LA TÉCNICA INMUNOHISTOQUÍMICA

Tomás García-Caballero
Inés Fernández Campos

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Complexo Hospitalario Universitario de Santiago de Compostela

Técnica histológica que permite detectar proteínas (“antígenos”)

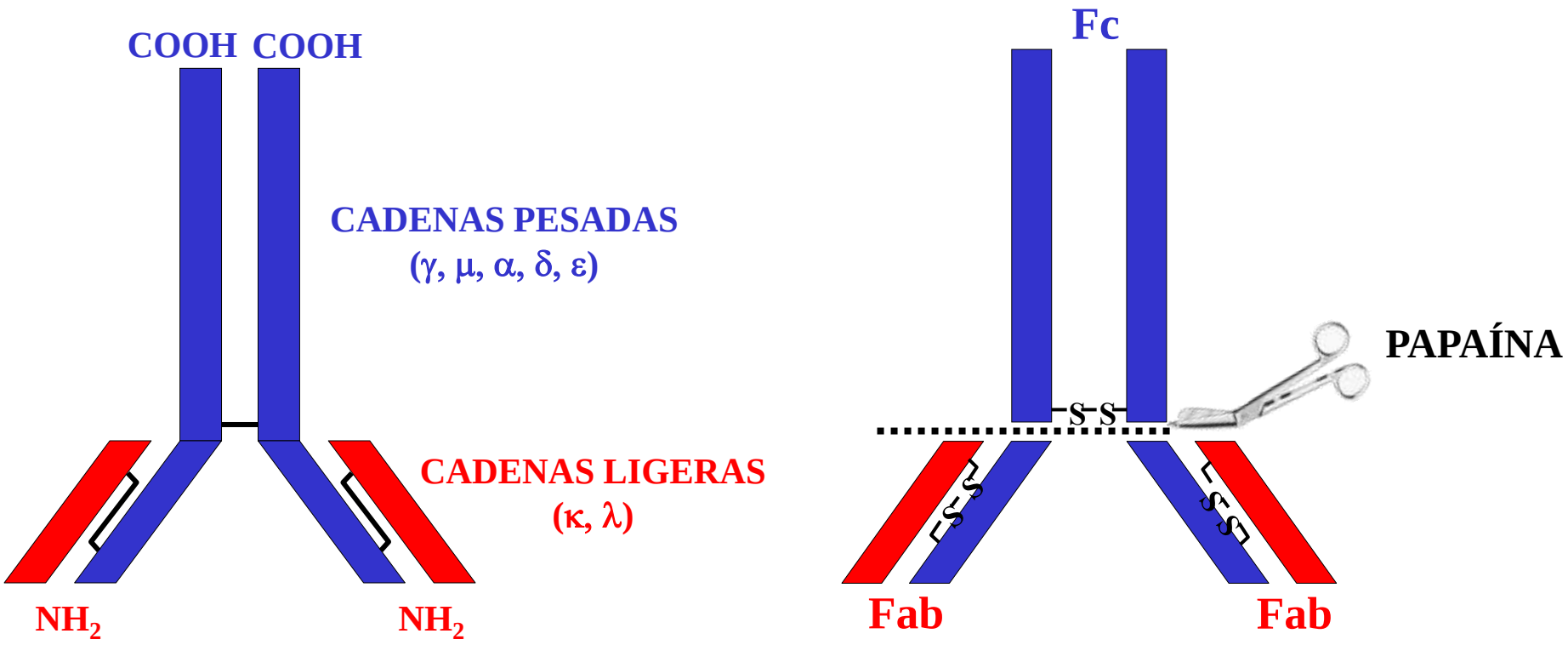
por medio de anticuerpos específicos y sistemas de detección enzimáticos

Concepto IHQ

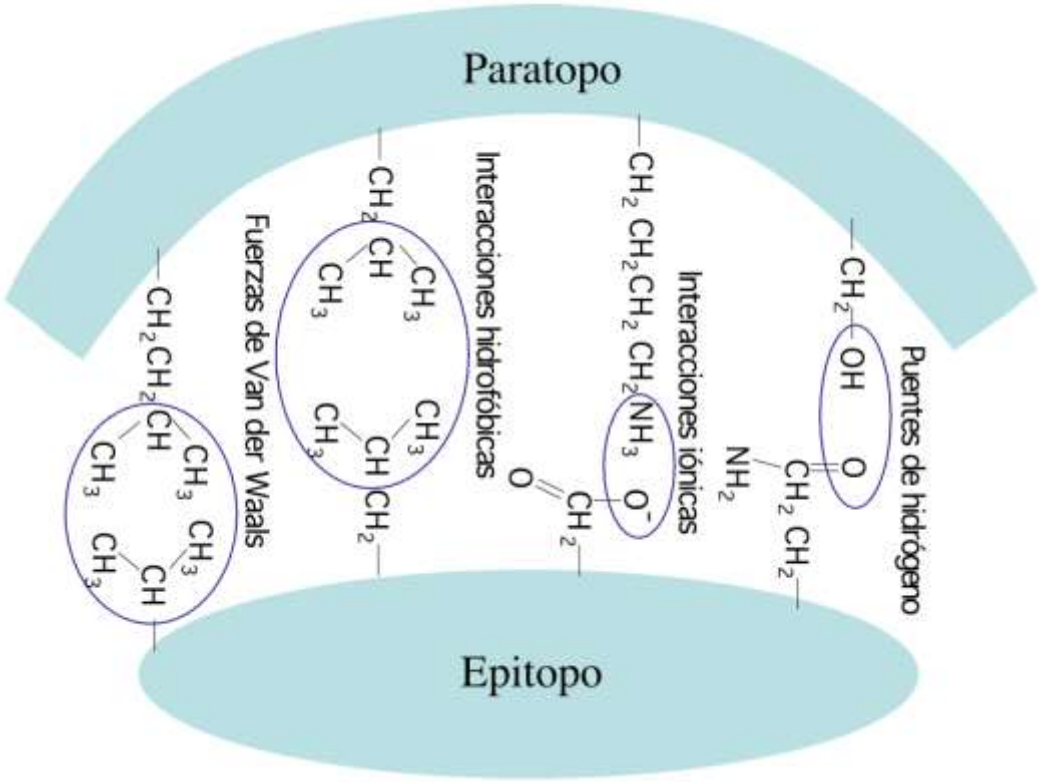
- **Batería de anticuerpos monoclonales**
- **Sensibilidad de los sistemas de detección**
- **Recuperación antigénica**

