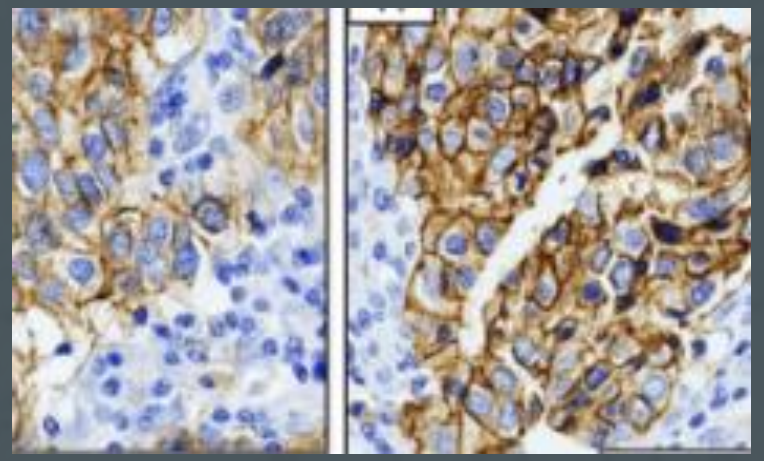


ROLE OF CONTROL IN IHC

MARIAMMAH SIMMANCHALAM

103369

MLS1

INTRODUCTION

- Immunohistochemistry is widely used in biomedical research to localize specific epitopes of molecules in cells and tissues.
- The validity of interpretations based on immunohistochemistry requires appropriate positive and negative controls that are often not reported in publications.
- This omission may lead to incorrect interpretations and irreproducible results in the literature and contribute to wasted time, effort, and resources as well as erosion of confidence in scientific investigation by the general public, legislative bodies and funding agencies.
- Valid interpretation immunohistochemical assays cannot be made in the absence of minimally appropriate controls.

WHY ARE CONTROLS USED IN HISTOCHEMICAL?

- To confirm that the observed staining pattern is true, accurate and reliable.
- To support the validity of the staining pattern.
- To exclude experimental artefacts.
- To ensuring consistent performance as variation in experimental conditions

Controls are essential component of experimental design in all scientific investigations.

ANTIGEN (TISSUE) CONTROLS

Positive control

- A section from a tissue known to express the protein of interest.
- A positive result from the positive control, even if the samples are negative, will indicate that the procedure is working and optimized.
- It will verify that any negative results are valid.

