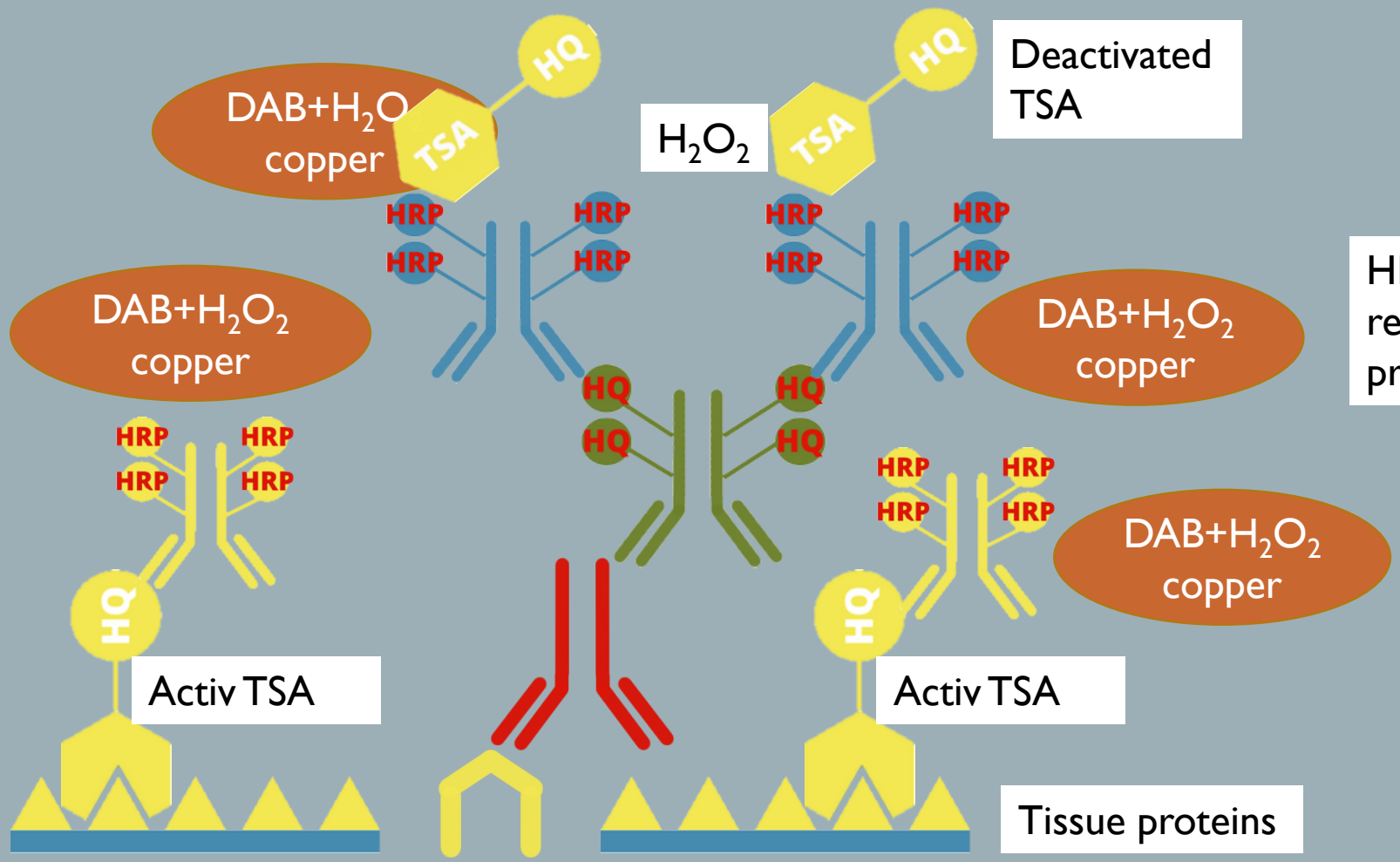
HRP multimer

Linker med HQ

OptiView HRP Multimer is a monoclonal mouse anti HQ-labeled HRP antibody

OptiView HQ Universal Linker is a cocktail of HQ-labeled (HQ is a hapten which covalent binds to goat antibodies) antibodies (goat anti-mouse IgG, goat mouse IgM, and goat anti-rabbit)

OPTIVIEW + AMP



HRP catalysis the oxidation of tyramide to reactive frie radicals, which binds to tissue proteins

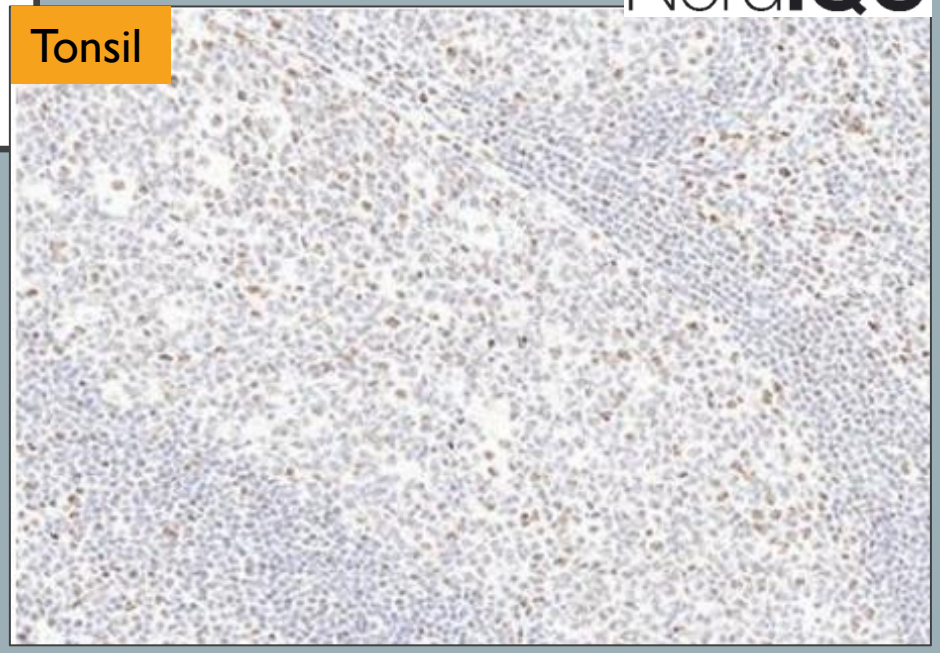
Introducing Bap-1

Important marker to identify mutation of the BRCA1 protein 1. Mutation will present itself as a negative nuclear reaction in 77-84% of metastatic melanomas and 50-70% of mesotheliomas.

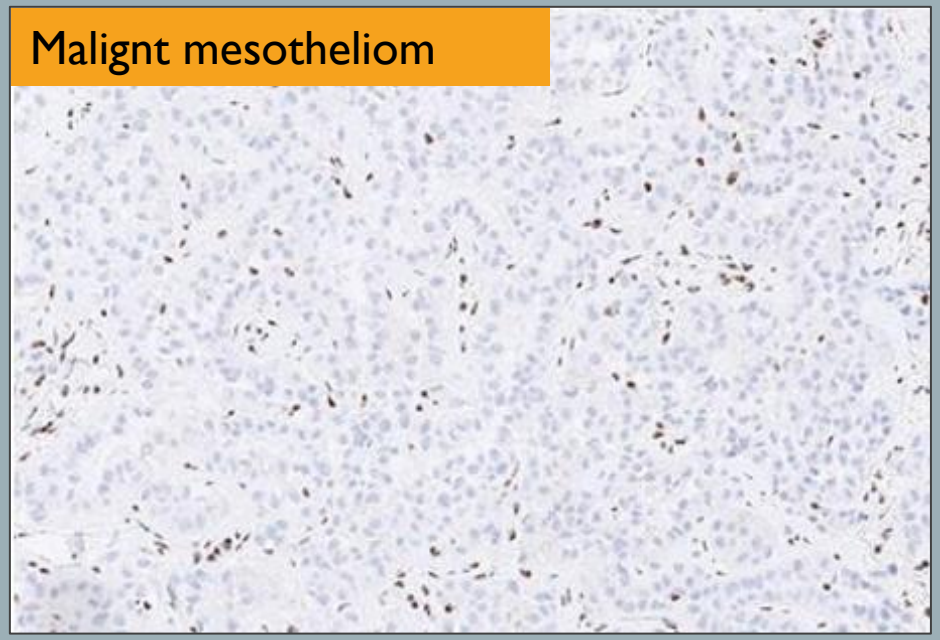
Criteria

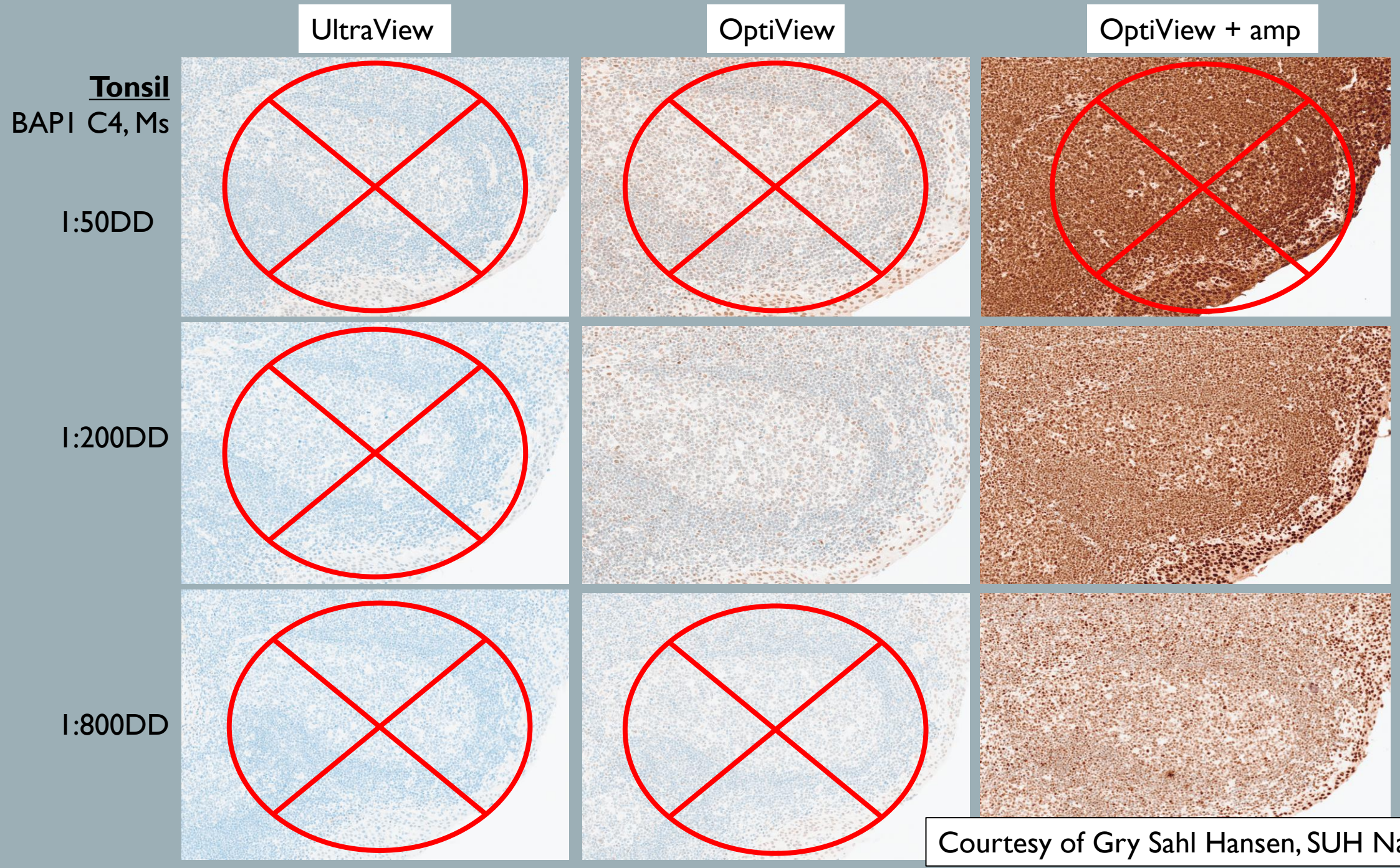
- At least weak nuclear staining in the majority of mantle zone lymphocytes in the tonsil.
- A weak to moderate nuclear staining in virtually all germinal center lymphocytes.
- At least weak to moderate nuclear staining of most stromal cells in both malignant mesotheliomas.
- No nuclear staining of neoplastic cells in malignant mesotheliomas. A weak cytoplasmic reaction is acceptable.