Desarrollo

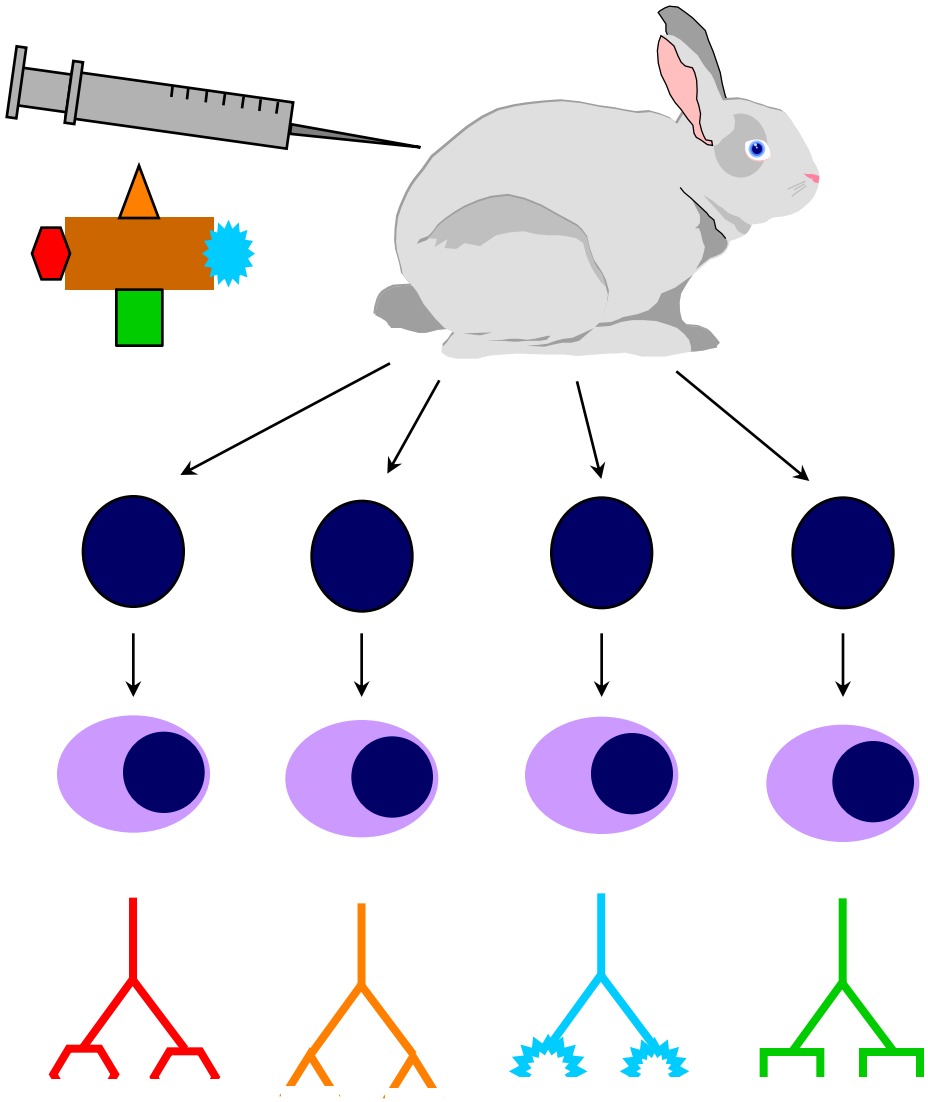
1. Batería de anticuerpos

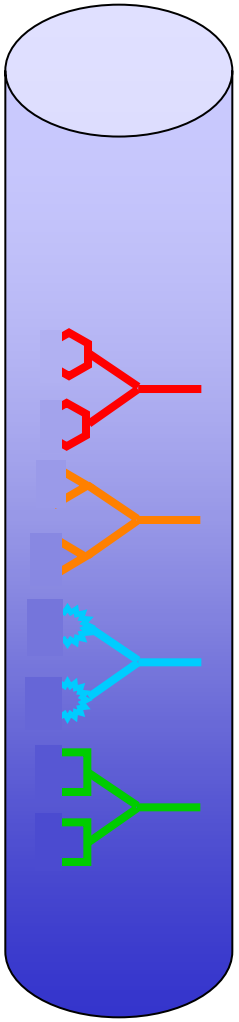


Paratopo / Epitopo

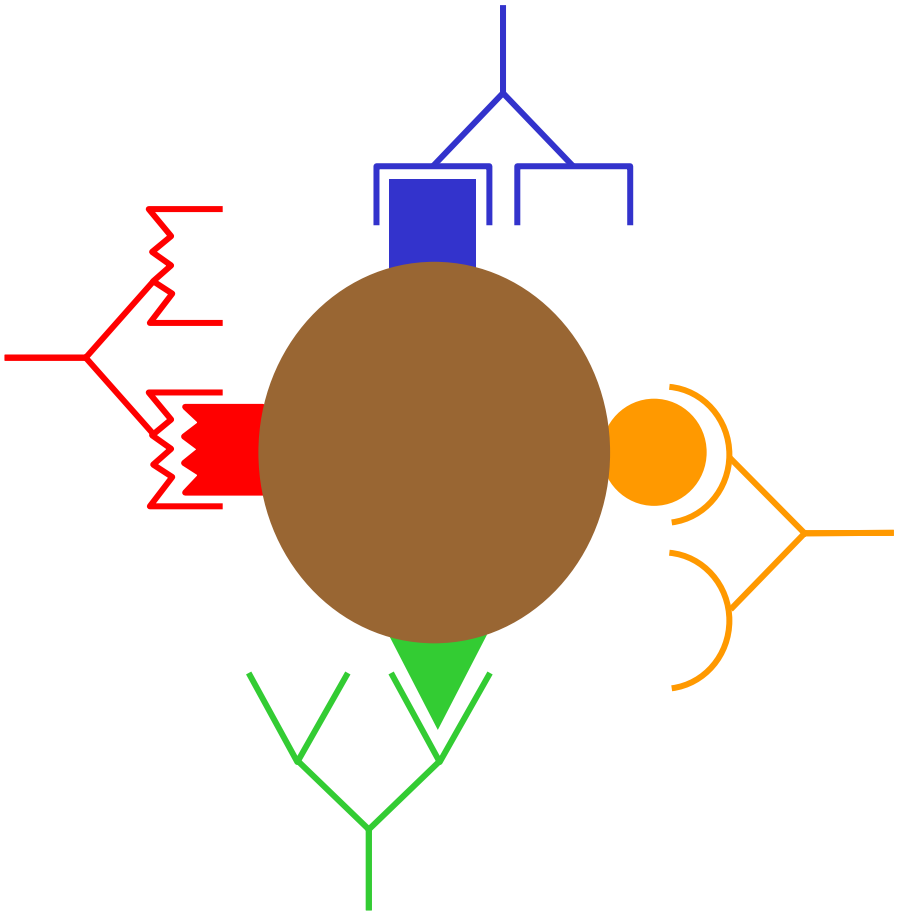


Anticuerpo policlonal





Anticuerpo - especificidad



Anticuerpo policlonal

Ac. monoclonales

Köhler G, Milstein C (1975)