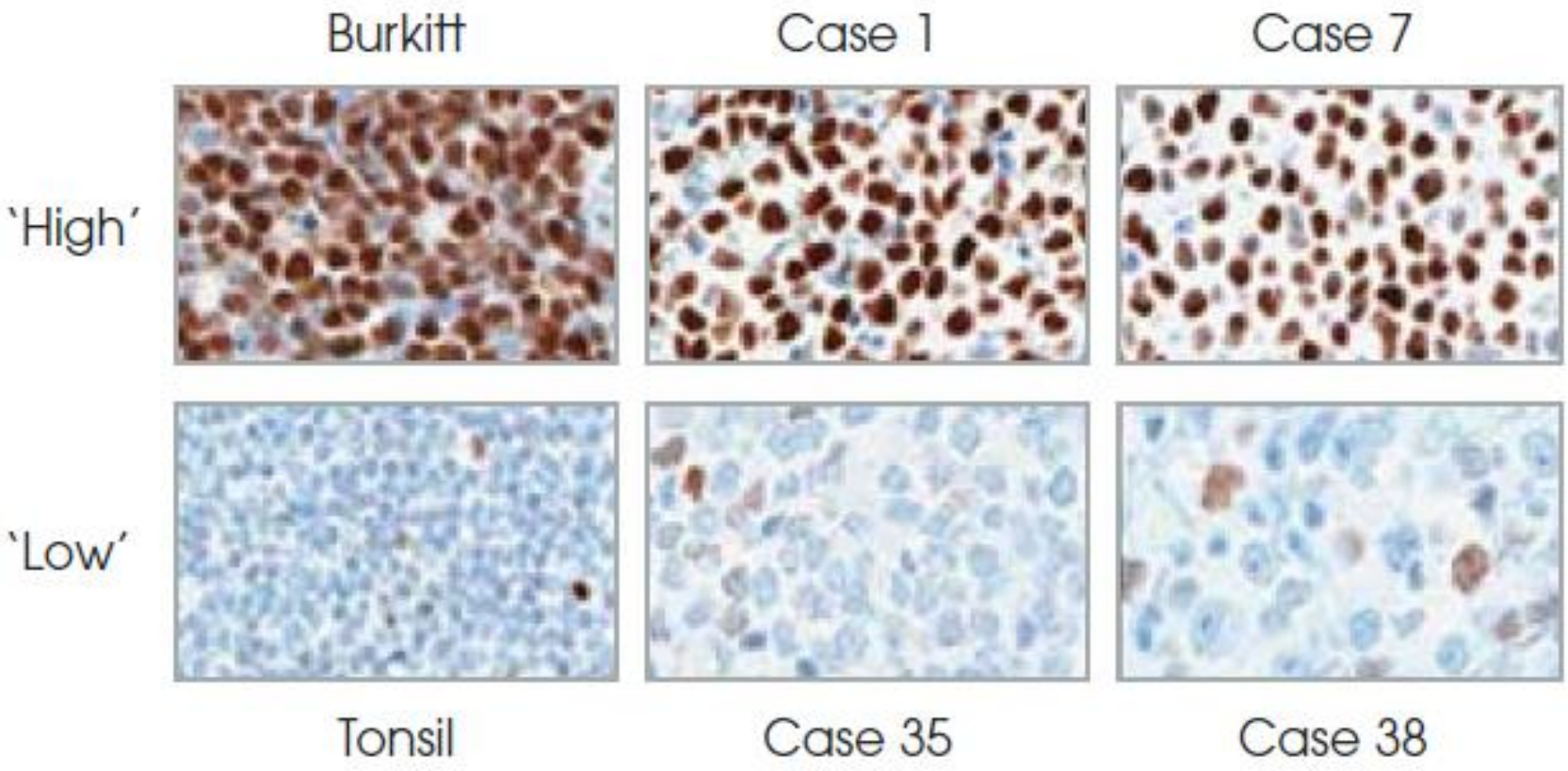
Negative control

- A section from a tissue known not to express the target antigen.
- This is to check for non-specific signal and false positive results.

ANTIGEN (TISSUE) CONTROLS
CONTINUE…

Endogenous tissue background control

- A section from the tissue before applying the primary antibody.
- Certain tissues have inherent properties that result in background staining, which could affect the interpretation of results.
- For example, certain tissues contain endogenous fluorescent molecules that could be confused for positive staining during fluorescent IHC.
- The tissue should be checked under the microscope using either fluorescent or bright-field illumination (for fluorescent or chromogenic labels, respectively) **to ensure that there is no endogenous background**.



IHC staining of select tumors and reactive tissue stained for c-Myc with anti-c-Myc antibody (Y69) (ab32072) from Kluk MJ et al. PLoS One (2012) 7(4): e33813. Burkitt lymphoma with a confirmed MYC translocation is positive control (high), negative control (low) is healthy tonsil.

FIGURE 1

HOW TO CHOOSE THE PERFECT CONTROL FOR YOUR IMMUNOHISTOCHEMISTRY

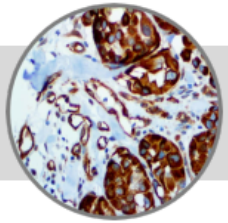
- Controls constitute a ubiquitous part of a well-designed scientific experiment.
- Selecting proper controls for immunohistochemistry (IHC) is essential to get reliable and reproducible results as a basis for good scientific practice.
- Primary antibody controls intend to verify the specificity of the primary antibody binding to the antigen of interest
- Controls for the primary antibody are applied to confirm the correctness of the preanalytical IHC phase related to sample preparation, such as fixation, post-fixation, storage, and processing
- Primary antibody controls must be included for each new antibody, protocol, and application that you test.

PI 2005, Rev. D DCN: 3269

Nestin, RMab

Clone: EP287

Rabbit Monoclonal



CE IVD Bio SB BIOSCIENCE FOR THE WORLD

www.biosb.com

Inset: IHC of Nestin on a FFPE Melanoma Tissue

Intended Use

For In Vitro Diagnostic Use.

This antibody is intended for use in Immunohistochemical applications on formalin-fixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations. Interpretation of results should be performed by a qualified medical professional.

* The Nestin antibody, clone EP286, has been manufactured using Epitomics RabMab® technology covered under Patent No.'s 5,675,063 and 7,402,409.

Immunogen

Cyclic peptide corresponding to C-terminus of human Nestin.

Summary and Explanation

Nestin is a type VI intermediate filament protein; they are expressed mostly in nerve cells where they are implicated in the radial growth of the axon. Nestin is expressed in dividing cells during the early stages of development in the Central Nervous System (CNS), Peripheral Nervous System (PNS) and in myogenic and other tissues. Nestin is expressed by many types of cells during development, although its expression is

Presentation

Myoglobin is a rabbit monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

Catalog No.	*Antibody Type*	*Dilution*	*Volume/Qty*
BSB 2000	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 2001	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 2002	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 2003	Concentrated	1:50 - 1:200	0.1 mL
BSB 2004	Concentrated	1:50 - 1:200	0.5 mL
BSB 2005	Concentrated	1:50 - 1:200	1.0 mL

Control Slides Available

Catalog No.	*Quantity*
BSB 2006	5 slides

Summary and Explanation

Nestin is a type VI intermediate filament protein; they are expressed mostly in nerve cells where they are implicated in the radial growth of the axon. Nestin is expressed in dividing cells during the early stages of development in the Central Nervous System (CNS), Peripheral Nervous System (PNS) and in myogenic and other tissues. Nestin is expressed by many types of cells during development, although its expression is usually transient and does not persist into adulthood. Nestin is however expressed in the neuronal precursor cells of the subgranular zone in adult organisms. Its expression is also reinduced in the adult during pathological situations, such as the formation of the glial scar after CNS injury and during regeneration of injured muscle tissue.

It has been reported that Nestin expression is significantly increased in melanoma and correlated with more advanced stages of the disease. It has also been reported in tumors of the CNS, including astrocytoma, ependymoma, oligodendroglioma, glioblastoma, and primitive neureoctodermal tumors, as well as in carcinomas such as prostatic adenocarcinoma, pancreatic ductal carcinoma, thyroid carcinoma, and in mesenchymal tumors. In breast carcinoma subtypes, Nestin is highly expressed in basal breast cancer but not in the HER2 subtype or luminal epithelial phenotype. In normal skin, Nestin is expressed in endothelial cells and the bulge area of hair follicles.