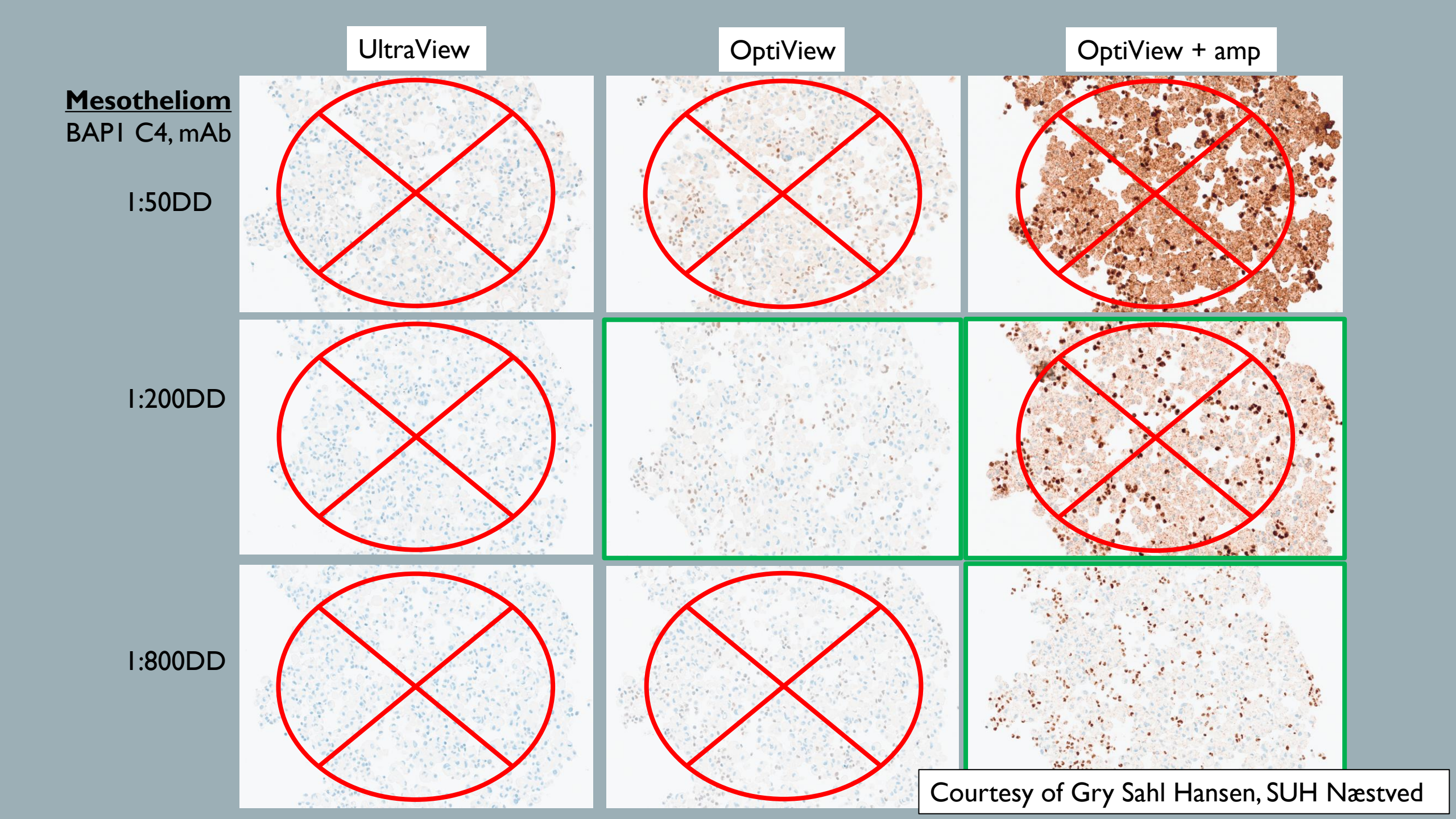
Tonsil



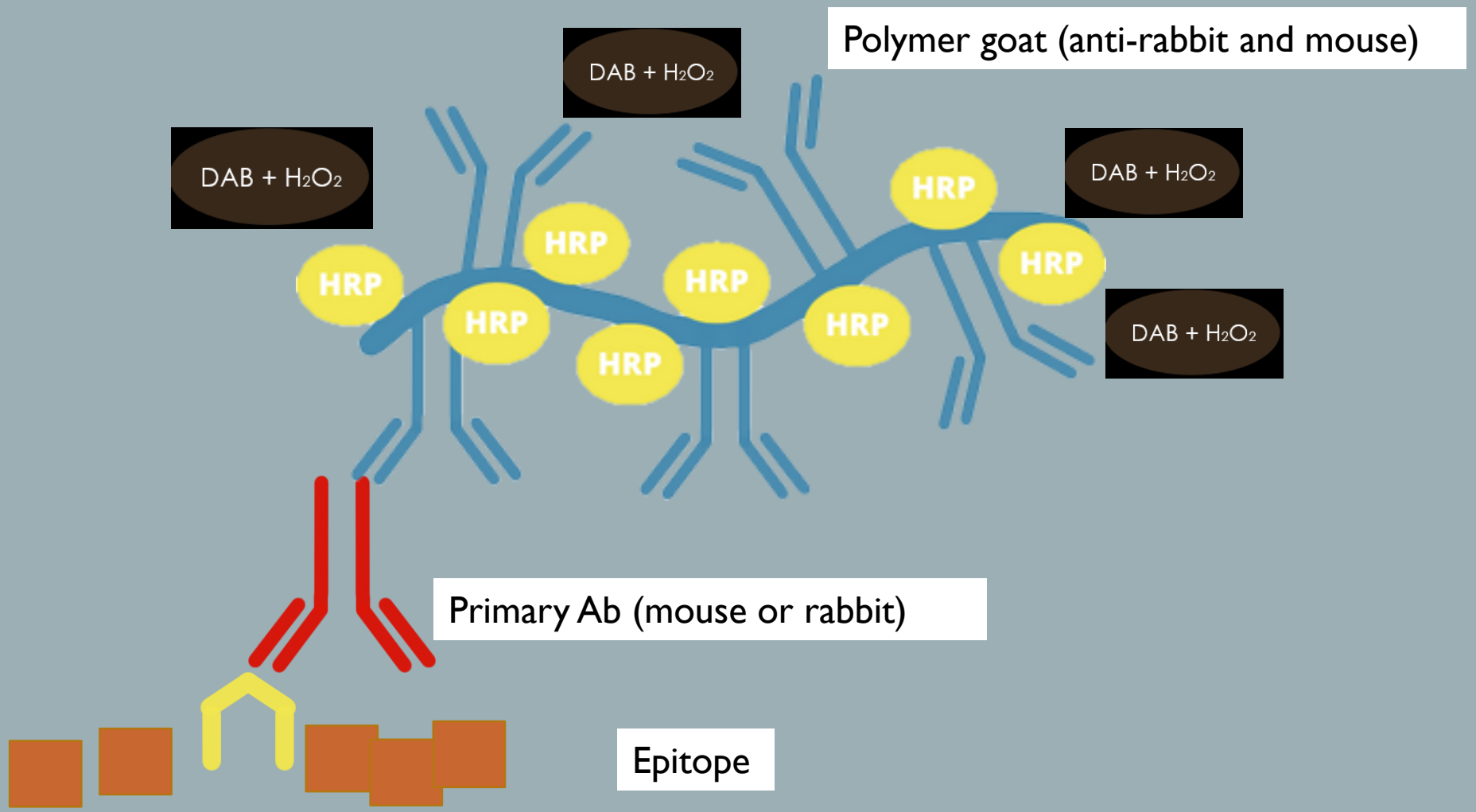
Malignt mesotheliom




UltraView
OptiView
OptiView + amp
Tonsil
BAP1 C4, Ms
1:50DD
1:200DD
1:800DD
Courtesy of Gry Sahl Hansen, SUH Næstved

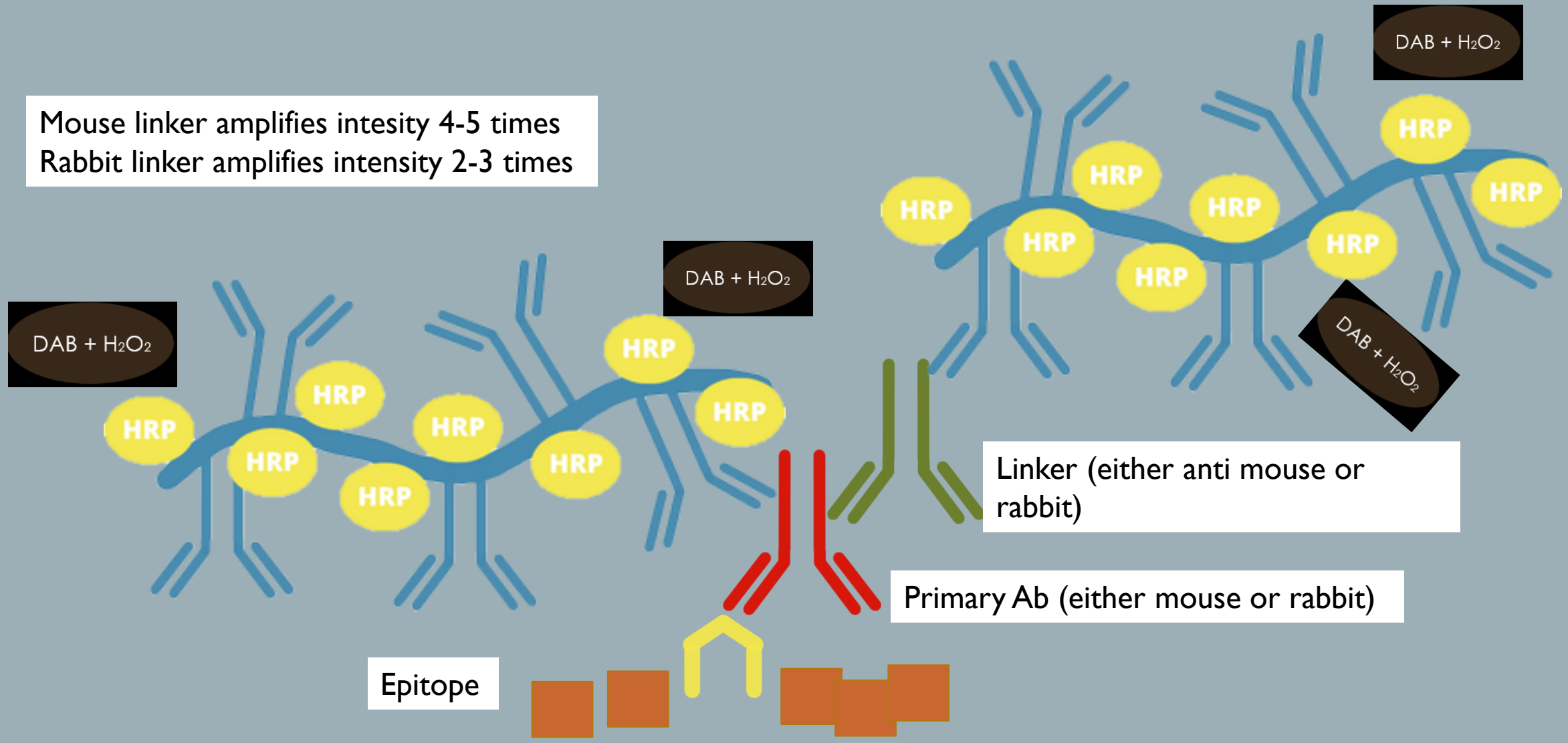

UltraView
OptiView
OptiView + amp
Mesotheliom
BAP1 C4, mAb
1:50DD
1:200DD
1:800DD
Courtesy of Gry Sahl Hansen, SUH Næstved

Envision Flex – 2 layer system

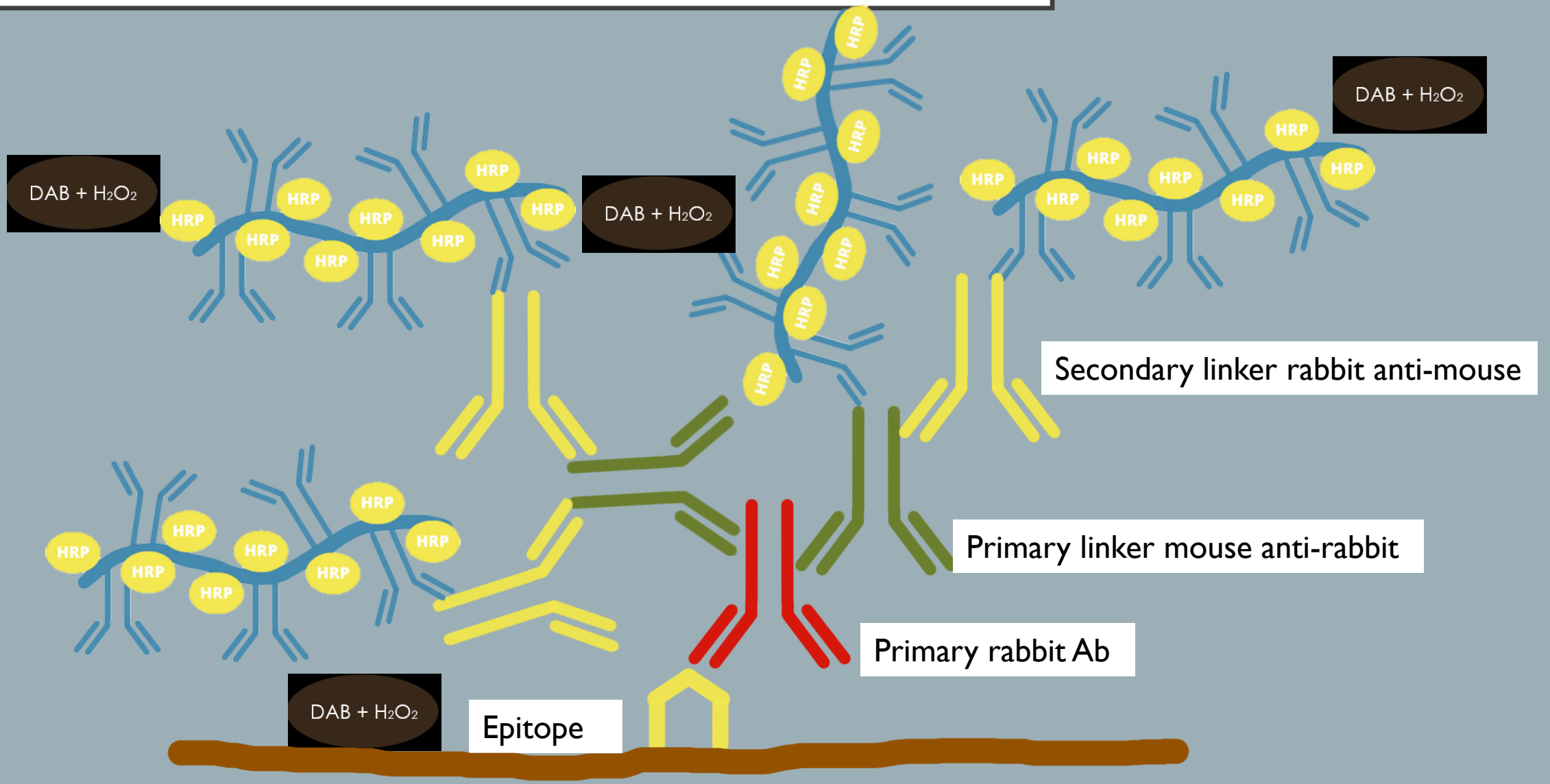


Envision Flex+ - 3 layer system

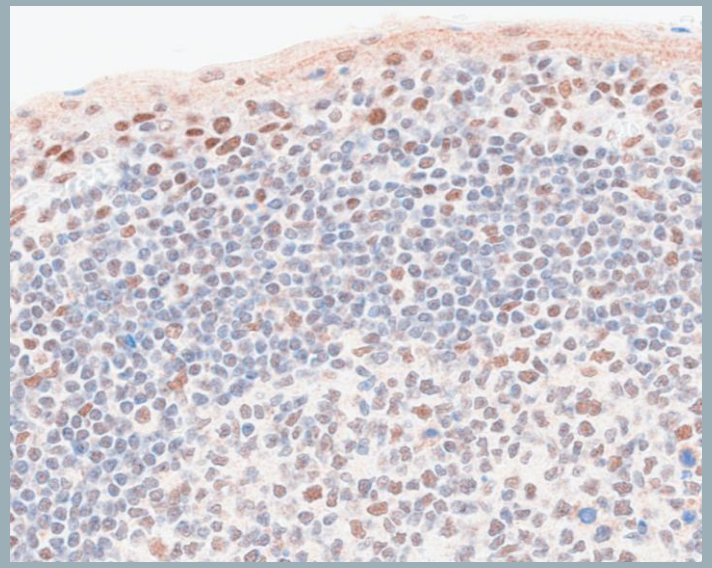
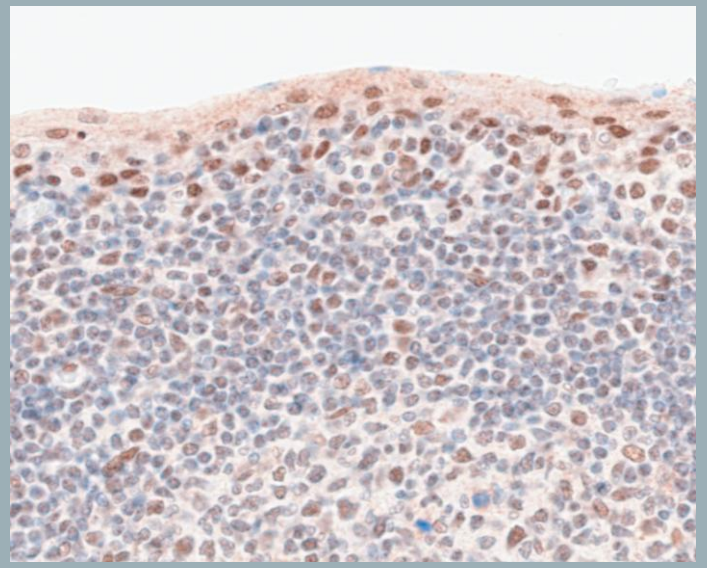
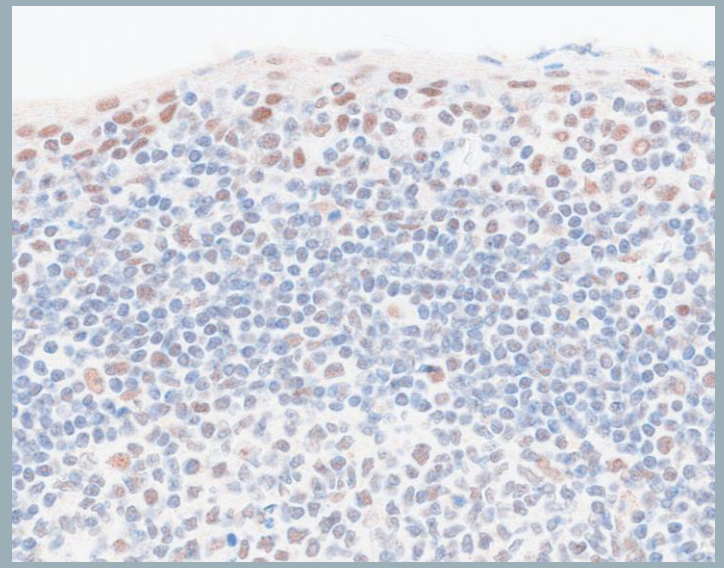
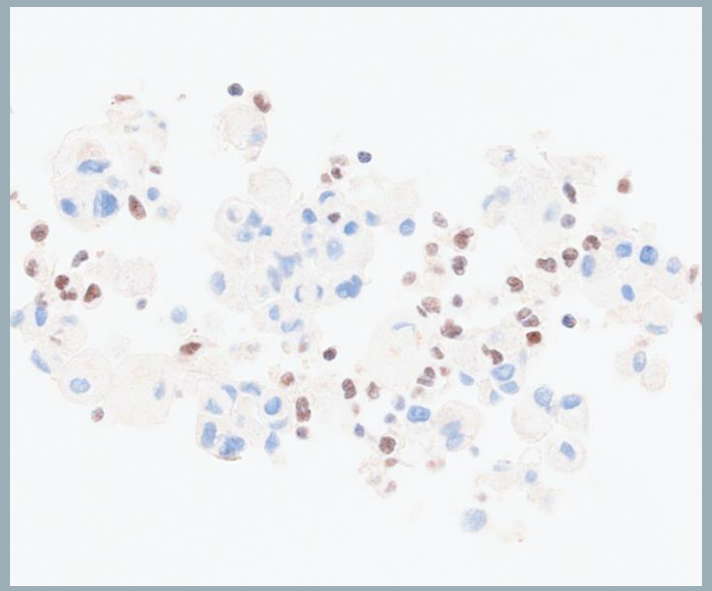
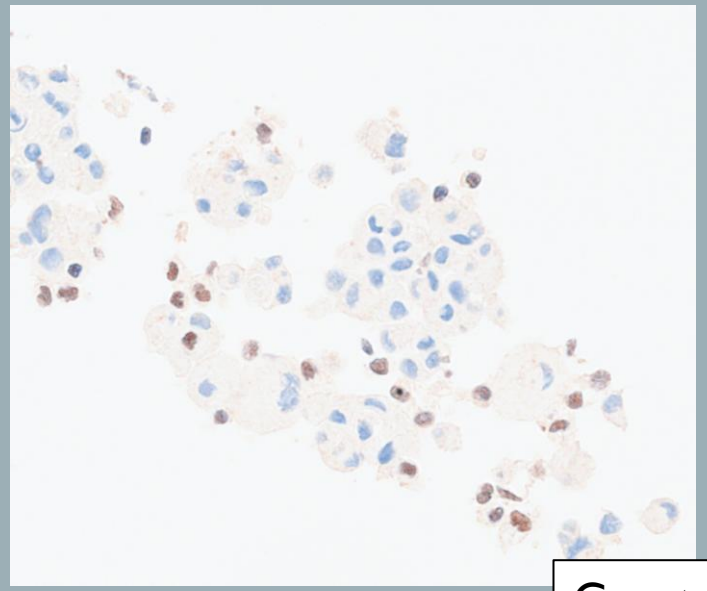
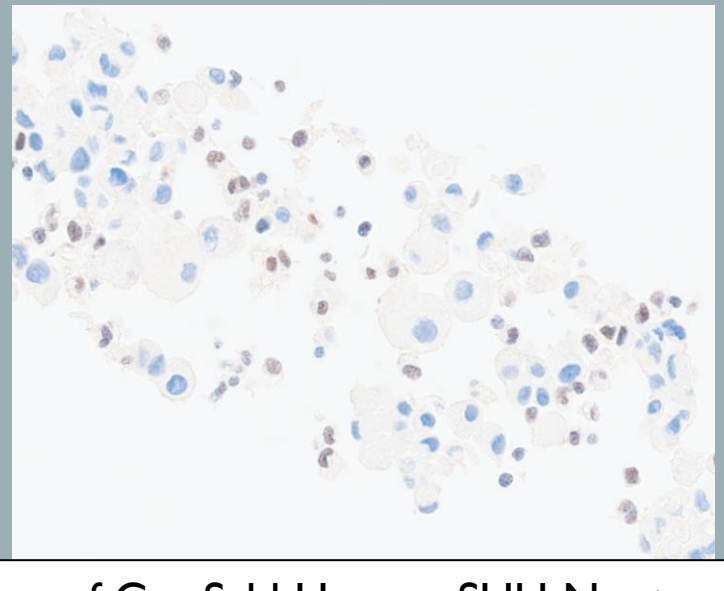
Mouse linker amplifies intesity 4-5 times
Rabbit linker amplifies intensity 2-3 times