Continuous cultures of fused cells secreting antibody of predefined specificity

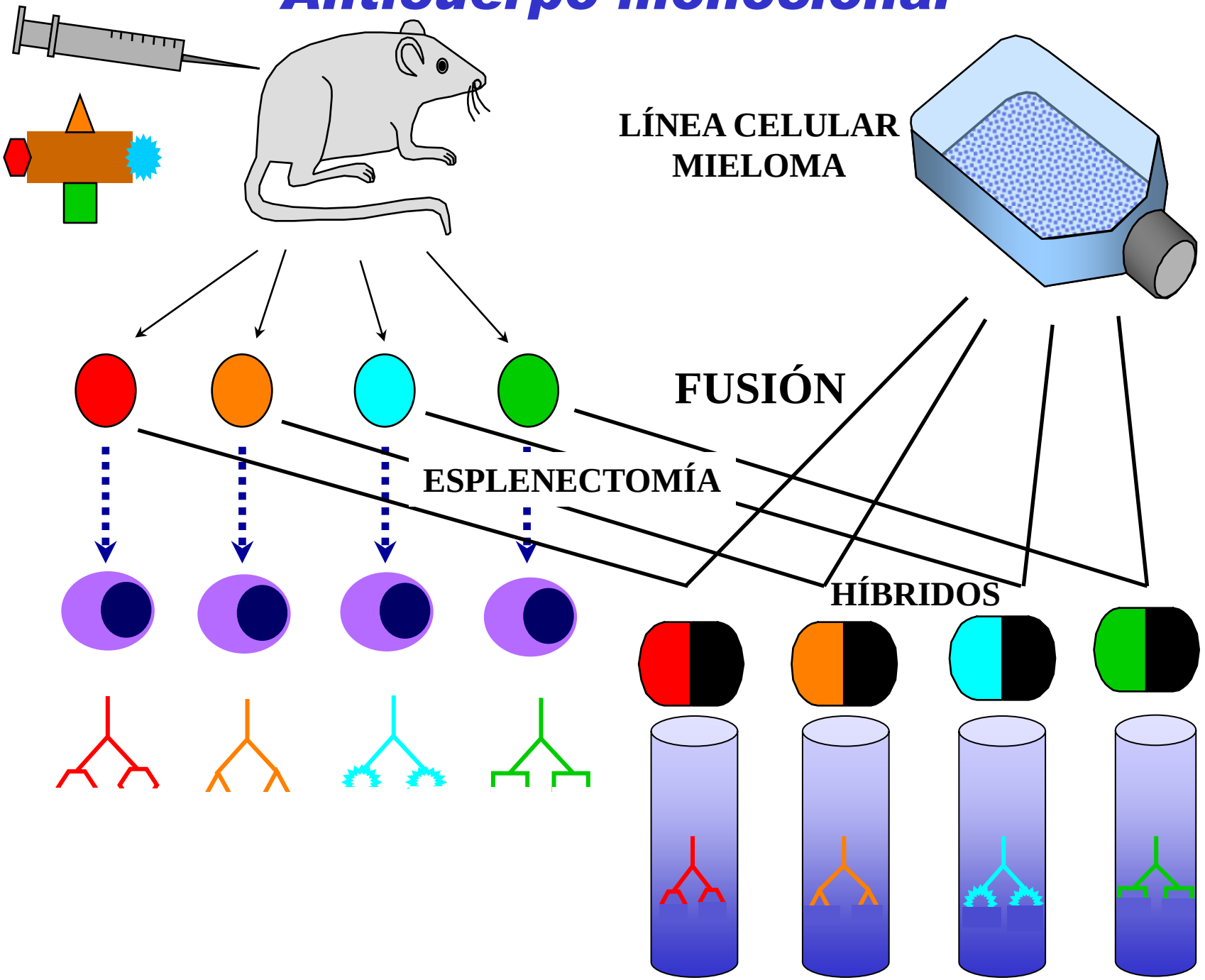
Nature 256:495-7

Ac. monoclonales

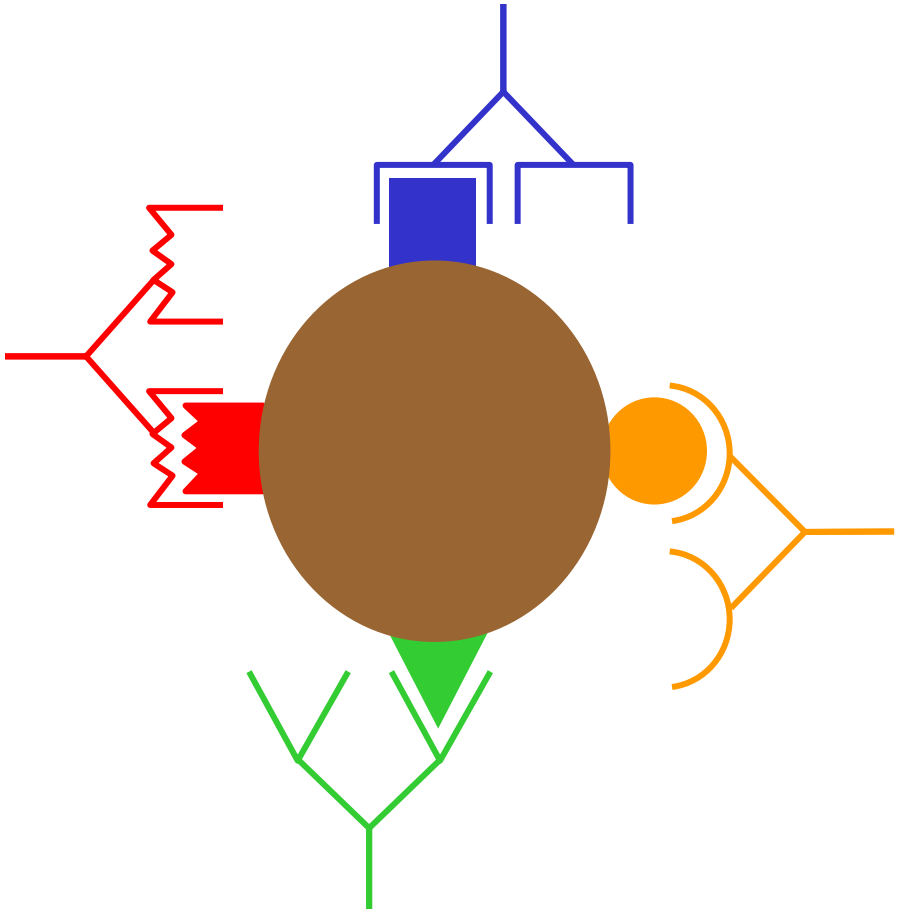
César Milstein y George Köhler

(Premio Nobel 1984)