Antibody Type	Rabbit Monoclonal	**Clone**	EP287
Isotype	IgG	**Reactivity**	Paraffin, Frozen
Localization	Cytoplasmic	**Control**	Kidney, Breast, Adrenal, Myometrium, Liver Carcinoma
	Species Reactivity	Human, Mouse	

Control Slides Available

Catalog No.	*Quantity*
BSB 2006	5 slides

Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN_3) as a preservative. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as laboratory coat, goggles and gloves when handling reagents.
4. Dispose of unused solution with copious amount of water.
5. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
7. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
8. For additional safety information refer to Safety Data Sheet for this product.
9. For complete recommendations for handling biological specimens, please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories" (see References in this document).

Storage Store at 2-8°C (Control Slides: Store at 20-25°C)

Stability

This product is stable up to the expiration date on the product label. Do not use after expiration date listed on package label. Temperature fluctuations should be avoided. Store appropriately when not in use, and avoid prolonged exposure to room temperature conditions.

PACKAGE INSERT

FIGURE 2

PROPER CONTROL & VALIDATION

- As per mention in the package insert, choose an appropriate control for the selected IHC.
- Run a validation test on the selected control and antibody
- Send the validation test slide to pathologist , get their approval.

- Refer to the package insert.
- Know the staining pattern of the selected control and antibody.
- Repeat the validation with new control, if the previous one is not approved.

STAINING PATTERN IN IHC CONTROL

- Immunohistochemistry is an efficient and accurate method of detecting subcellular localization of a protein.

- Subcellular localization refers to the presence site of a protein in the cell, for example, in the nucleus, cytoplasm or cell membrane

CYTOPLASMIC STAINING

- If a protein locates in the cytoplasm, the cytoplasm will be stained, while other locations will not be stained.

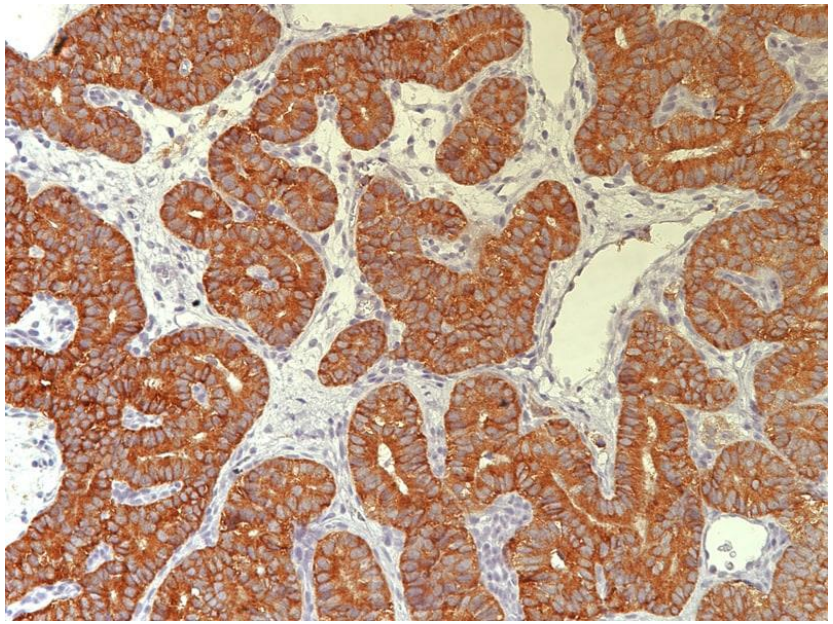


Figure 3 : Cytoplasmic staining pattern (inhibin): stain is diffuse within cytoplasm

EXAMPLES OF CYTOPLASMIC STAINING

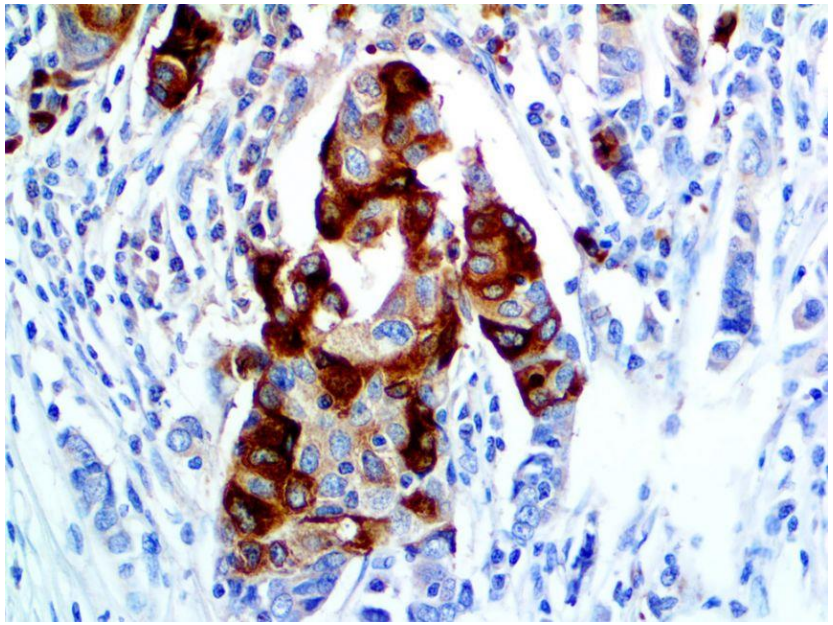


Figure 4 : Cytoplasmic staining pattern (mammaglobin)