Envision Flex++ - 4 layer system

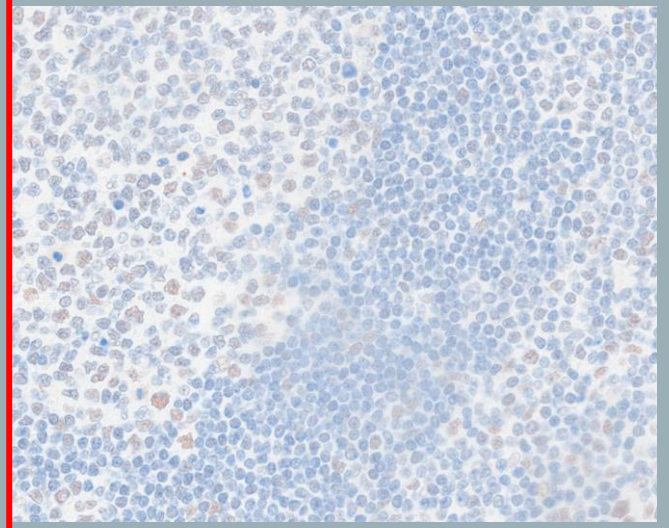
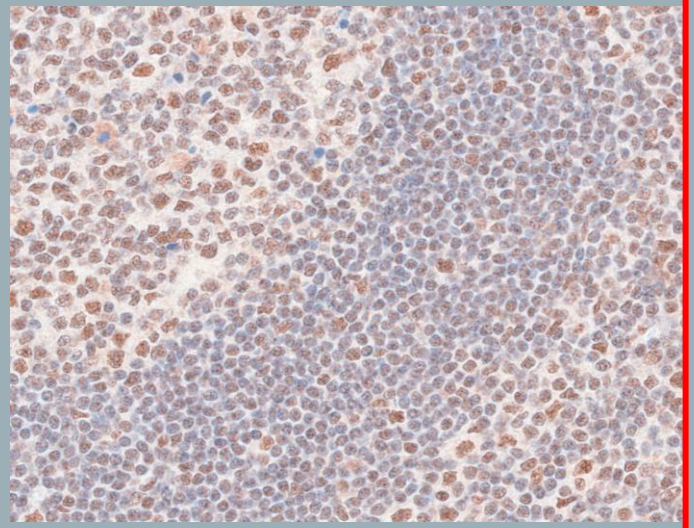
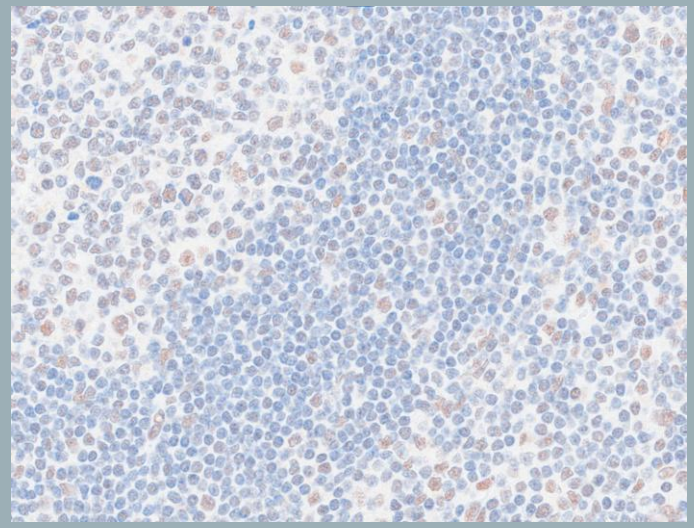
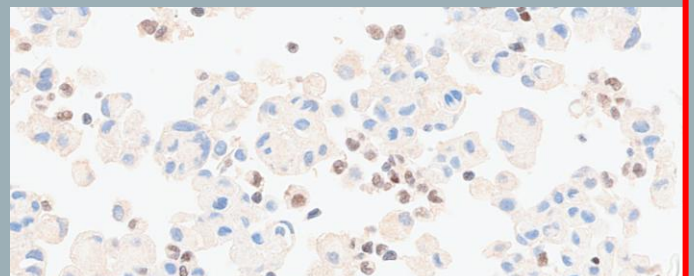
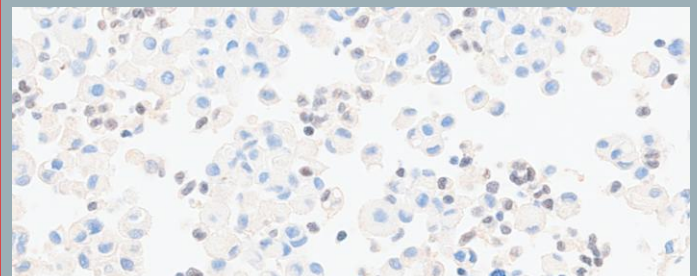


BAP1 C4, Santa Cruz mAb

	Flex+ 1:200DD	Flex++ 1:200DD	Flex++ 1:800DD
Tonsil			
Mesothelioma			

Courtesy of Gry Sahl Hansen, SUH Næstved

BAP1 EPR22826-65 Apcam rmAb

	Flex+ 1:200RR	Flex++ 1:200RR	Flex++ 1:800RR
Tonsil			
Mesothelioma			

Summary and Explanation

Dako EnVision FLEX+ detection system, has been designed to be a flexible system and gives an optimal staining on Autostainer Link instruments, when using the protocol recommended in this package insert. As a guideline, using EnVision FLEX+ Mouse (Code K8002) signal amplification increases **4-5 fold** and using EnVision FLEX+ Rabbit (Code K8009, optional reagent) increases signal amplification **2-3 fold.**

Courtesy of Gry Sahl Hansen, SUH Næstved

VALIDATION PROCESS

Recommendations for Improved Standardization of Immunohistochemistry

Neal S. Goldstein, MD, Stephen M. Hewitt, MD, PhD, Clive R. Taylor, MD, DPhil, Hadi Yaziji, MD, David G. Hicks, MD, and Members of Ad-Hoc Committee On Immunohistochemistry Standardization

Abstract: Immunohistochemistry (IHC) continues to suffer from variable consistency, poor reproducibility, quality assurance disparities, and the lack of standardization resulting in poor concordance, validation, and verification. This document lists the recommendations made by the Ad-Hoc Committee on Immunohistochemistry Standardization to address these deficiencies. Contributing factors were established to be underfixation and irregular fixation, use of nonformalin fixatives and ancillary fixation procedures divested from a deep and full understanding of the IHC assay parameters, minimal or absent IHC assay optimization and validation procedures, and lack of a standard system of interpretation and reporting. Definitions and detailed guidelines pertaining to these areas are provided.

Key Words: immunohistochemistry, pathology, assay, oncology, standardization, procedure, tissue, fixation

(*Appl Immunohistochem Mol Morphol* 2007;15:124–133)

IHC assay standardization was vital for reproducible and reliable results. Agencies, including the Biologic Stain Commission, CLSI (previously NACCLS), FDA, and the manufacturing sector established guidelines, standards, and recommendations for reagents and package inserts. These efforts have resulted in consistent, high-quality assay components and instruments on which contemporary IHC is performed.[1–4] It has also allowed the development and use of so-called black box IHC stainers in which IHC assays have preset parameters set by the manufacturer.[5]

Despite the improvements of reagents and automation, authors over the years have consistently noted the inconsistent quality of IHC assays.[6–11] Unlike previous IHC-epochs, most of the causative responsibility rests with the individual laboratory performing the IHC and specifically, the lack of standardization and attention to quality assurance programs.[12,13] Prior consensus conferences identified the likely causative factors (Table 1).[14]

Principles of Analytic Validation of Immunohistochemical Assays

Guideline From the College of American Pathologists Pathology and Laboratory Quality Center

Patrick L. Fitzgibbons, MD; Linda A. Bradley, PhD; Lisa A. Fatheree, BS, SCT(ASCP); Randa Alsabeh, MD; Regan S. Fulton, MD, PhD; Jeffrey D. Goldsmith, MD; Thomas S. Haas, DO; Rouzan G. Karabakhtsian, MD, PhD; Patti A. Loykasek, HT(ASCP); Monna J. Marolt, MD; Steven S. Shen, MD, PhD; Anthony T. Smith, MLS; Paul E. Swanson, MD

• ***Context.***—**Laboratories must validate all assays before they can be used to test patient specimens, but currently there are no evidence-based guidelines regarding validation of immunohistochemical assays.**

Objective.—**To develop recommendations for initial analytic validation and revalidation of immunohistochemical assays.**

Design.—**The College of American Pathologists Pathology and Laboratory Quality Center convened a panel of pathologists and histotechnologists with expertise in immunohistochemistry to develop validation recommendations. A systematic evidence review was conducted to address key questions. Electronic searches identified 1463 publications, of which 126 met inclusion criteria and were extracted. Individual publications were graded for quality, and the key question findings for strength of evidence. Recommendations were derived from strength of evidence, open comment feedback, and expert panel consensus.**

Results.—**Fourteen guideline statements were established to help pathology laboratories comply with validation and revalidation requirements for immunohistochemical assays.**

Conclusions.—**Laboratories must document successful analytic validation of all immunohistochemical tests before applying to patient specimens. The parameters for cases included in validation sets, including number, expression levels, fixative and processing methods, should take into account intended use and should be sufficient to ensure that the test accurately measures the analyte of interest in specimens tested in that laboratory. Recommendations are also provided for confirming assay performance when there are changes in test methods, reagents, or equipment.**

(*Arch Pathol Lab Med.* 2014;138:1432–1443; doi: 10.5858/arpa.2013-0610-CP)

Accepted for publication February 3, 2014.
Published as an Early Online Release March 19, 2014.

AARHUS TEST PANEL

Omnis	Test 1	Test 2	Test 3	Test 4 (take a chance)
Dilution:	1:50	1:100	1:200	Optional
A (Standard)	TRS high 30, Ab 20	TRS high 30, Ab 20	TRS high 30, Ab 20	Optional
B (Standard)	TRS low 30, AB 20	TRS low 30, AB 20	TRS low 30, AB 20	Optional
C (Standard+)	TRS High 30, AB 20, Linker	TRS High 30, AB 20, Linker	TRS High 30, AB 20, Linker	Optional
D (Secondary)	Flex ++	Flex ++	Flex ++	Flex ++
E (Secondary)				

Ventana	Test 1	Test 2	Test 3	Test 4 (take a chance)
Dilution:	1:50	1:100	1:200	Optional
A (Standard) OptiView	HIER CC1 32, Ab 32	HIER CC1 32, Ab 32	HIER CC1 32, Ab 32	Optional
B (Standard) OptiView	HIER CC1 64, AB 32	HIER CC1 64, AB 32	HIER CC1 64, AB 32	Optional
C (Secondary)				P1 8 min, Ab 32
D (Secondary)				P3 4 min, CC1 32, AB 32

On our Omnis we try to use only one HIER time, which is 30 minutes, to ensure that as many slides can fit together as possible. We therefore only adjust;

- HIER pH
- Concentration
- Ab incubation
- Detectionslayer.

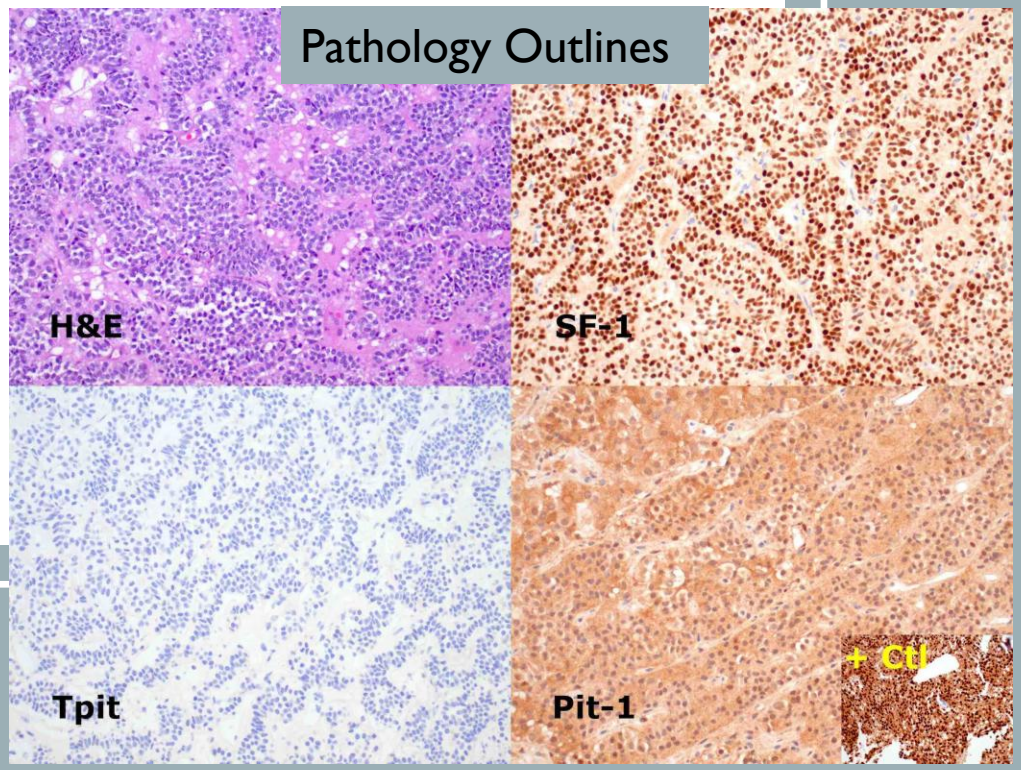
Ab that cannot function under these conditions are changed to another platform or replaced with other clones

A few Ab require enzymatic pretreatment to function properly, but this is not standard in the laboratory. Less than 8% of our 353 Ab use proteolysis, and only 3% as a stand-alone pretreatment.