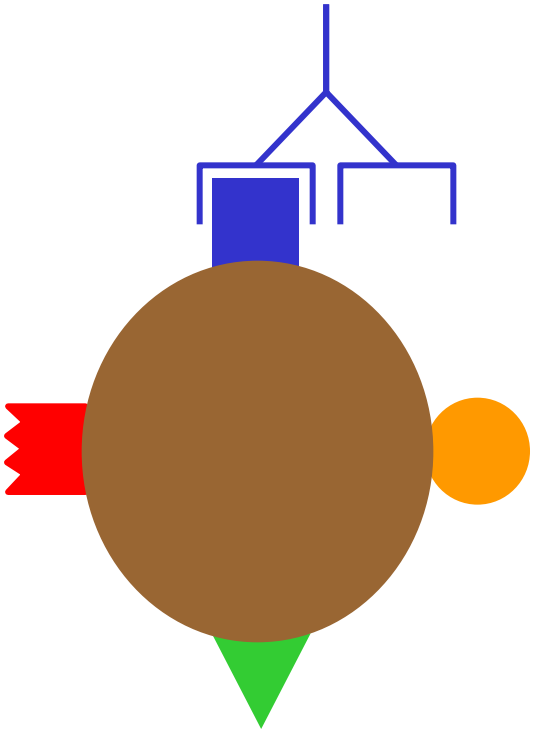
Anticuerpo monoclonal



Anticuerpo - especificidad



Anticuerpo policlonal



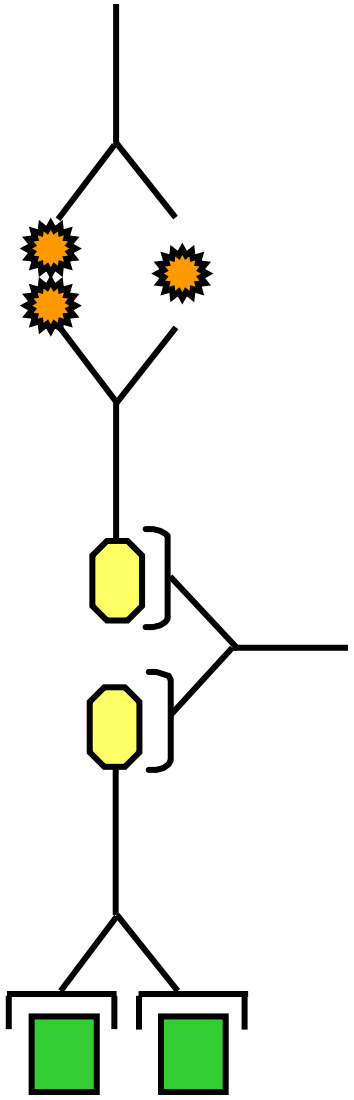
Anticuerpo monoclonal

2. Sensibilidad

Sternberger LA, Hardy PH, Cuculis JJ, Meyer HG (1970)