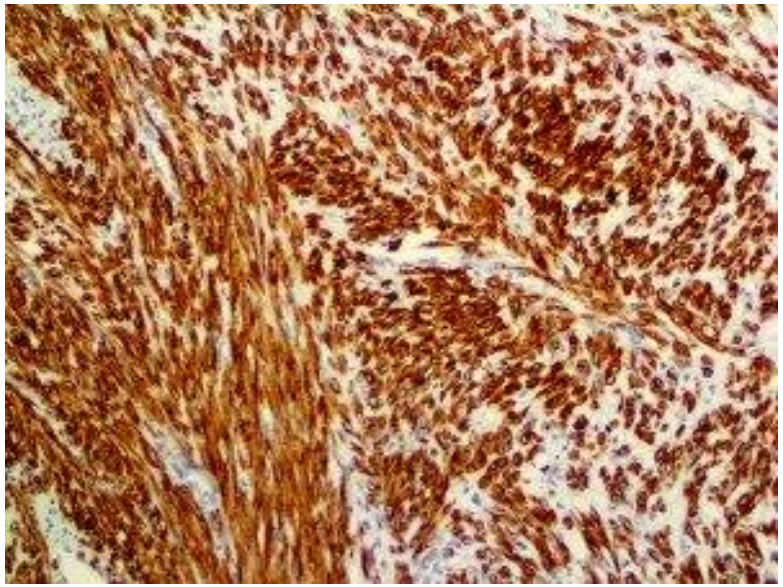


Figure 5 : Cytoplasmic staining pattern (desmin)

MEMBRANOUS STAINING

- If a protein locates in the cell membrane, the membrane will be stained, while other locations will not be stained

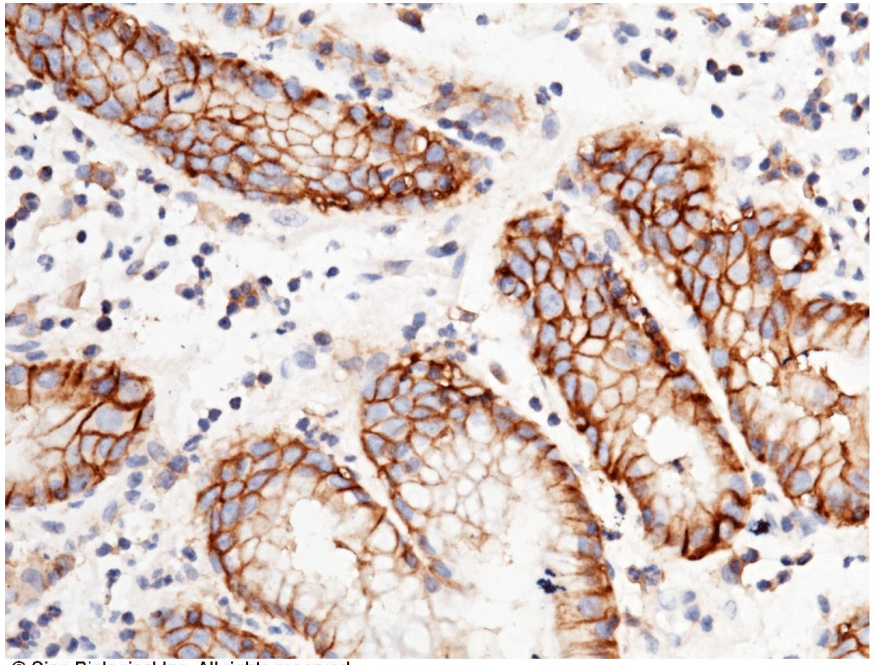


Figure 6 : Immunochemical staining of human CD147 in human gastric cancer with mouse monoclonal antibody (1:100, formalin-fixed paraffin embedded sections). The image showing membrane staining of epithelium cell.