Introducing SF-1

- steroidogen faktor 1

- New WHO guidelines require a panel of, among others, SF-1, TPIT and PIT-1 for classification of pituitary adenomas.
- SF-1 detects adenomas in the pituitary gland that arise from gonadotroph cells
- SF-1 can also be used to differentiate adrenal cortical lesions and renal clear cell carcinomas
- Detects testicular tumors that arise from Leydig cells and Sertoli cells.



Modern Pathology (2018) 31:900–909
https://doi.org/10.1038/s41379-018-0016-8

Epidemiology and biomarker profile of pituitary adenohypophysial tumors

Ozgur Mete[1,2] · Amber Cintosun[1,2] · Irwin Pressman[3] · Sylvia L. Asa[1,2]

Introducing SF-1
- steroidogen faktor 1

THE HUMAN PROTEIN ATLAS

RESOURCES ABOUT NEWS LEARN DATA H

NR5A1

Steroidogenic factor 1 Search

Search result (4 genes): **NR5A1** | SOX9 | ESRRG ...

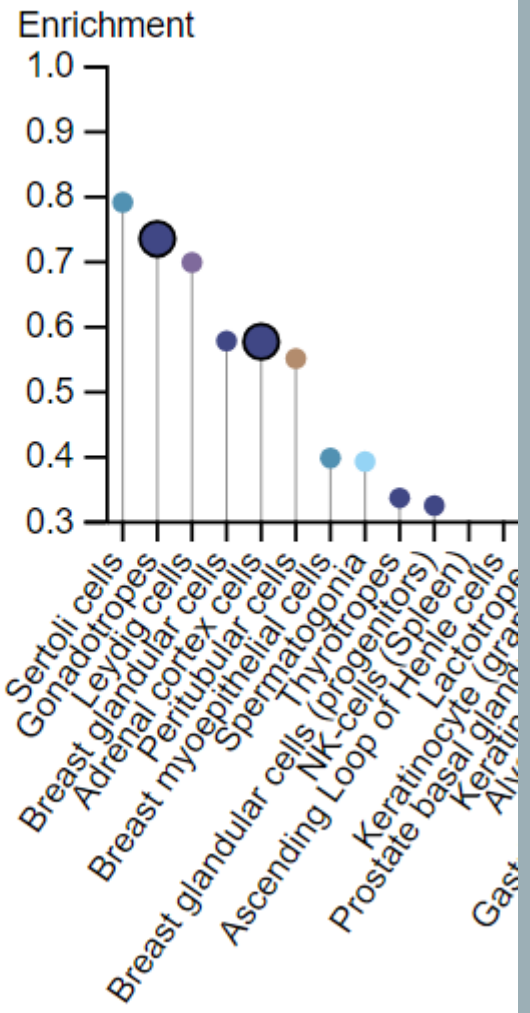
SUMMARY TISSUE BRAIN SINGLE CELL SUBCELL CANCER BLOOD CELL LINE STRUCT & INT

PROTEIN SUMMARY
GENE INFORMATION
RNA DATA
ANTIBODY DATA

CELL TYPE RNA EXPRESSION

Single cell type specificity[i]	Group enriched (Granulosa cells, Ovarian stromal cells, Leydig cells, Sertoli cells, Peritubular cells)
Single cell type expression cluster[i]	Ovarian stromal cells - Transcription (mainly)
Tissue cell type classification[i]	Cell type enriched (Adrenal gland - Adrenal cortex cells, Pituitary gland - Gonadotropes, Spleen - Enc cells)
Immune cell specificity[i]	Not detected in immune cells
Immune cell expression cluster[i]	Not detected - no cluster assigned

Tissue specific cell types[i]



Manifacture	Clone	IVD - status
Invitrogen	mmAb N1665	RUO
Cell Marque	RmAb EP434	IVD(-R)

I WANT TO IMPLEMENT SF-1 IHC STAINING WHAT SHOULD I TEST ON?

https://chatgpt.com

1. **Run-to-run and initial validation controls**
   - **Positive control block(s)**:
     - *Normal adrenal cortex* (best single positive control). SF-1 shows strong nuclear staining in zona fasciculata/glomerulosa. cellmarque.com Oxford Academic
     - *Normal testis* (Leydig cells / Sertoli elements) or *ovary* (granulosa/theca cells) as additional positive controls if available. cellmarque.com
     - **Include an adrenocortical carcinoma or adenoma** slide in your validation set (if available) because SF-1 is used diagnostically for adrenal cortical origin. PubMed PMC
   - **Negative control block(s)**:
     - Tissue known to lack SF-1 (examples: colonic mucosa, skin epidermis, many non-endocrine carcinomas). Also include an **isotype or no-primary** control slide for each run. PMC
2. **Validation tumor / mimic panel** — include representative cases so you can see performance across likely clinical scenarios:
   - **Adrenocortical adenoma and carcinoma** (several cases, varying expression). PubMed
   - **Sex cord–stromal tumors** (granulosa cell tumor, Sertoli–Leydig, Leydig cell tumor) — SF-1 is often positive. cellmarque.com
   - **Common mimics/metastases to adrenal**: renal cell carcinoma, hepatocellular carcinoma, metastatic carcinoma (lung, colon) — include negative and borderline cases to ensure specificity. PMC
   - **Other endocrine tissues** if relevant (pituitary/adrenal medulla as negative controls where appropriate).
3. **Case mix and numbers**
   - For a **routine non-predictive** marker: aim for **≥10 positive + 10 negative** validation cases (include

https://www.perplexity.ai

For implementing SF-1 (Steroidogenic Factor 1) IHC staining, the most recommended positive control tissues are **adrenal cortex**, **testis** (particularly Sertoli and Leydig cells), and **adrenocortical carcinoma**. biogenex +2

Recommended Positive Controls

- **Adrenal cortex:** Shows strong, consistent nuclear SF-1 staining and is routinely used as a positive control. pubmed.ncbi.nlm.nih
- **Testis:** Sertoli and Leydig cells in normal, atrophic, or immature testes reliably express SF-1. pubmed.ncbi.nlm.nih
- **Adrenocortical carcinoma and adenoma:** High frequency of SF-1 positivity, useful as controls, especially when tumor tissue is available. academic.oup +1

Negative Controls

- Use renal cell carcinoma, especially papillary and chromophobe subtypes, or other non-steroidogenic tissues as negative controls, as they do not typically express SF-1. pubmed.ncbi.nlm.nih
- Run negative controls each time alongside positive ones to assess background and specificity. biogenex

AI SEARCHING FOR CLONES

www.rndsystems.com
Human SF-1/NR5A1 MAb (Clone N1665) - R&D...

Bio SB, Inc.
SF-1 / Steroidogenic Factor 1 (BSB-149), MMab - Bio SB

abcepta
SF1 Antibody (clone 2E12) - Buy Now! - Abcepta

abcam.com
Steroidogenic factor 1/SF-1 antibody [EPR19744]...

The most commonly used clones for SF-1 immunohistochemistry in the literature are **N1665** and **EP434**, with additional clones like **EPR19744** and **2E12** also appearing in published work. `rndsystems +3`

Frequently Used SF-1 IHC Clones

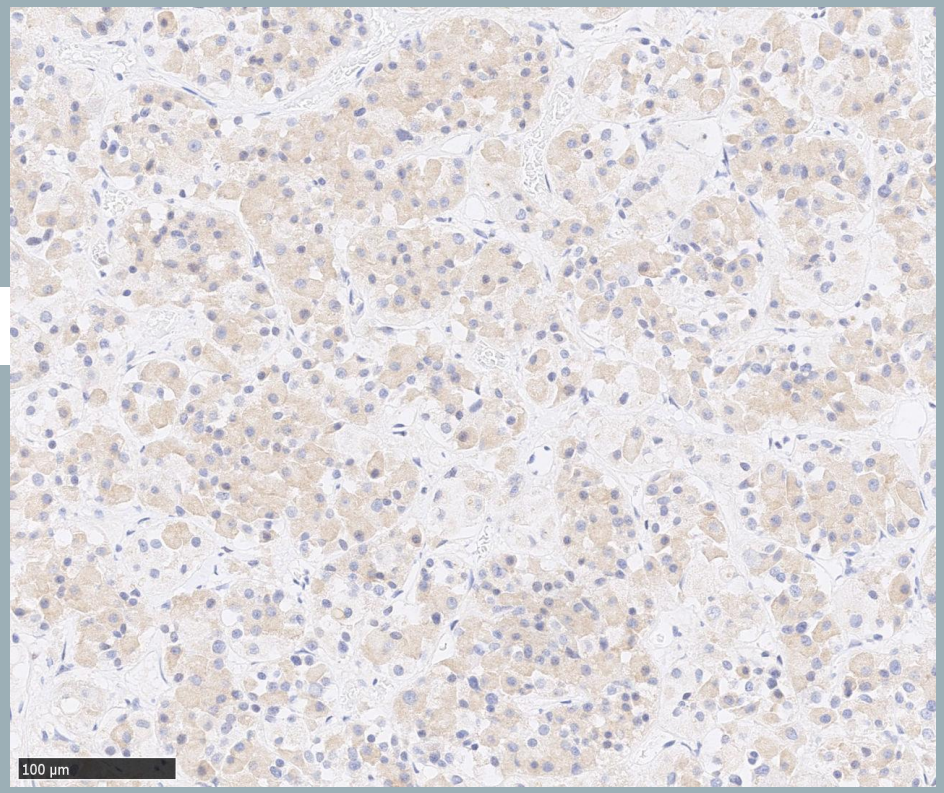
- **N1665:** Mouse monoclonal; widely used in diagnostic and research studies for strong nuclear staining of steroidogenic tissues. `rndsystems`
- **EP434:** Rabbit monoclonal; established in routine pathology for adrenocortical and gonadal tumors. `biosb`
- **EPR19744:** Rabbit monoclonal from Abcam; validated for IHC-paraffin and cited in literature for tissue specificity. `abcam`
- **2E12:** Mouse monoclonal; available t… applications. `abcepta`

Manifacture	Clone	IVD - status
Invitrogen	mmAb N1665	RUO
Cell Marque	RmAb EP434	IVD(-R)

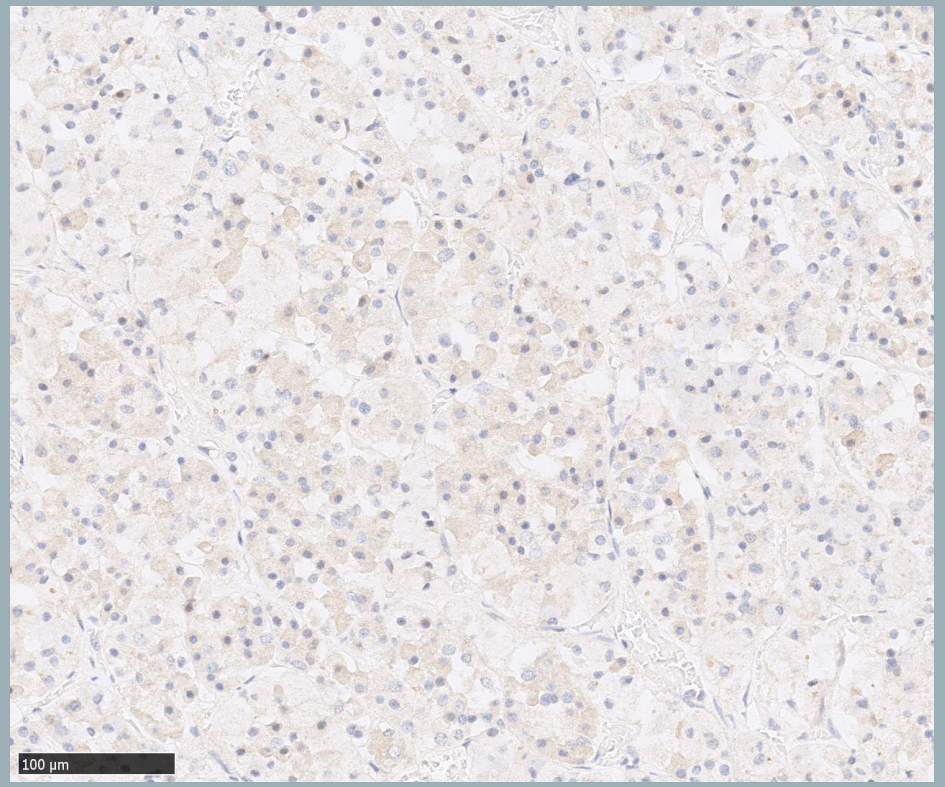
SF-1
- INVITROGEN N1665

Pituitary gland

1:100 CC1 48 min, Ab 32 min

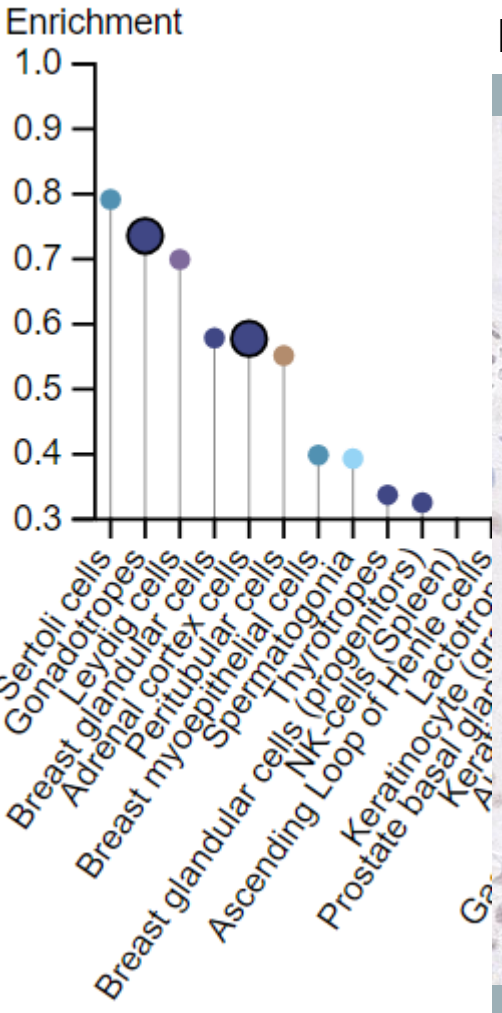


1:50 CC1 48 min, Ab 32 min

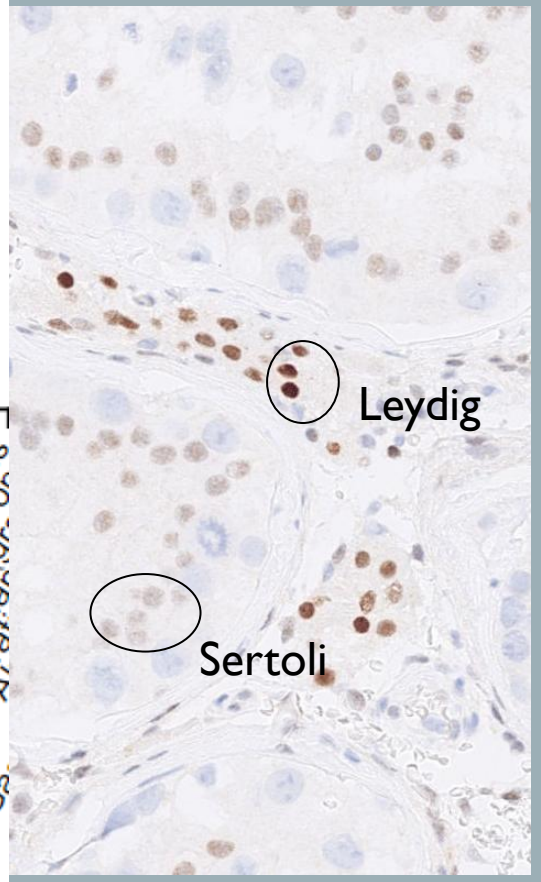


SF-1
- CELL MARQUE EP434

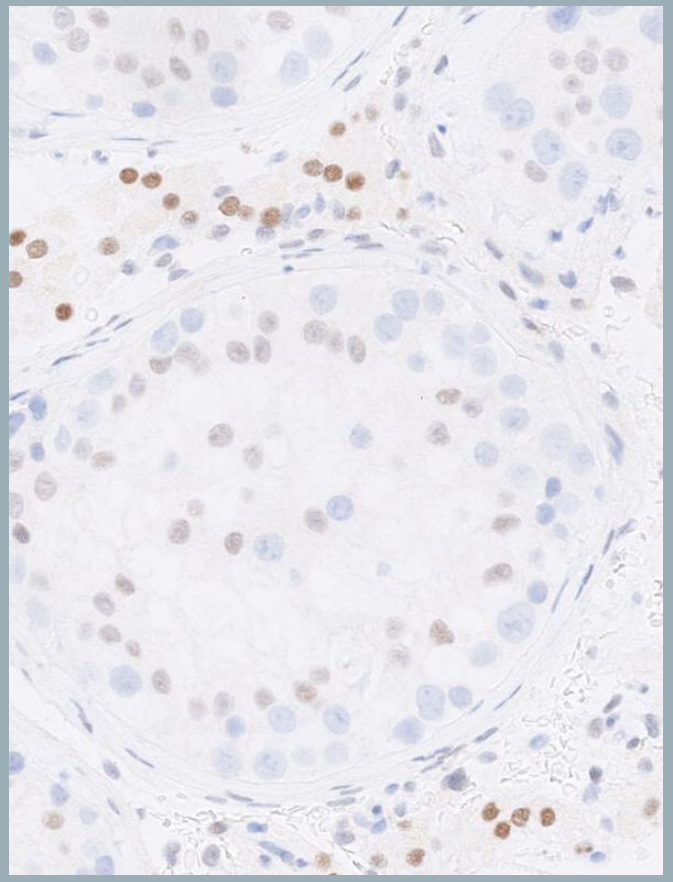
ific cell types[i]