The unlabeled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (**horseradish peroxidase-antihorseradish peroxidase**) and its use in identification of spirochetes

J Histochem Cytochem 18:315-33



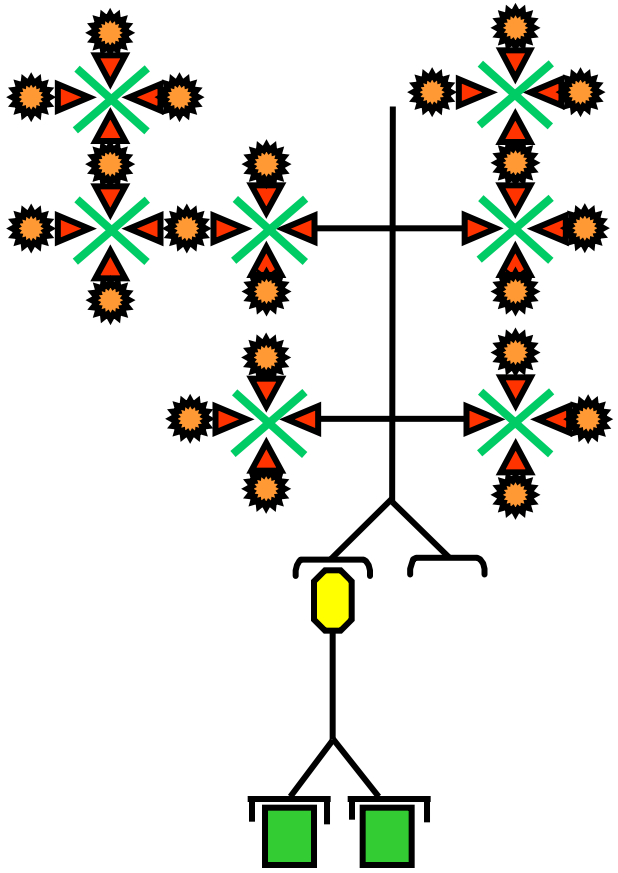
Sensibilidad

Hsu SM, Raine L, Fanger H

(1981)

Use of avidin-biotin-peroxidase complex (**ABC**) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures

J Histochem Cytochem 29:577-80

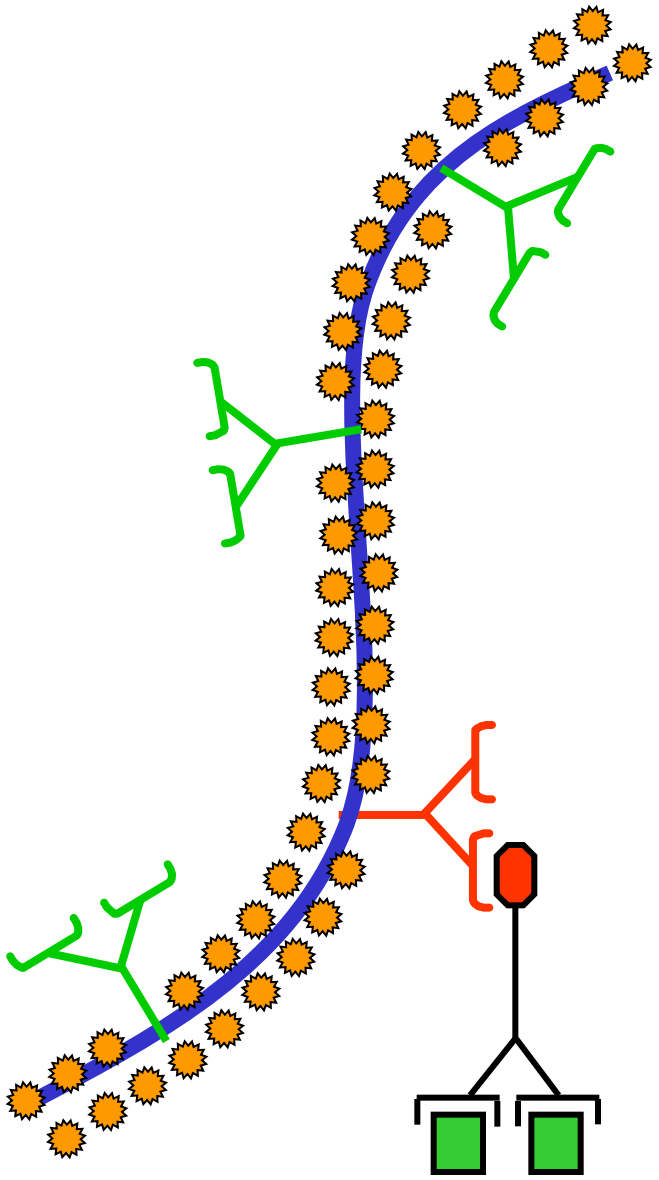


Sensibilidad

Bisgaard K, Lihme A, Rolsted H, Pluzek K-J (1993)