EXAMPLES OF MEMBRANOUS STAINING

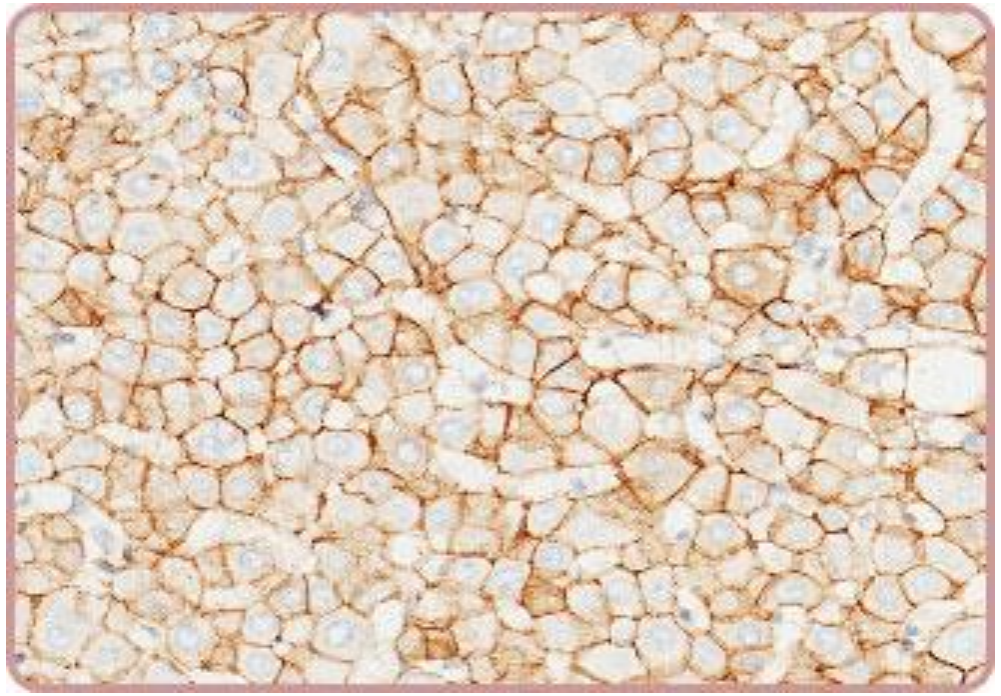


Figure 7 : Immunochemical staining of human E-cad in human liver with rabbit polyclonal antibody (1 µg/mL, formalin-fixed paraffin embedded sections).The image showing cytomembrane staining.

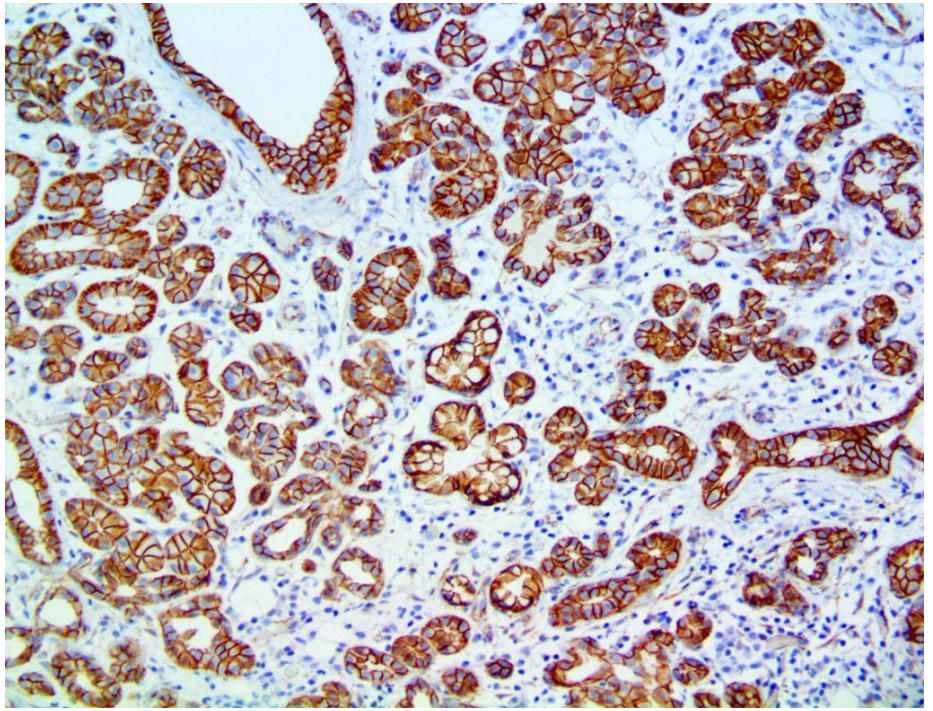


Figure 8 : Immunochemical staining of human Beta catenin in salivary gland tissue. The image showing cytomembrane staining.

NUCLEAR STAINING

- If a protein locates in the nucleus, the nucleus will be stained, while other locations will not be stained

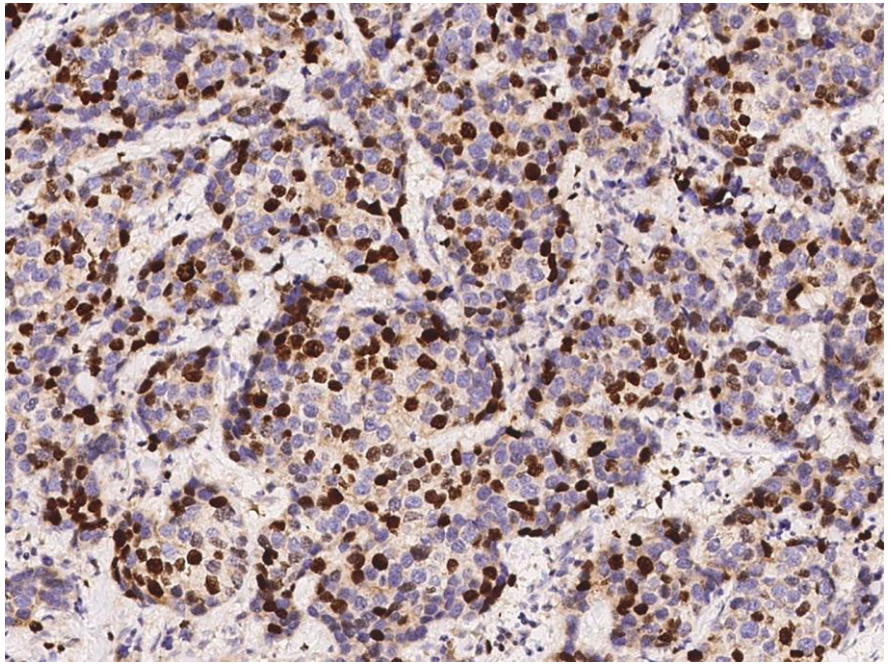


Figure 9: Immunochemical staining of human KI-67 in human breast carcinoma with mouse monoclonal antibody