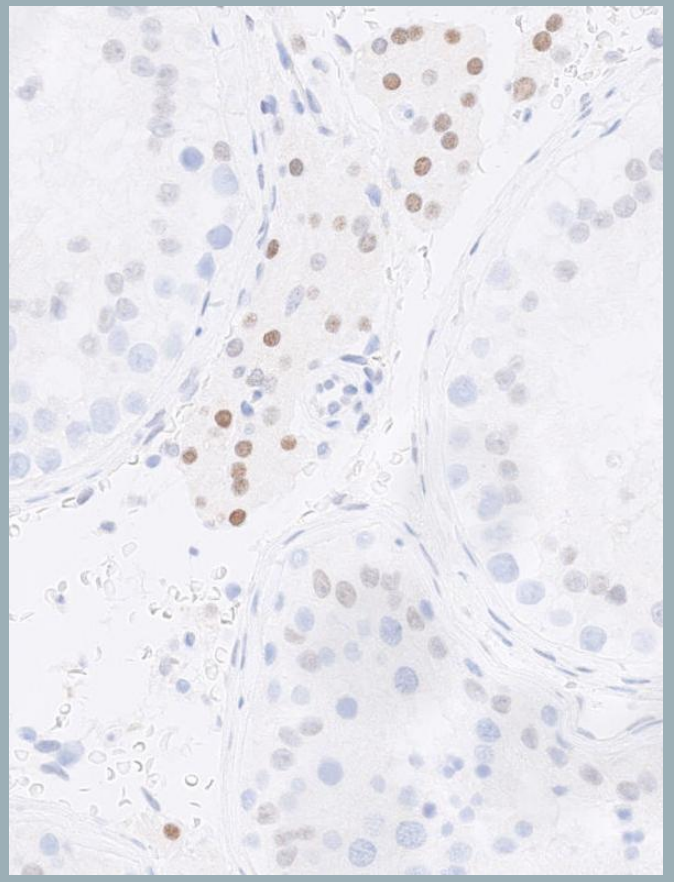
1:25 CC1 64 AB 32



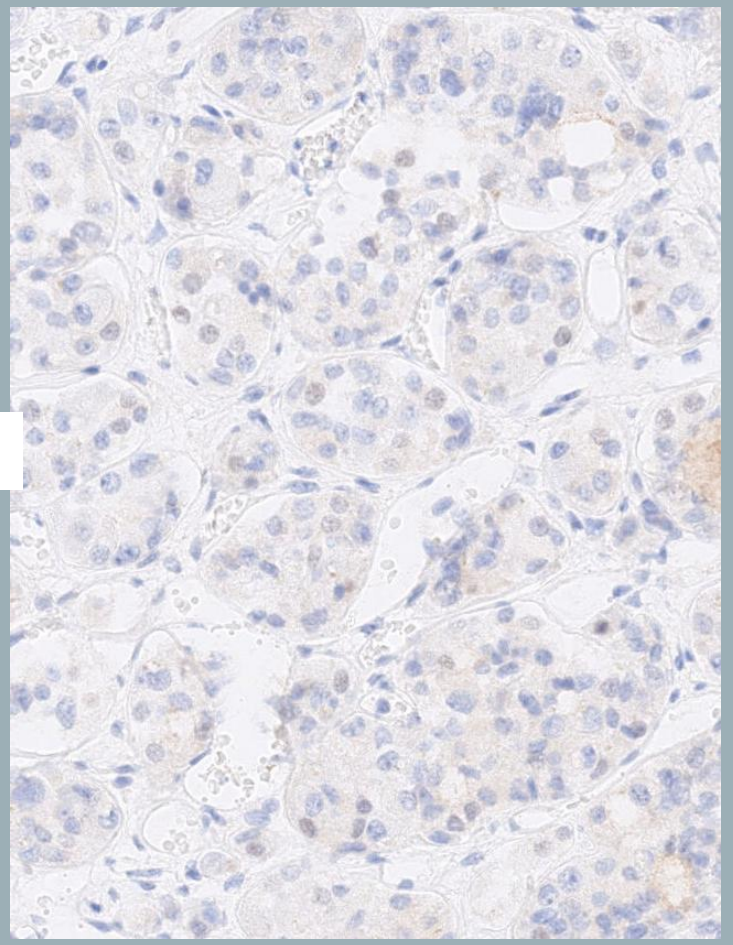
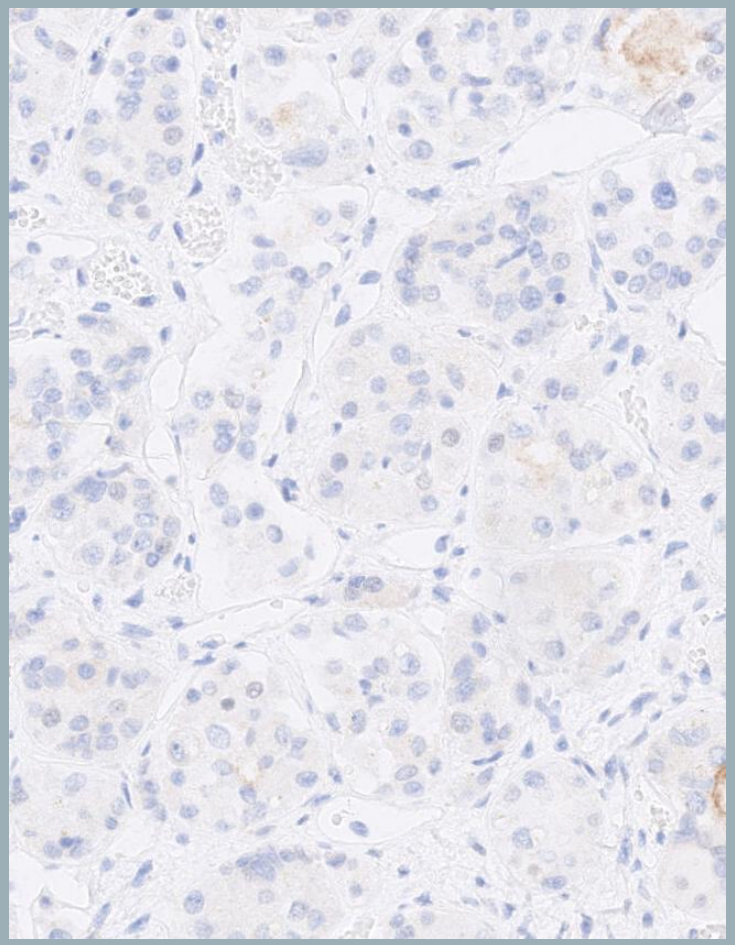
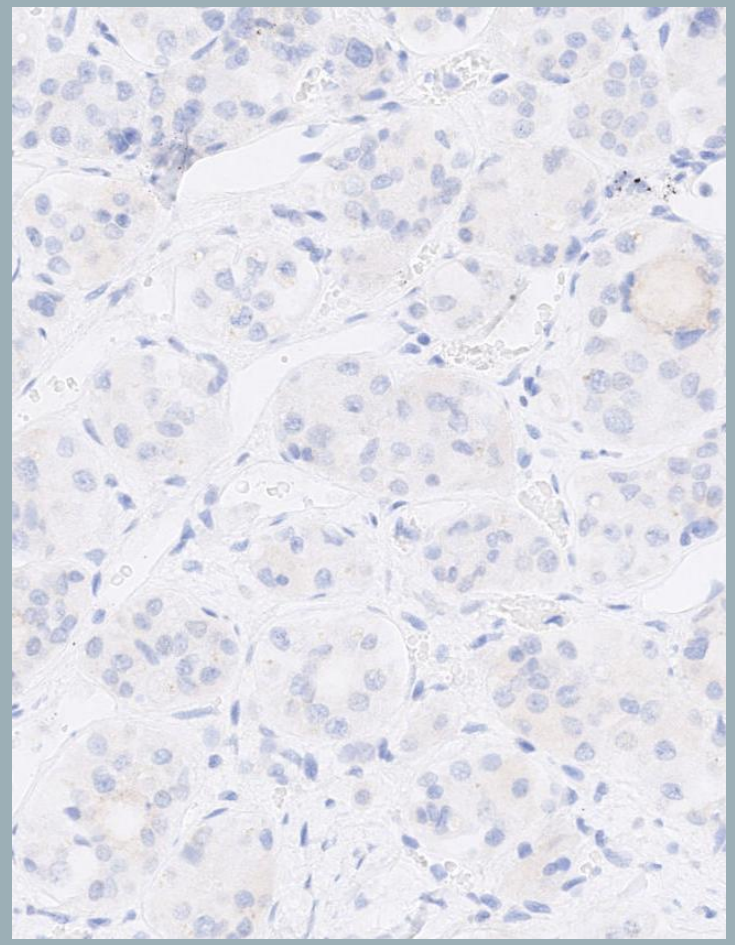
1:50 CC1 64 AB 32



1:100 CC1 64 AB 32



SF-1
- CELL MARQUE EP434

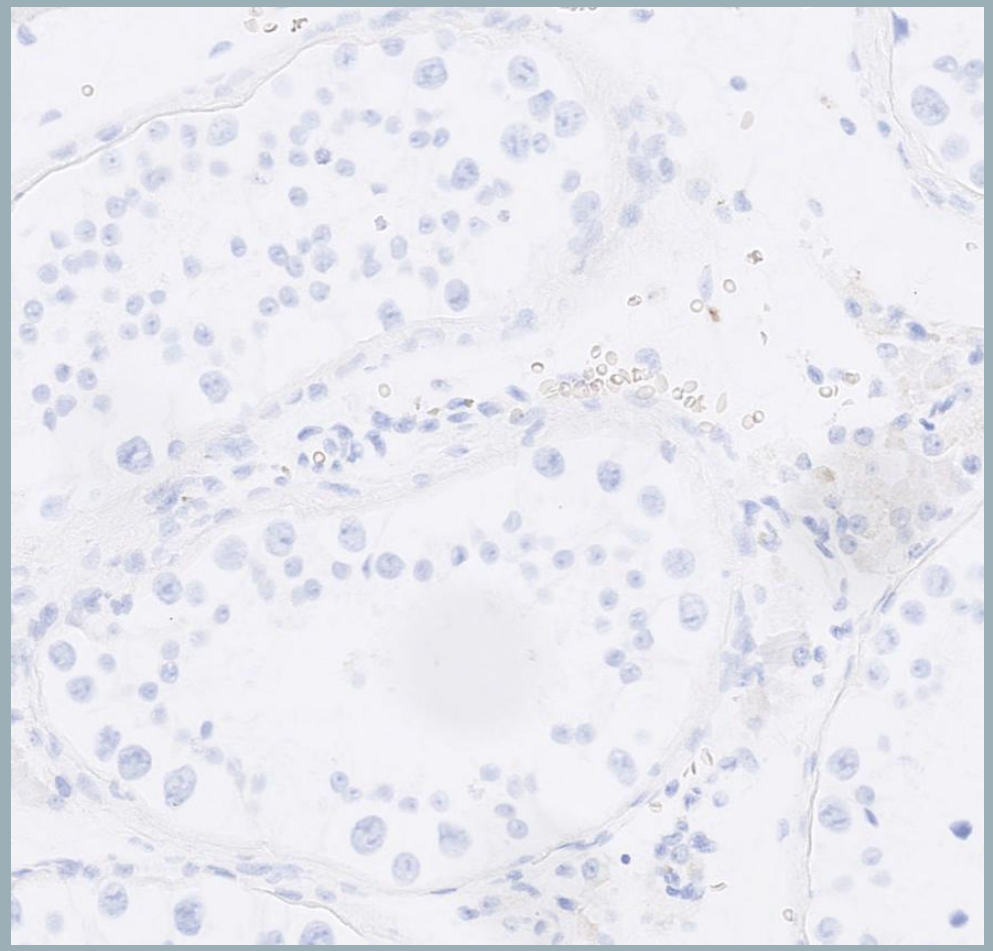
	1:25 CC1 64 AB 32	1:50 CC1 64 AB 32	1:100 CC1 64 AB 32
Pituitary gland			