Polymeric conjugates for enhanced signal generation in enzyme immunoassays

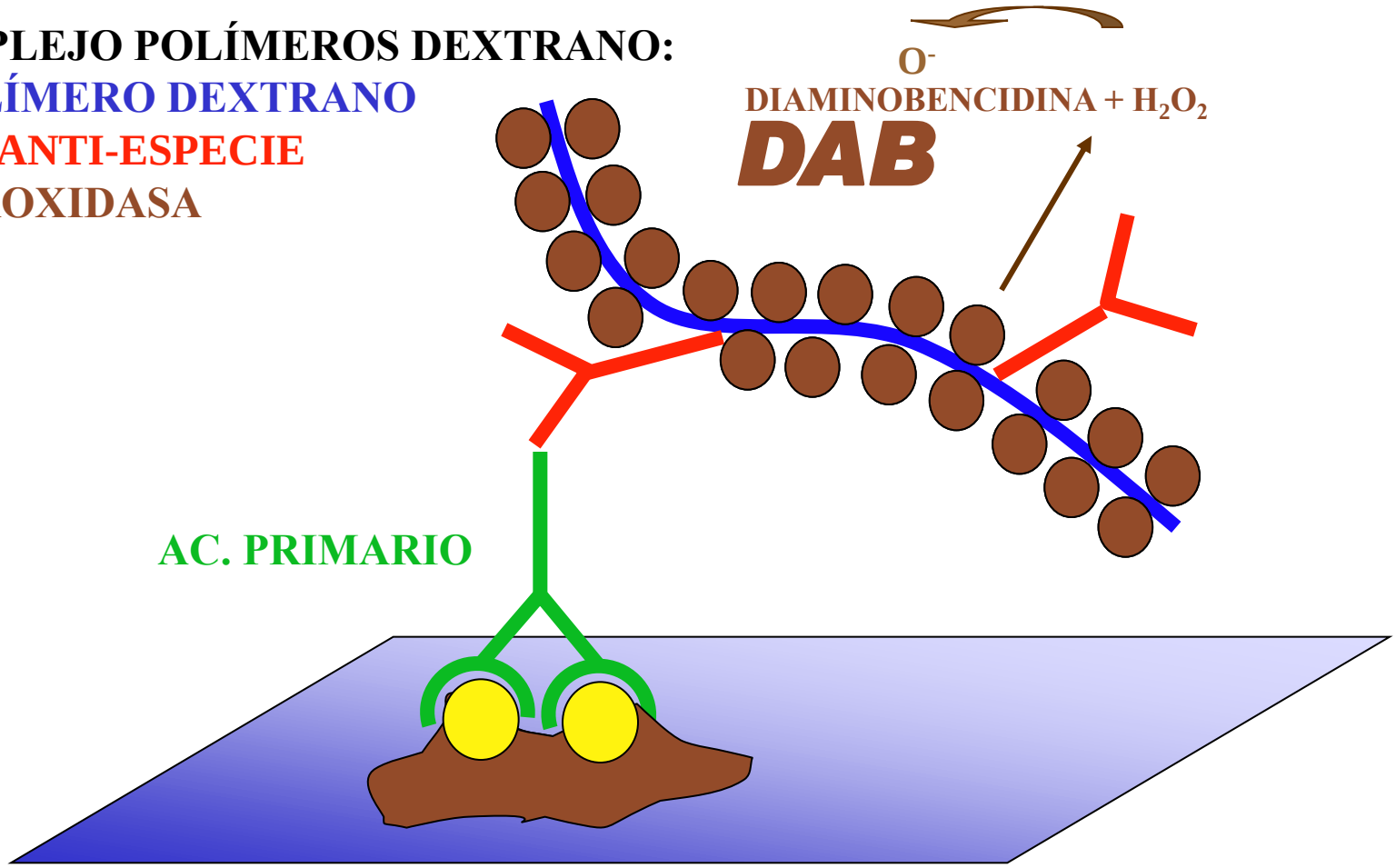
Scand Soc Immunol XXIV Annual Meeting. University Aarhus



Sensibilidad

COMPLEJO POLÍMEROS DEXTRANO:

- **POLÍMERO DEXTRANO**
- **AC. ANTI-ESPECIE**
- **PEROXIDASA**

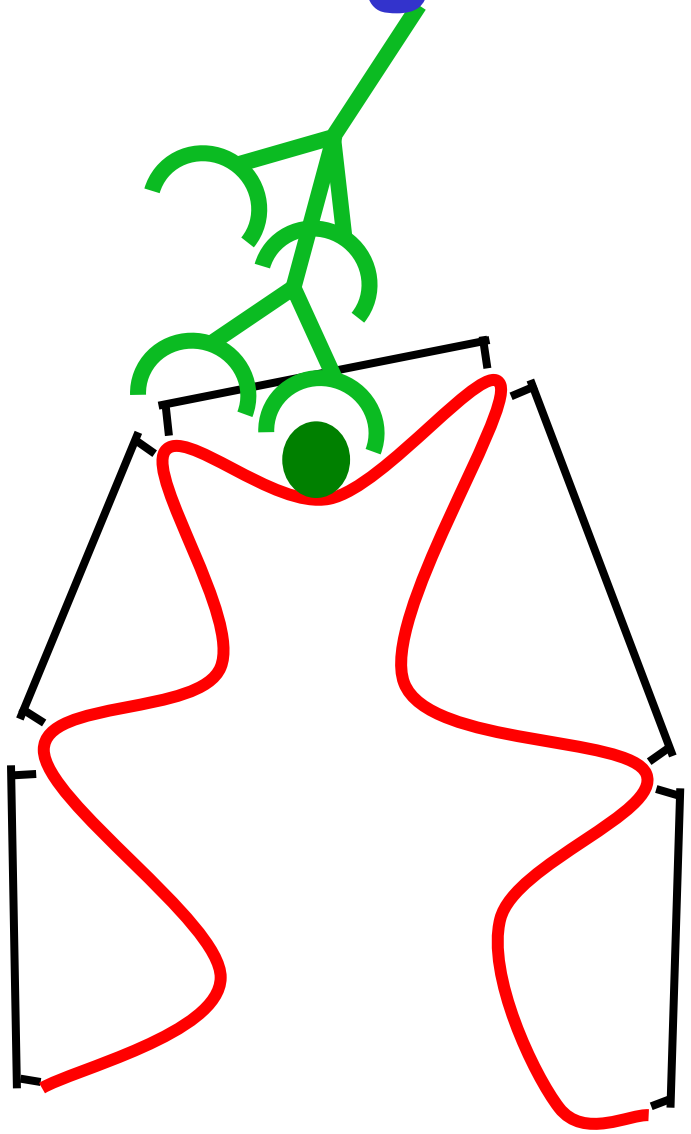


3. Recuperación antigénica

Shi S-R, Key ME, Kalra KL (1991)