EXAMPLES OF NUCLEAR STAINING

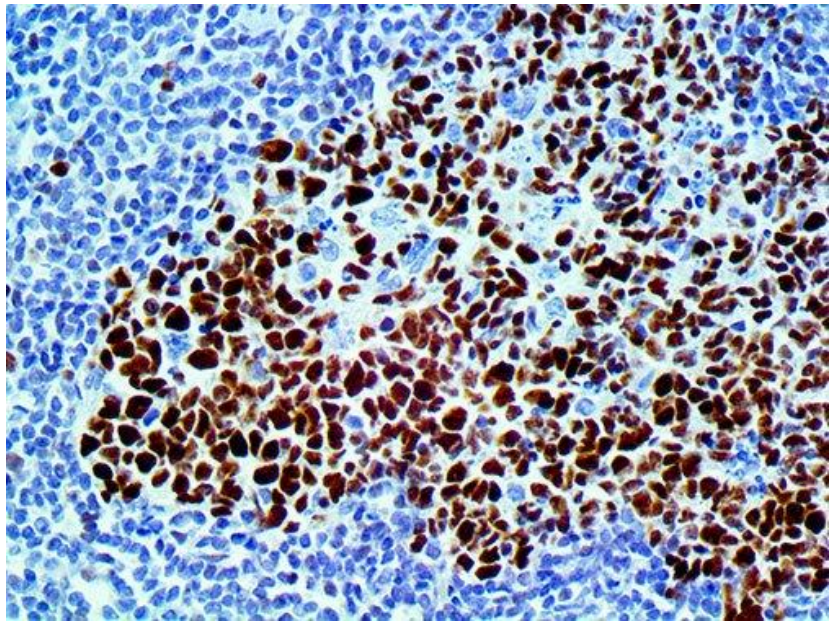


Figure 10: Immunochemical staining of human BCL 6 in human tonsil with mouse monoclonal antibody

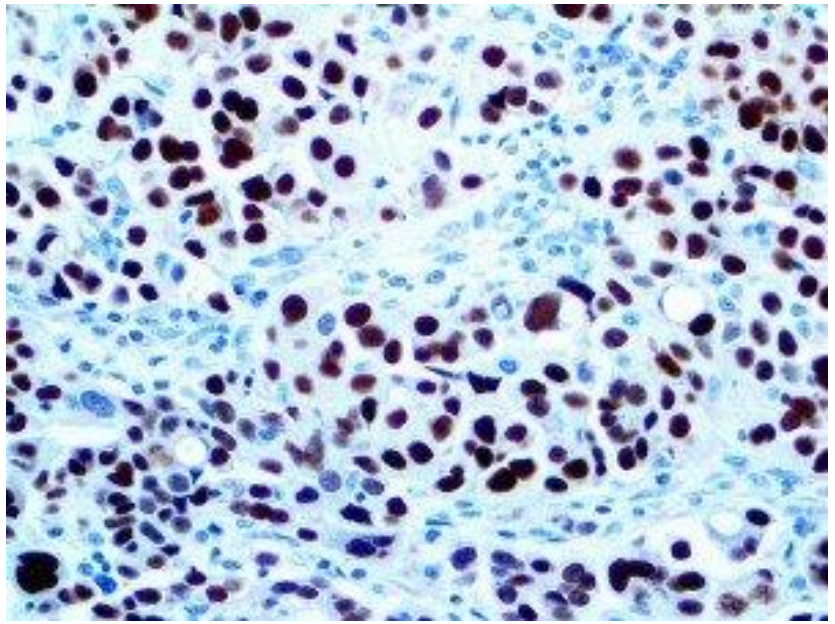


Figure 11: Immunochemical staining of human Pax 8 in human ovarian carcinoma with rabbit monoclonal antibody

CONTROL SELECTION

- Refer package insert
- Always try to find control from a normal tissue instead of carcinoma tissues that are tested positive for IHC,
- Make sure the tissues are fully fixed
- Avoid taking necrotic or calcified tissue as control
- Avoid taking over fixed tissue

BASIC CONTROL USED IN SJMC HISTOPATHOLOGY LAB

Monoclonal Mouse Anti-Human

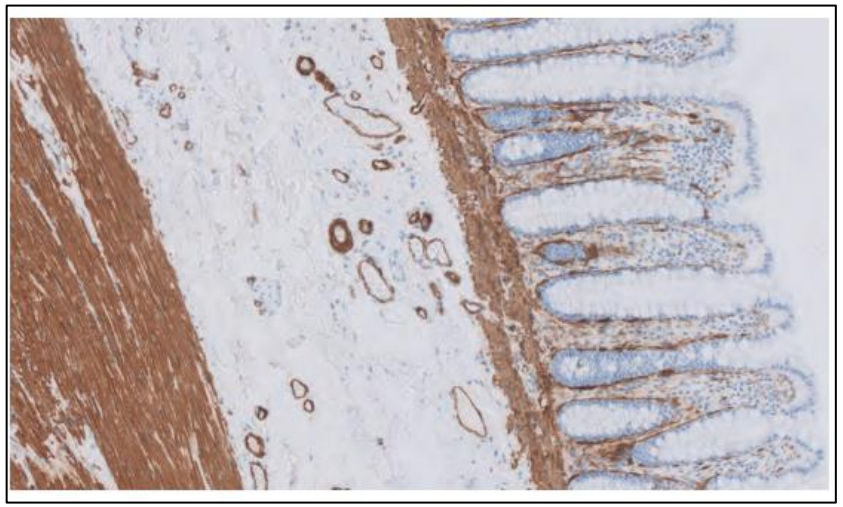
Antibody: Actin (Smooth Muscle)