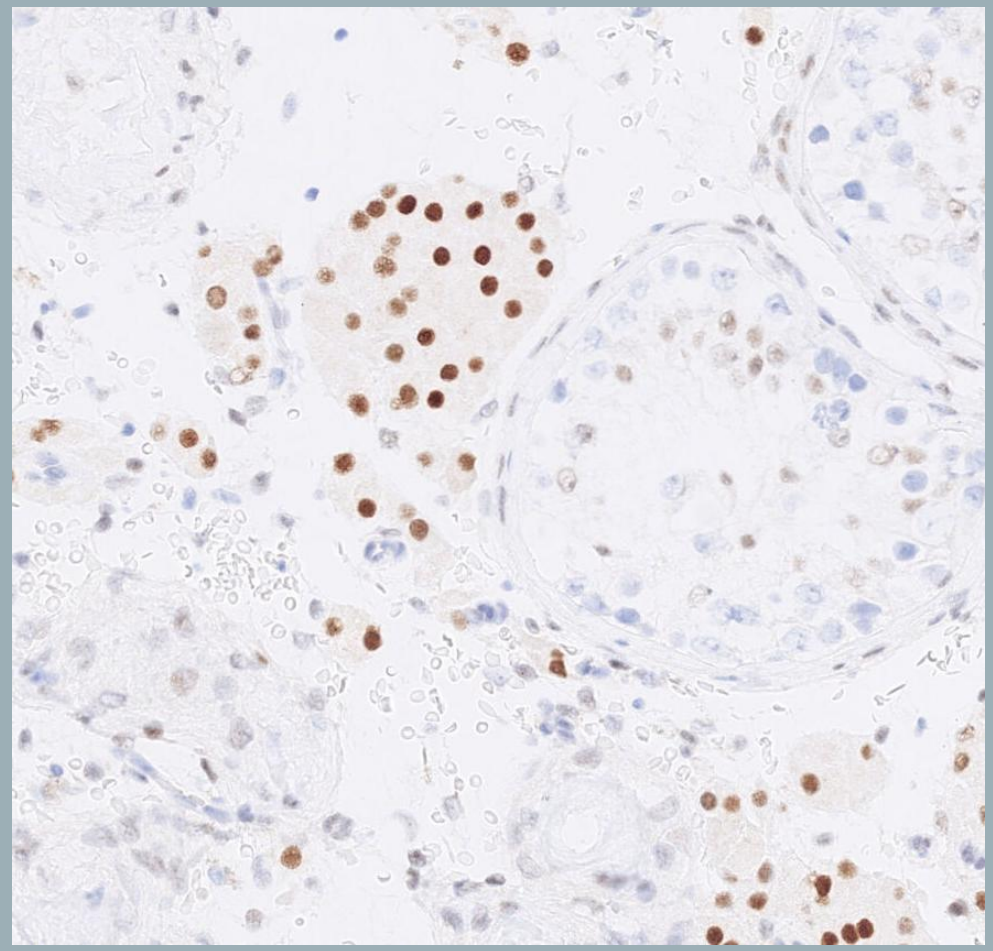
SF-1
- CELL MARQUE EP434

1:25 Protease 1 AB 32

1:25 Protease 3 + CC1 32 AB 32

Testis

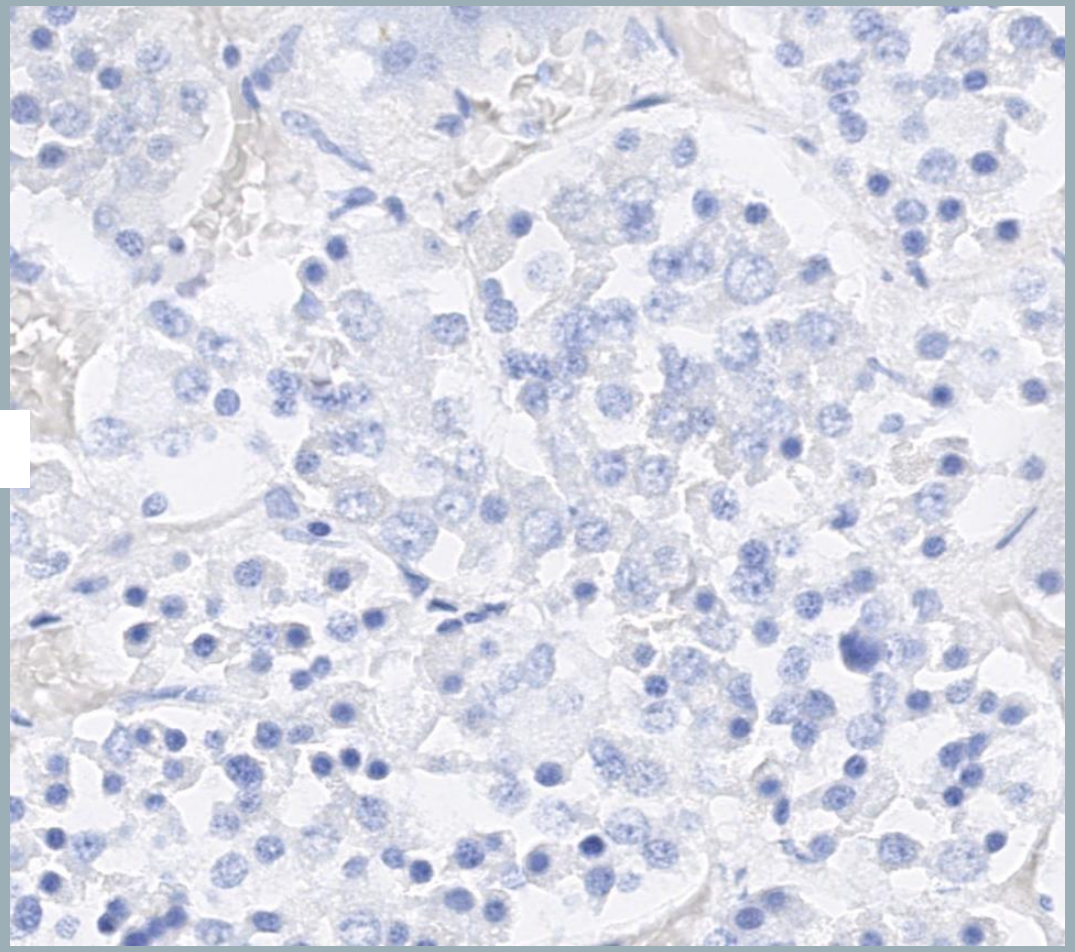




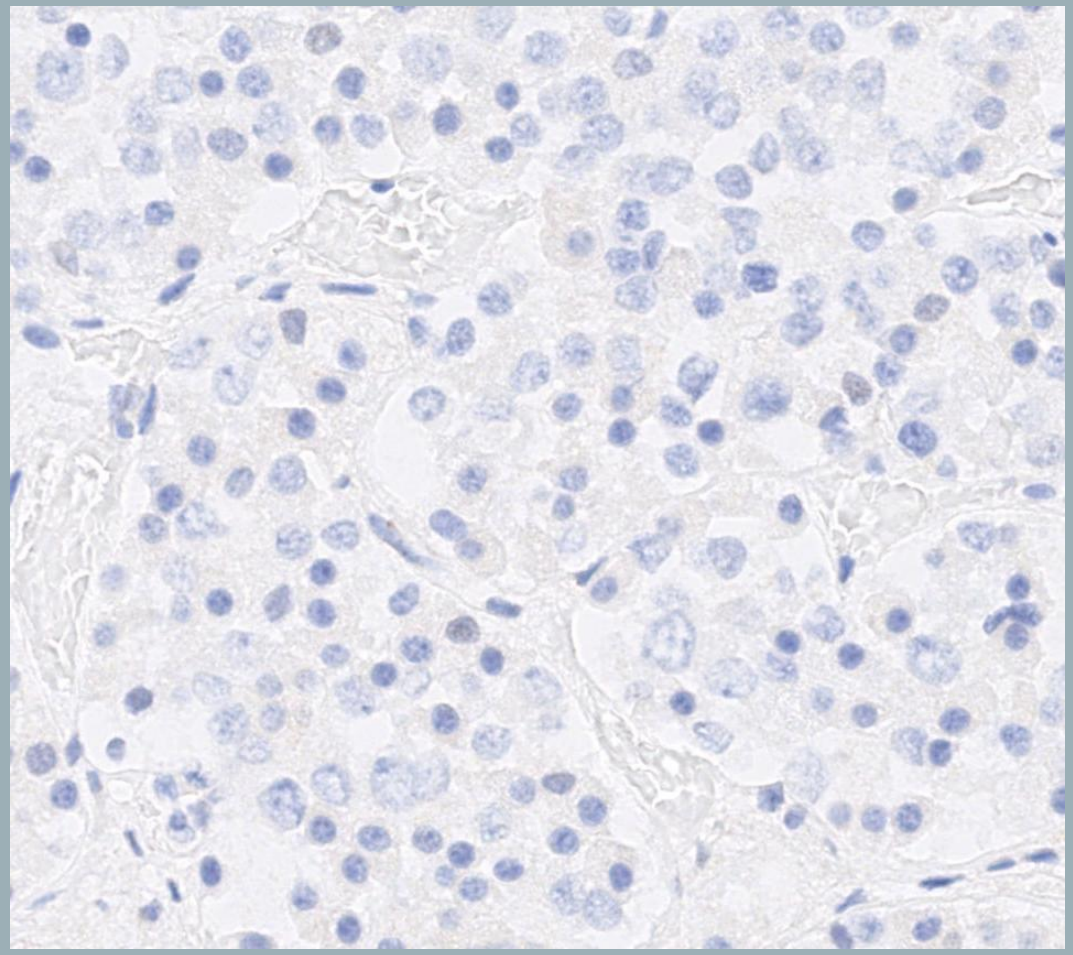
SF-1
- CELL MARQUE EP434

Pituitary gland

1:25 Protease 1 AB 32



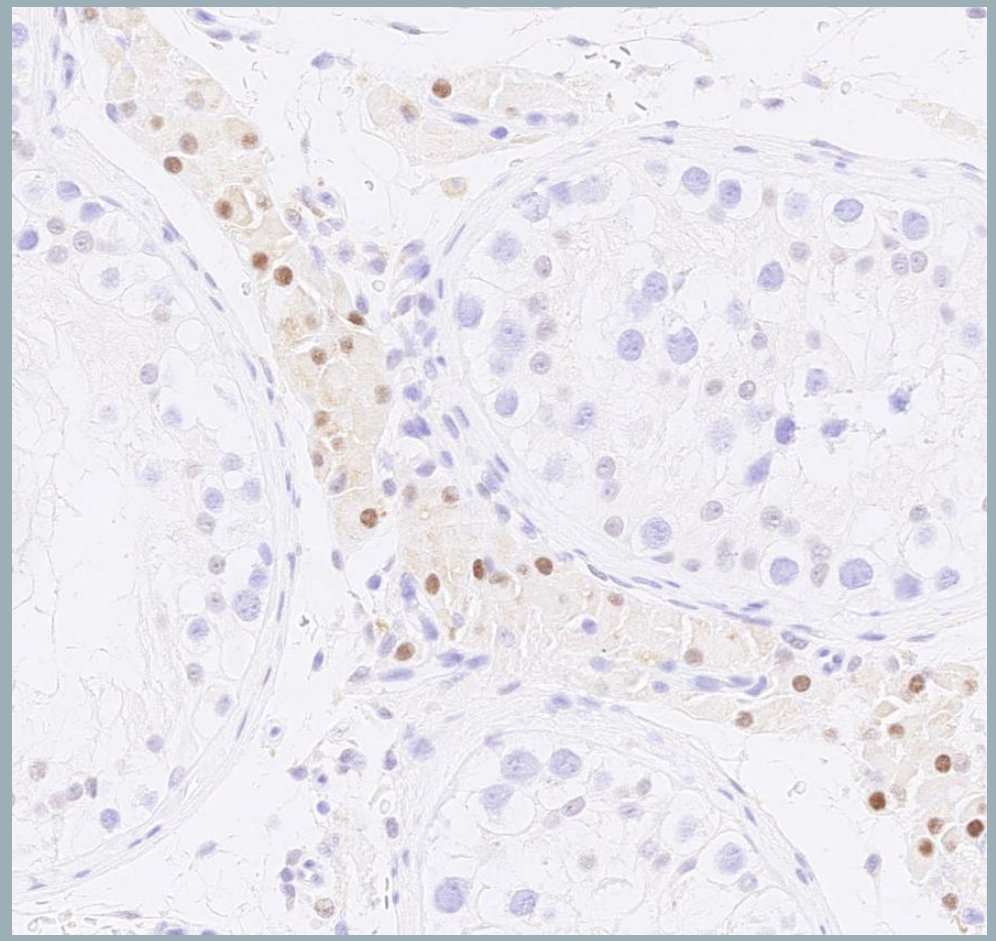
1:25 Protease 3 + CC1 32 AB 32



SF-1
- CELL MARQUE EP434

Testis

1:50 Flex+ (30/20)



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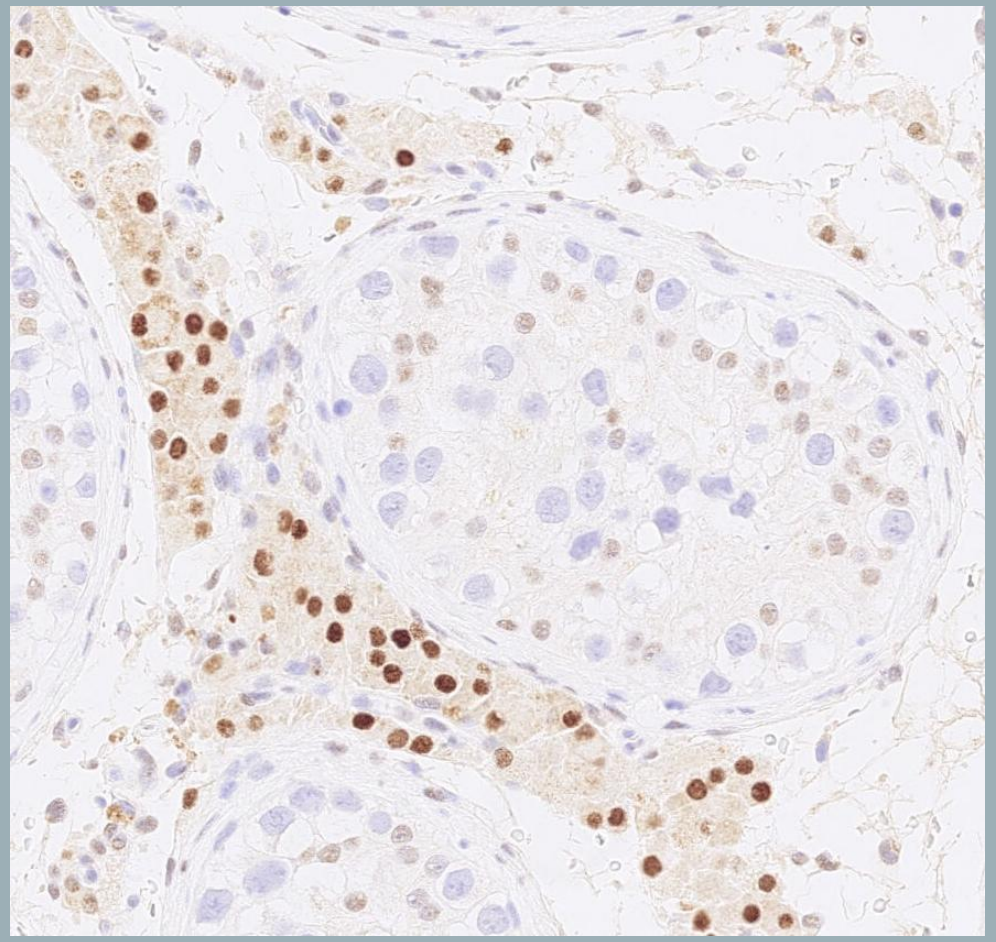
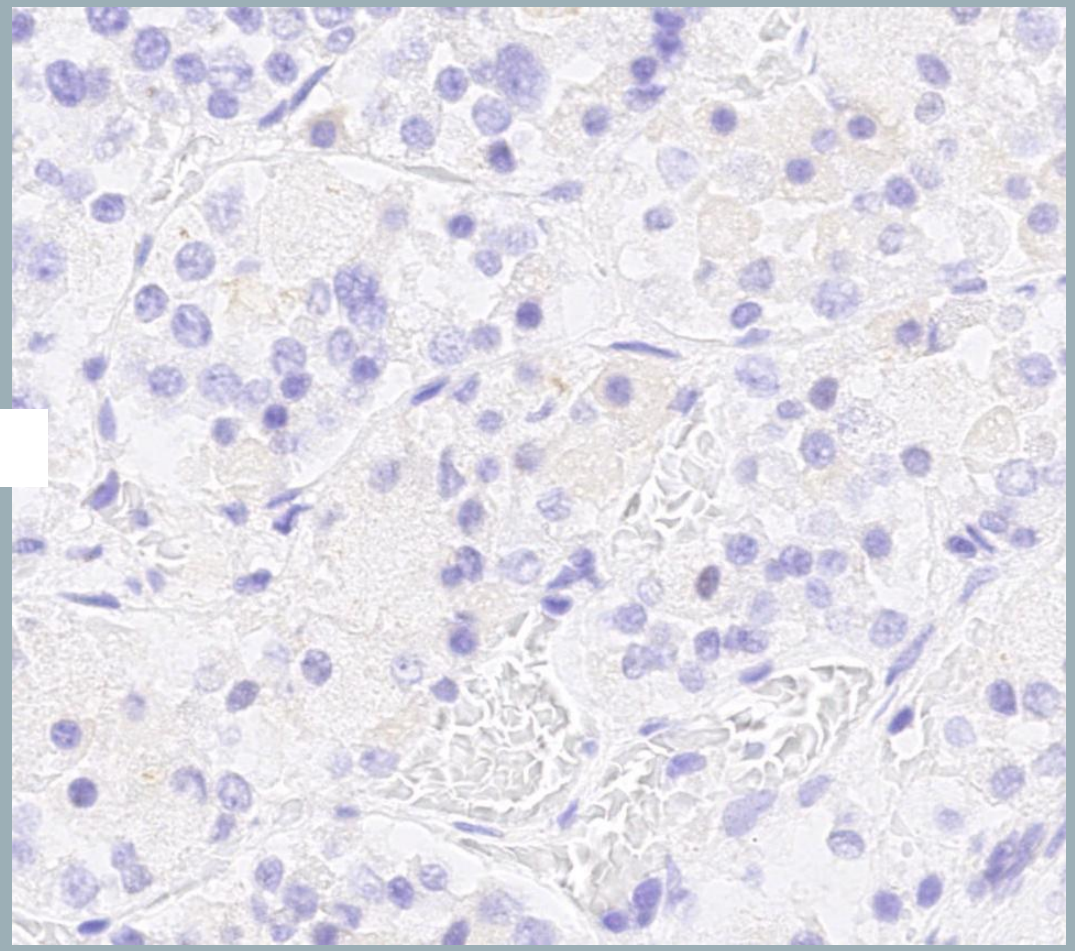