Antigen retrieval in formalin-fixed paraffin embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections

J Histochem Cytochem 39:741-8



Recuperación antigénica

Shi S-R, Key ME, Kalra KL (1991)

Antigen retrieval in formalin-fixed paraffin embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections

J Histochem Cytochem 39:741-8

Recuperación antigénica

Norton AJ, Jordan S, Yeomans P (1994)

Brief, high-temperature heat denaturation (**presure cooking**): a simple and effective method of antigen retrival for routinely processed tissues

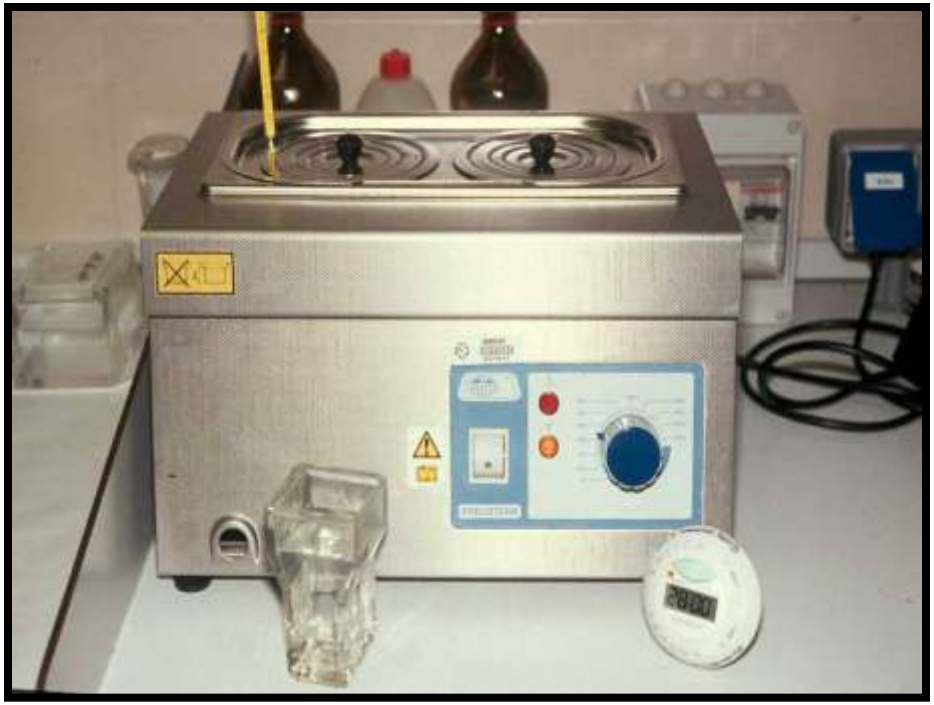
J Pathol 173:371-9

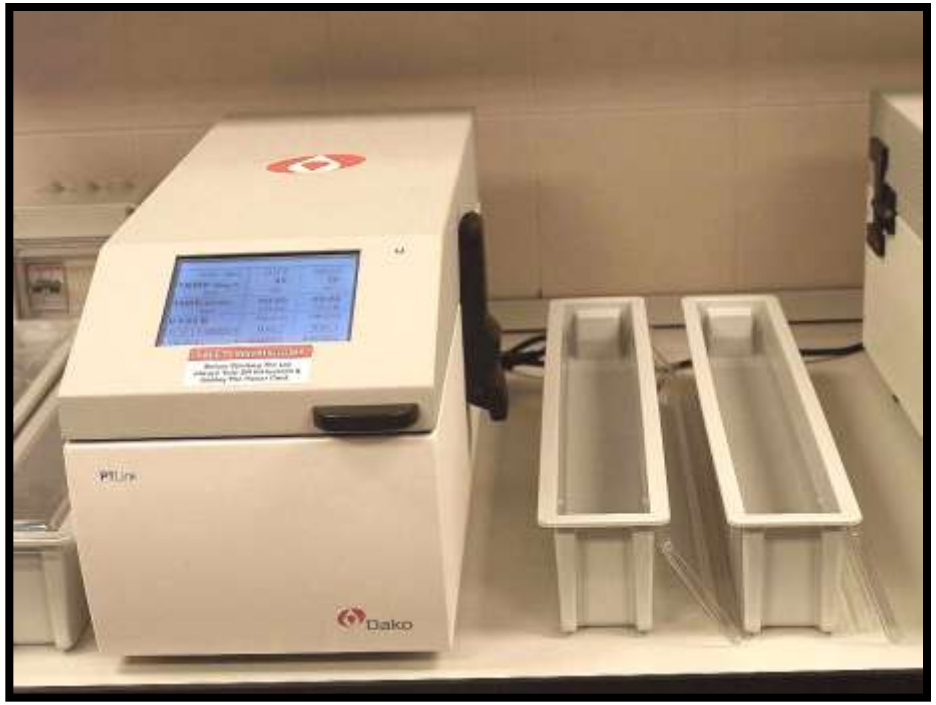
Recuperación antigénica

Kawai K, Serizawa A, Hamana T, Tsutsumi Y (1994)

Heat-induced antigen retrieval of proliferating cell nuclear antigen and p53 protein in formalin-fixed, paraffin-embedded sections.