Clone: 1A4

Code: GA611 | IR611

Reaction Location	Cytoplasm	
Quality Control	**Colon/Appendix**	**Liver**
High Expression	Smooth muscle cells in the lamina muscularis mucosa show a moderate to strong staining reaction.	Smooth muscle cells in large vessels show a moderate to strong staining reaction.
Low Expression	NA	Smooth muscle cells lining the liver sinusoids show a weak to moderate staining reaction.
Non-expression	Epithelial cells.	Hepatocytes

Note: No staining reaction should be seen in appendiceal columnar epithelial cells, lymphocytes or liver cells.



Colon 20X

BASIC CONTROL USED IN SJMC HISTOPATHOLOGY LAB

Monoclonal Mouse Anti-Human

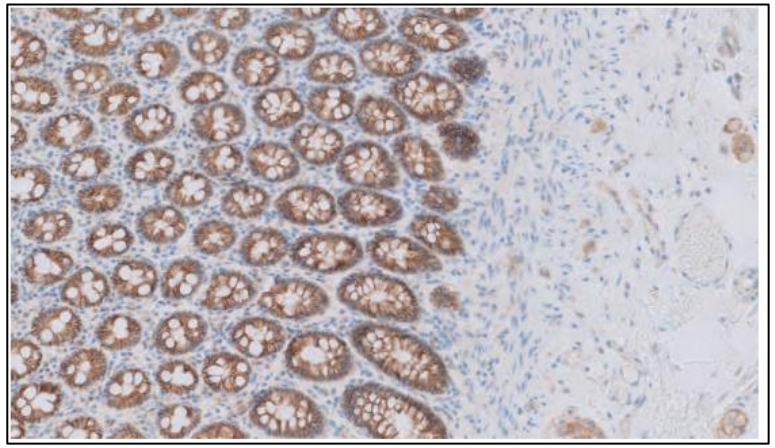
Antibody: Beta-Catenin

Clone: β-Catenin-1

Code: GA702 | IR702

Reaction Location	Membrane	
Quality Control	**Colon/Appendix**	**Liver**
High Expression	Epithelial cells show a moderate to strong staining reaction.	Epithelial cells of bile ducts show a moderate to strong staining reaction.
Low Expression	NA	Hepatocytes show a weak to moderate staining reaction.
Non-expression	NA	NA

Note: A weak cytoplasmic reaction is accepted.



Colon 10X

BASIC CONTROL USED IN SJMC HISTOPATHOLOGY LAB

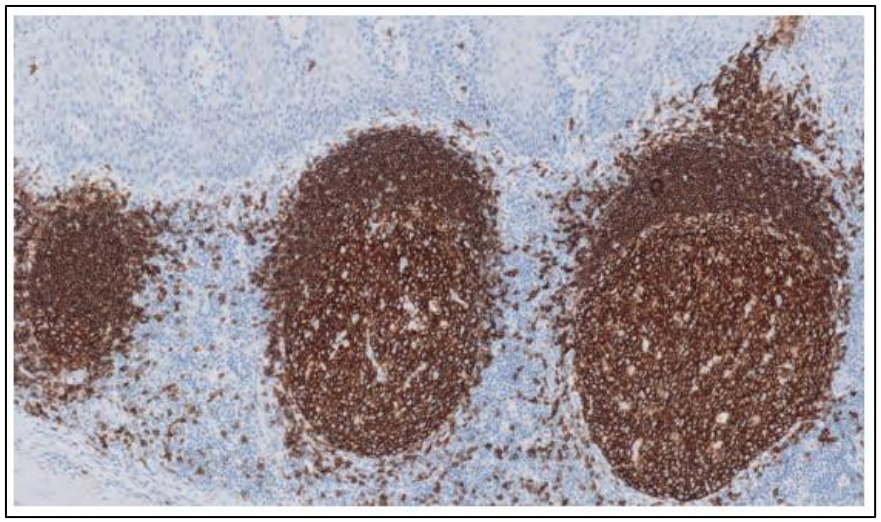
Monoclonal Mouse Anti-Human

Antibody: CD20cy

Clone: L26

Code: GA604 | IR604

Reaction Location	Membrane	
Quality Control	**Tonsil**	**Liver**
High Expression	Mantle zone and germinal center B cells show a moderate to strong staining reaction.	NA
Low Expression	NA	Isolated B cells show a weak to moderate staining reaction.
Non-expression	Squamous epithelial cells.	NA