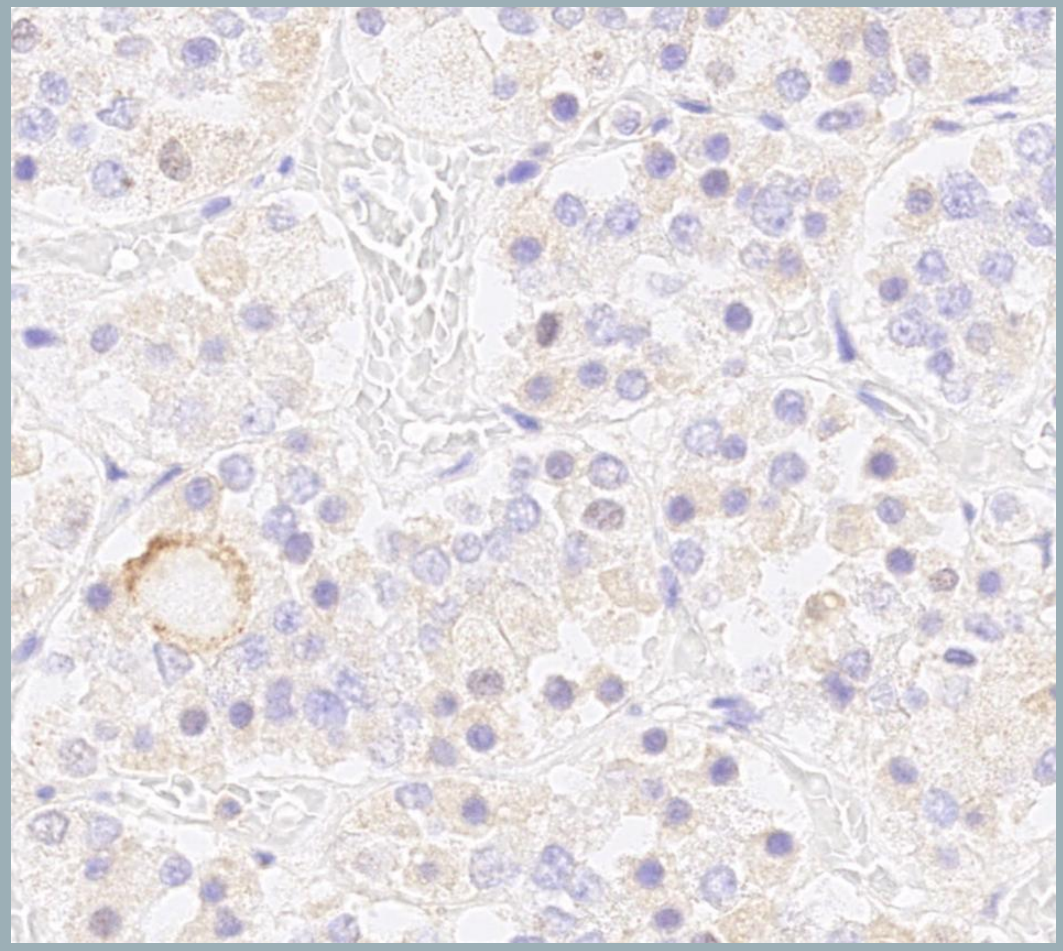
SF-1
- CELL MARQUE EP434

1:50 Flex+ (30/20)

1:50 Flex++ (30/20)

Pituitary gland

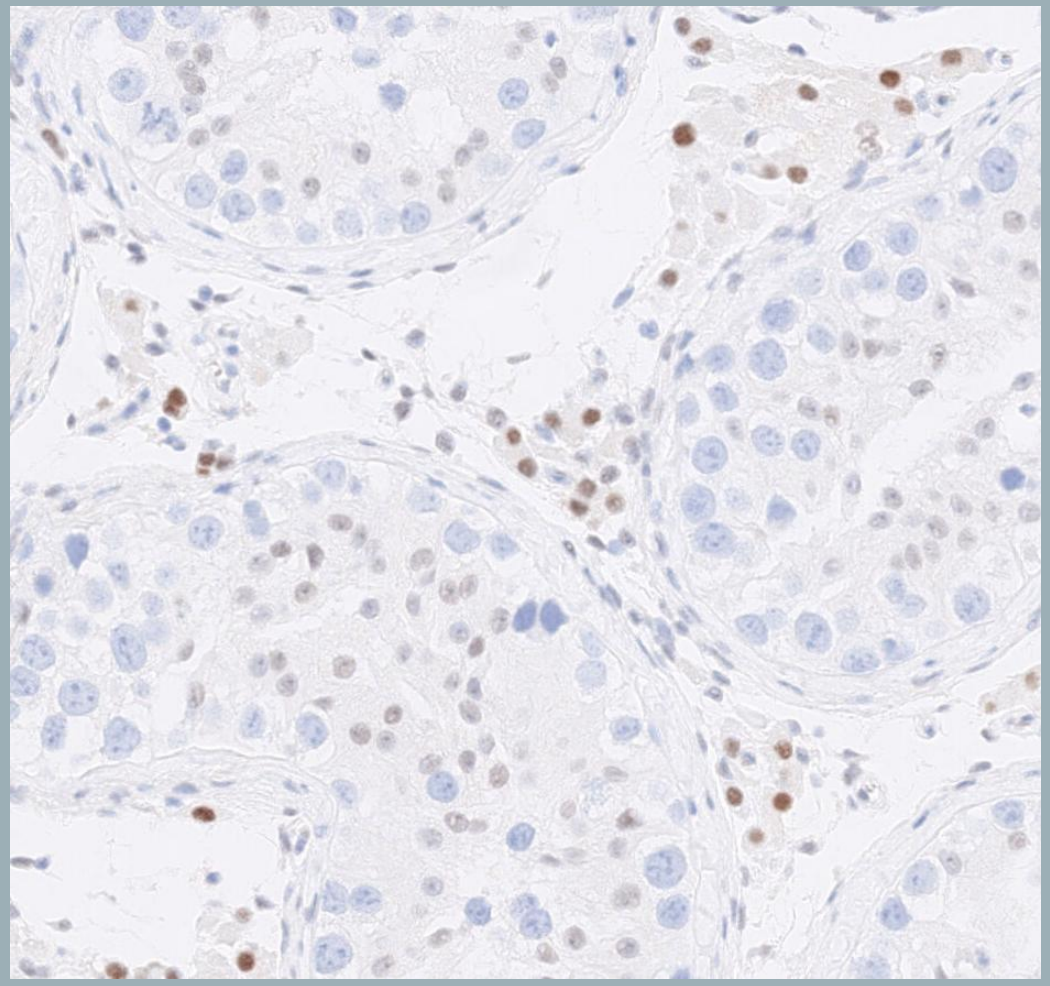


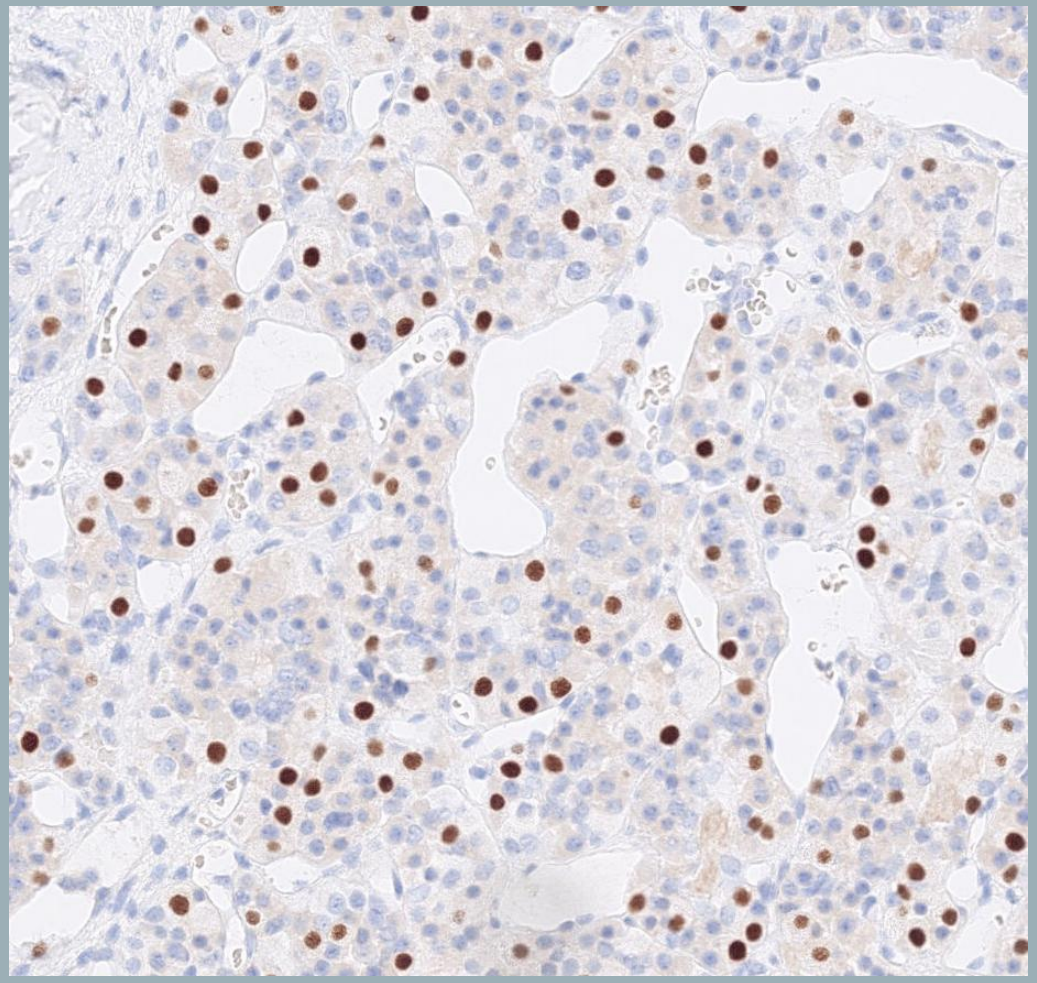


SF-1
- CELL MARQUE EP434

Testis

Pituitary gland





1:25 CC1 64 AB 32

SF-1 Conclusion

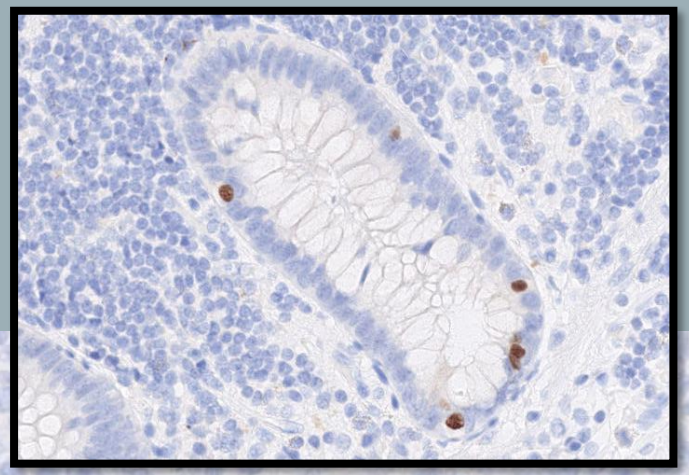
- SF-1 Clone selection is important.
- Detection system selection can be very important
- **It is important to know your marker in advance**, otherwise how do we select controls?

INSM1 – MRQ-70

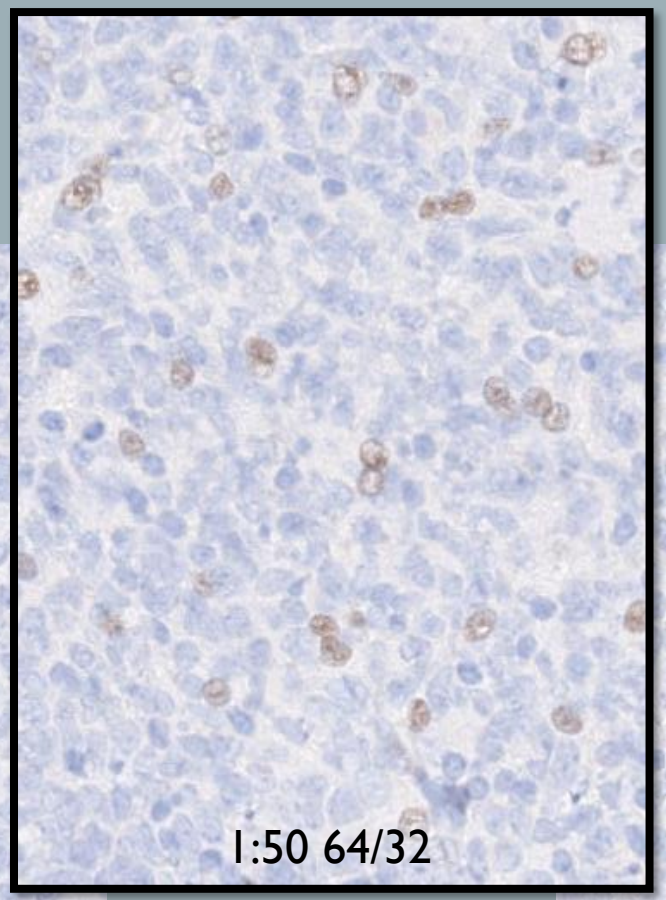
- Used diagnostically in the differentiation of neuroendocrine tumors

CELL TYPE RNA EXPRESSION	
Single cell type specificity[i]	Cell type enriched (Enteroendocrine cells)
Single cell type expression cluster[i]	Enteroendocrine cells - Hormone signaling (mainly)
Tissue cell type classification[i]	Cell type enriched in 7 tissues
Immune cell specificity[i]	Not detected in immune cells
Immune cell expression cluster[i]	Not detected - no cluster assigned

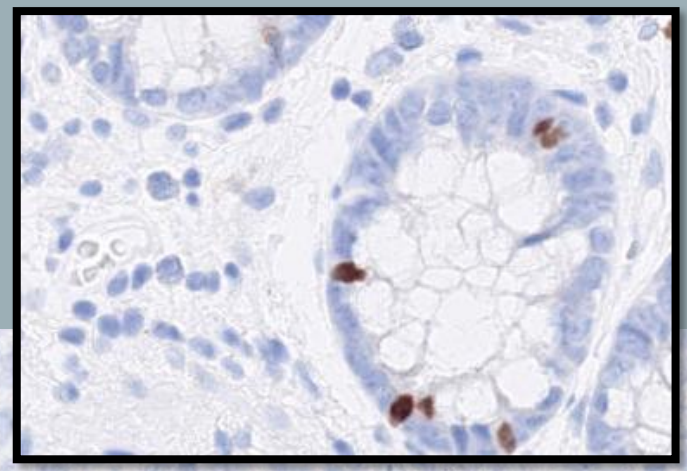
INSM1 – MRQ-70



1:100 64/32



1:50 64/32



1:200 64/32

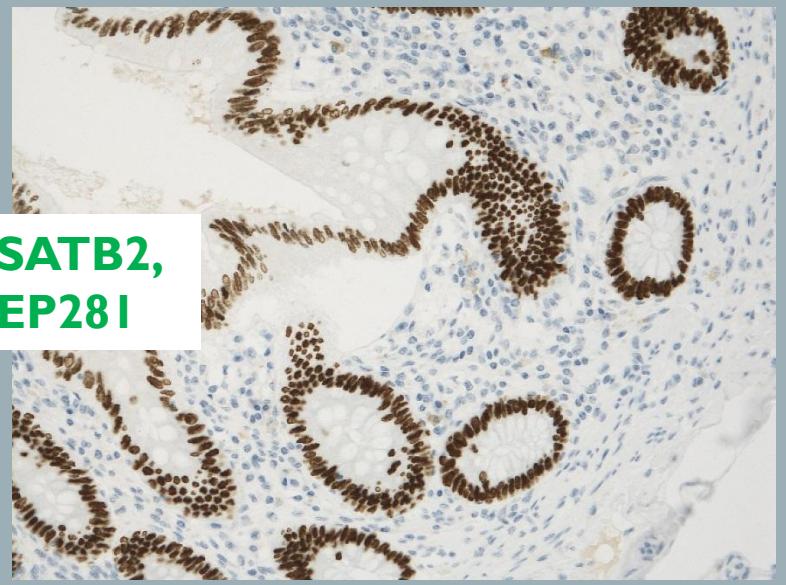
Appendix

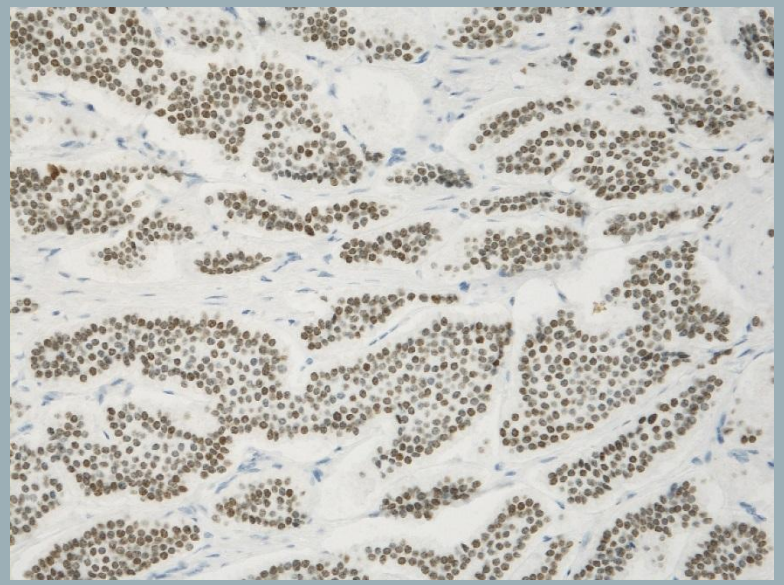
Neuroendocrine tumour (colon)

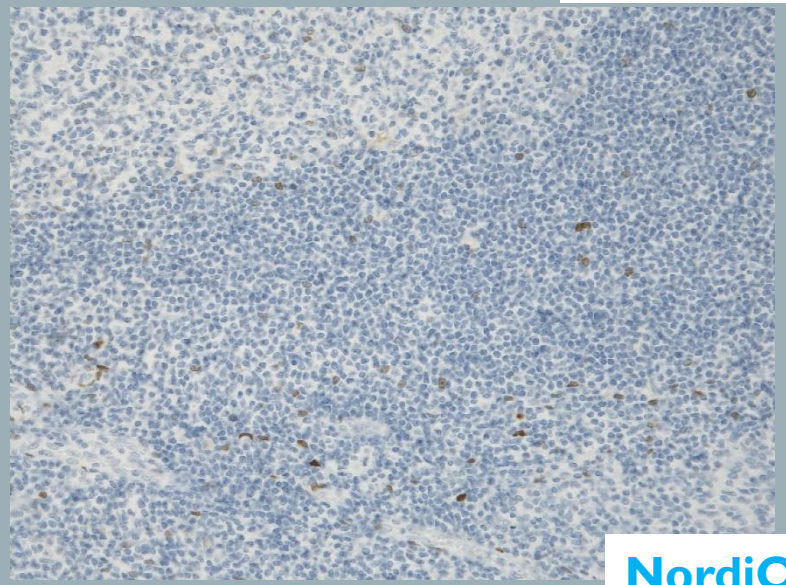
Tonsil

NordiQC

SATB2, EP281

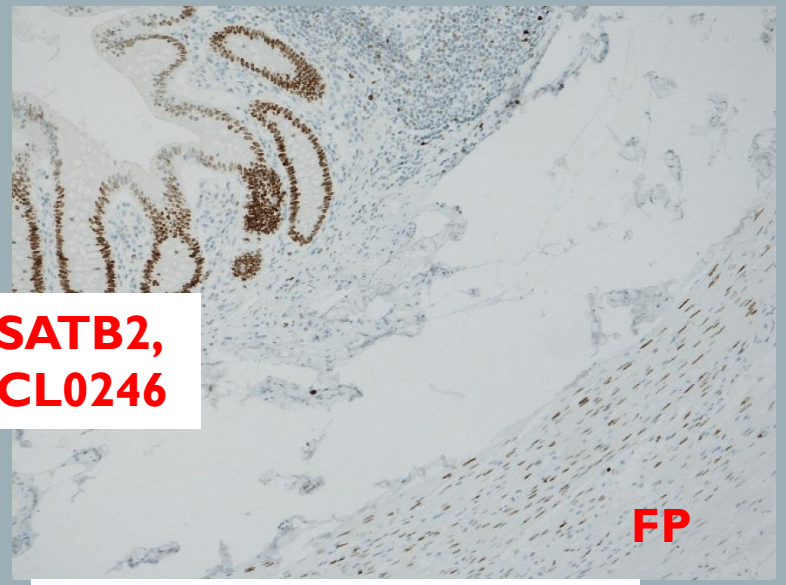


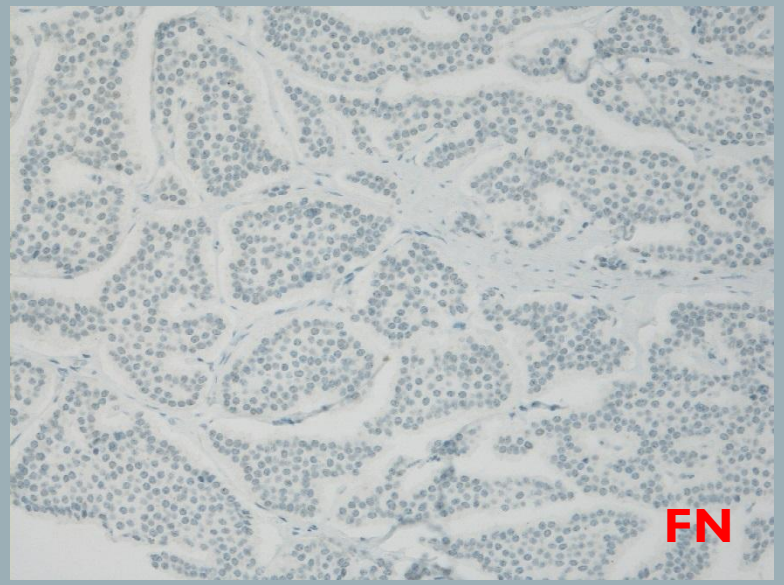


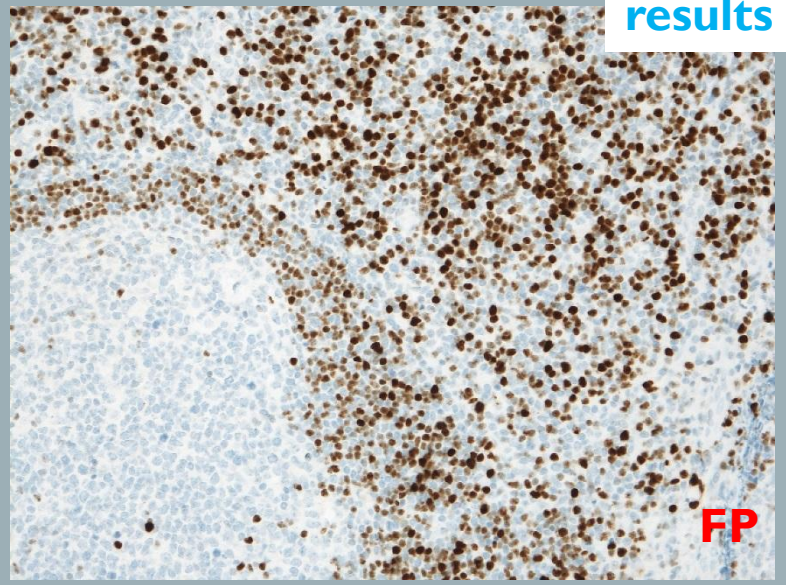


NordiQC results

SATB2, CL0246







Courtesy of Michael Bzorek

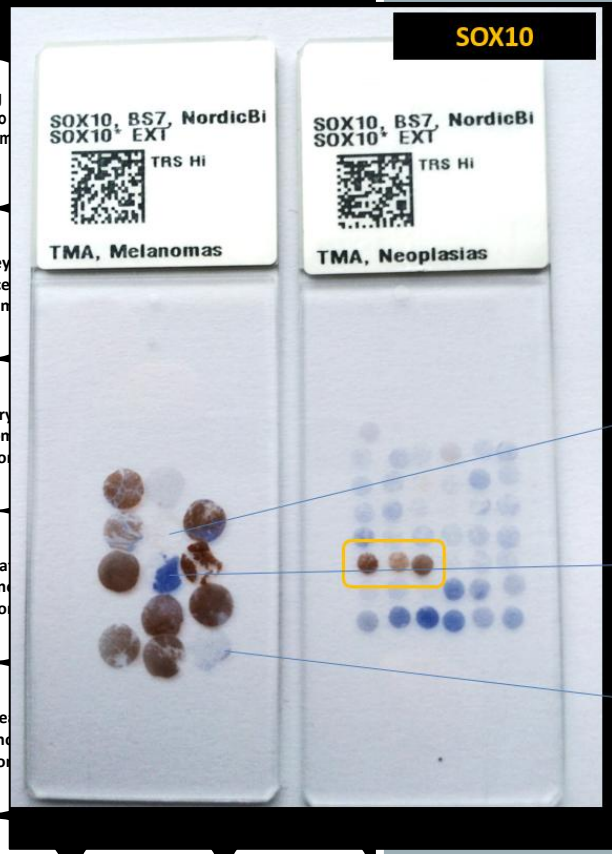
TMA
Malignant Melanomas

Melanoma 1
Kidney
Melanoma 2
Melanoma 3
Melanoma 4
Melanoma 5
Melanoma 6
Melanoma 7
Melanoma 8
Melanoma 9
Melanoma 10
Melanoma 11
Melanoma 12

TMA
Mixed tumors

SOX10

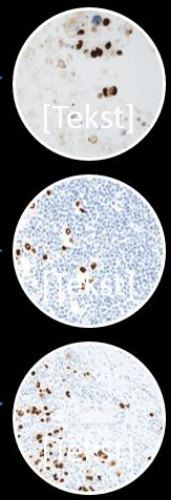
Liver
Mamma Ductal carcinoma
Mamma Ductal carcinoma
Mamma Lobular carcinoma
Lung Adeno carcinom
Colon Adeno carcinoma
Colon Adeno carcinoma
Kidney Clear cell carcinoma
Kidney Clear ce carcinon
Ovary Serous carcinoma
Ovary Serous carcinoma
Ovary Clear cell carcinoma
Ovary Endon carcino
Tonsil
Testis Seminoma
Testis Seminoma
Prosta Adeno carcino
Lymph n. Melanoma
Skin Melanoma
Lymph n. Melanoma
Pancre Adeno carcino
Colon GIST
Uterus Leiomyo sacrcoma
Testis Lipo Sarcoma
Hodgkin L. Classic
Hodgkin L. Mixed
Diffuse L. B Cell lymphoma
Diffuse L. B Cell lymphoma
CLL
Follicular B Cell lymph.
Mantle Cell Lymph.
Pheriph. T Cell lymph.
Large C. Anaplastic T Cell lymph.



SOX10, BS7:

15/15 Melanomas were positive

37/37 other neoplasm`s were negative



Courtesy of Michael Bzorek

WHY DO OPTIMAL SETTINGS GIVE INSUFFICIENT RESULTS?

HER2

PATHWAY® rmAb clone **4B5, 790-2991, (VRPS)**[3]	32	Ventana/Roche	25	7	0	0	100%	78%
PATHWAY® rmAb clone **4B5, 790-2991, (LMPS)**[4]	93	Ventana/Roche	70	20	3	0	97%	75%
HercepTest™, rmAb **DG44, GE001, (VRPS)**[3]	29	Dako/Agilent	24	5	0	0	100%	83%
HercepTest™, rmAb **DG44, GE001, (LPMS)**[4]	5	Dako/Agilent	3	2	0	0	100%	60%

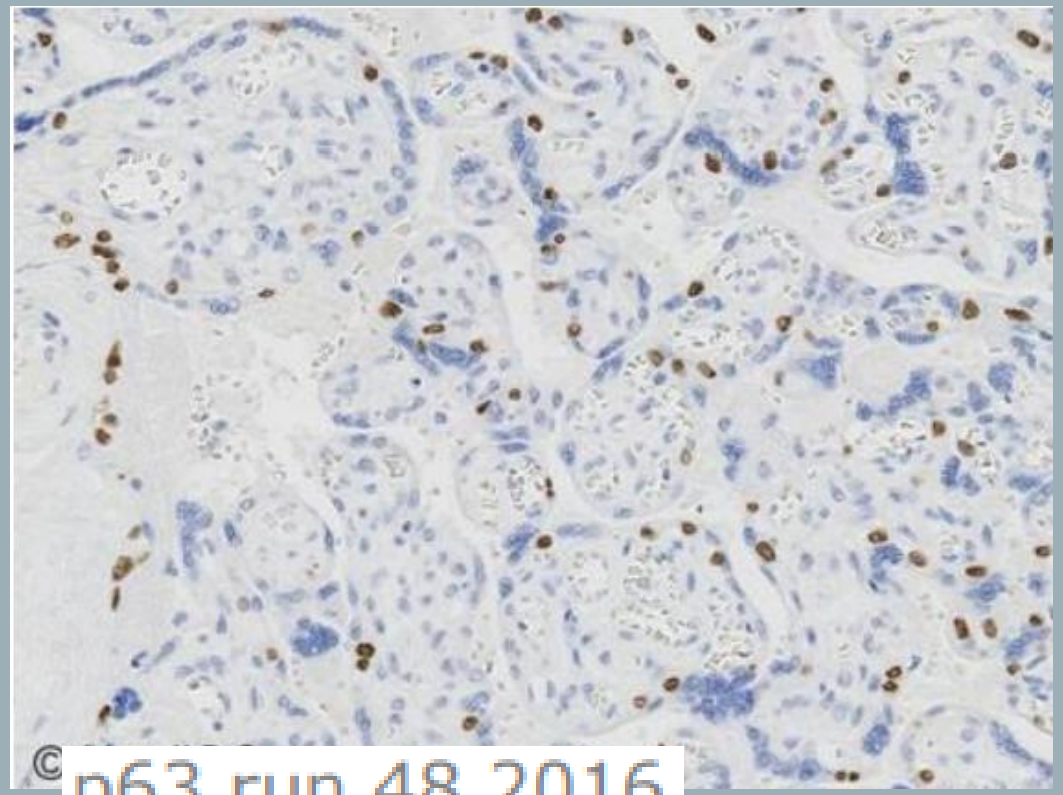
PD-L1

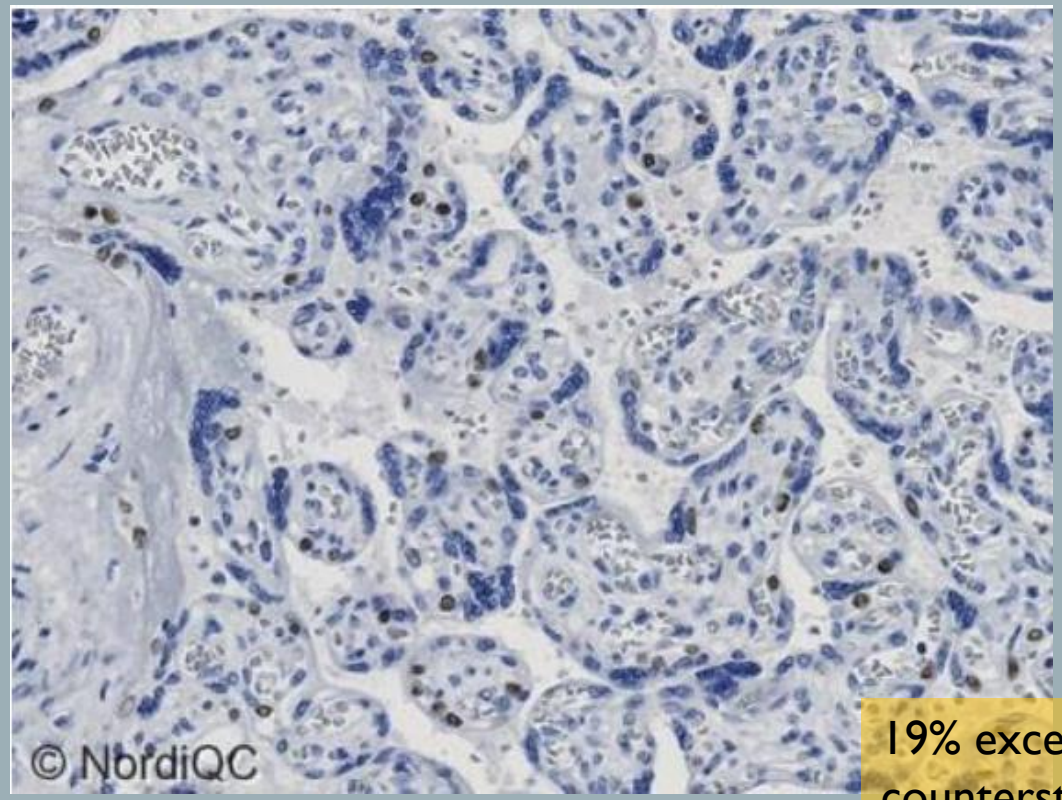
mAb clone 22C3 pharmDX, **GE006 (VRPS)**[3]	48	Dako/Agilent	34	14	-	-	100%	71%

PD-L1 TPS/CPS, Technical assessment

In order to account for heterogeneity of PD-L1 expression in the individual tumour cores included in the tissue micro array (TMA) blocks, ***reference slides*** were made throughout the blocks. The PD-L1 expression levels were thus characterized in ***every twenty-fifth*** slide and during the assessment.

WHY DO OPTIMAL SETTINGS GIVE INSUFFICIENT RESULTS?





19% excessive counterstain

mAb clone **DAK-p63 GA662**[3]	20	Dako/Agilent	5	12	3	-	85%	25%
mAb clone **DAK-p63 GA662**[4]	15	Dako/Agilent	9	6	-	-	100%	60%

Conclusion

- Immunohistochemistry is not only influenced by pre-analytical factors
- Choice of clone is extremely important
- The sensitivity of the analysis can determine whether we can trust the result
- You must be critical of sources when searching the literature
- You must be open to discovering new things
- You must find it really exciting, because it is a really complex task