Tonsil 10X

BASIC CONTROL USED IN SJMC HISTOPATHOLOGY LAB

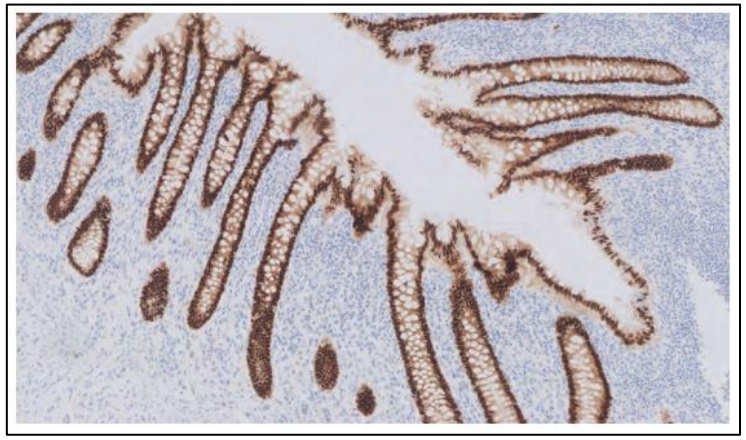
Monoclonal Mouse Anti-Human

Antibody: CDX-2

Clone: DAK-CDX-2

Code: GA080 | IR080

Reaction Location	Nucleus	
Quality Control	**Appendix**	**Pancreas**
High Expression	Columnar epithelial cells show a moderate to strong staining reaction.	NA
Low Expression	NA	Ductal and scattered intercalated epithelial cells show a weak to moderate staining reaction.
Non-expression	NA	NA

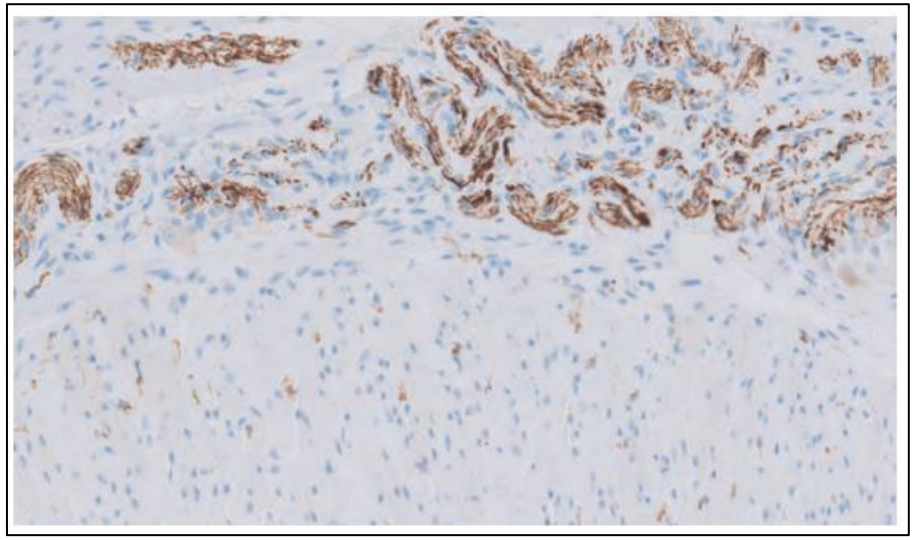


Appendix 10X

BASIC CONTROL USED IN SJMC HISTOPATHOLOGY LAB

Antibody:	Monoclonal Mouse Anti-Human Neurofilament Protein
Clone:	2F11
Code:	GA607 | IR607

Reaction Location	Cytoplasm
Quality Control	**Colon/Appendix**
High Expression	Large axons and ganglion cells in Auerbach's plexus show a moderate to strong staining reaction.
Low Expression	Isolated axons in the muscularis externa show a weak to moderate staining reaction.
Non-expression	Smooth muscle cells and epithelium



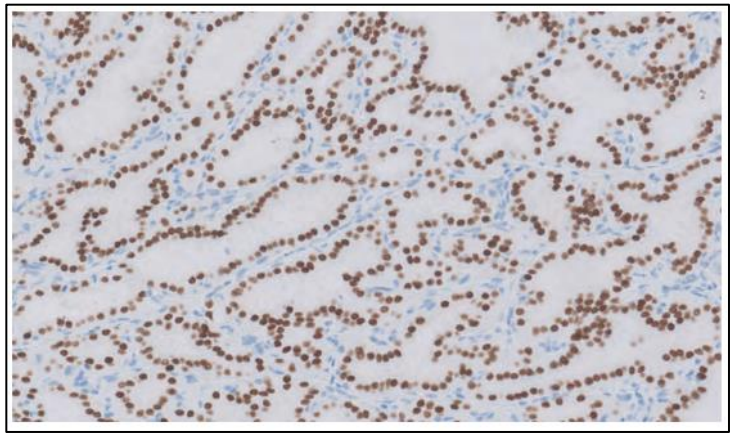
Colon 10X

BASIC CONTROL USED IN SJMC HISTOPATHOLOGY LAB

Antibody: Monoclonal Mouse Anti-Thyroid Transcription Factor
Clone: 8G7G3/1
Code: IR056

Reaction Location	Nucleus	
Quality Control	**Thyroid**	**Lung**
High Expression	Thyroid follicular epithelial cells show a moderate to strong staining reaction.	Type II pneumocyte cells lining the alveolar walls show a moderate to strong staining reaction.
Low Expression	NA	NA
Non-expression	NA	NA

Note: Occasionally, a weak staining reaction may be observed in columnar epithelial cells of the respiratory ducts.



Thyroid 20X

THANK YOU

PRESENTED BY MARIAMMAH SIMMANCHALAM

103369 (MLS 1)