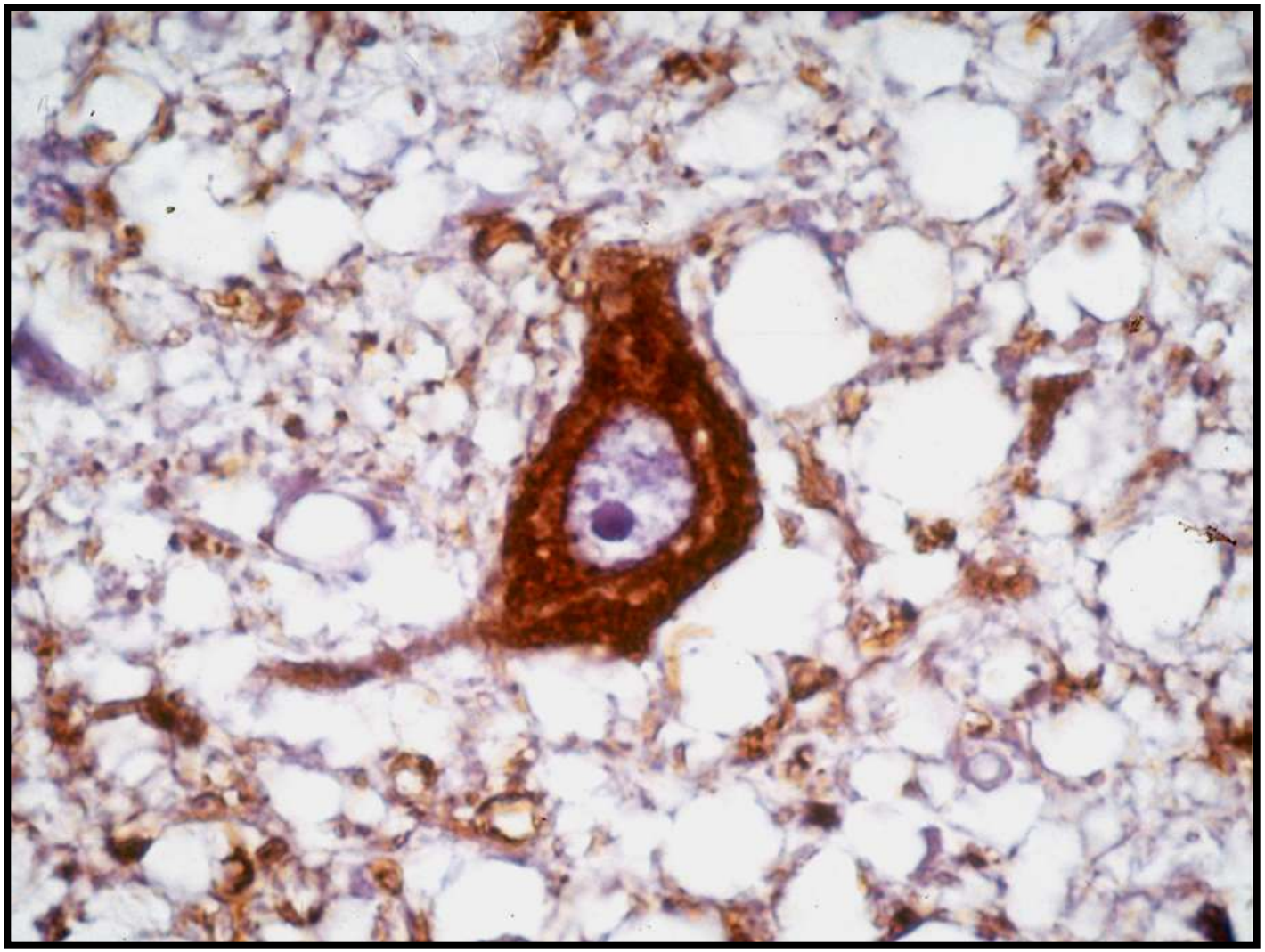
Pathol Int 44:759-64.



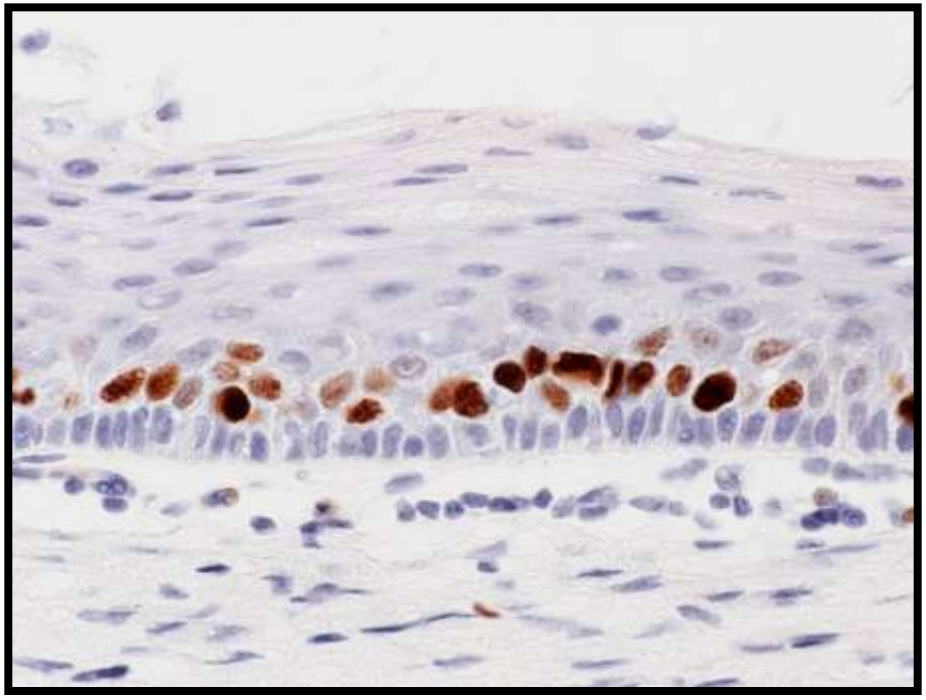


Patrones de tinción

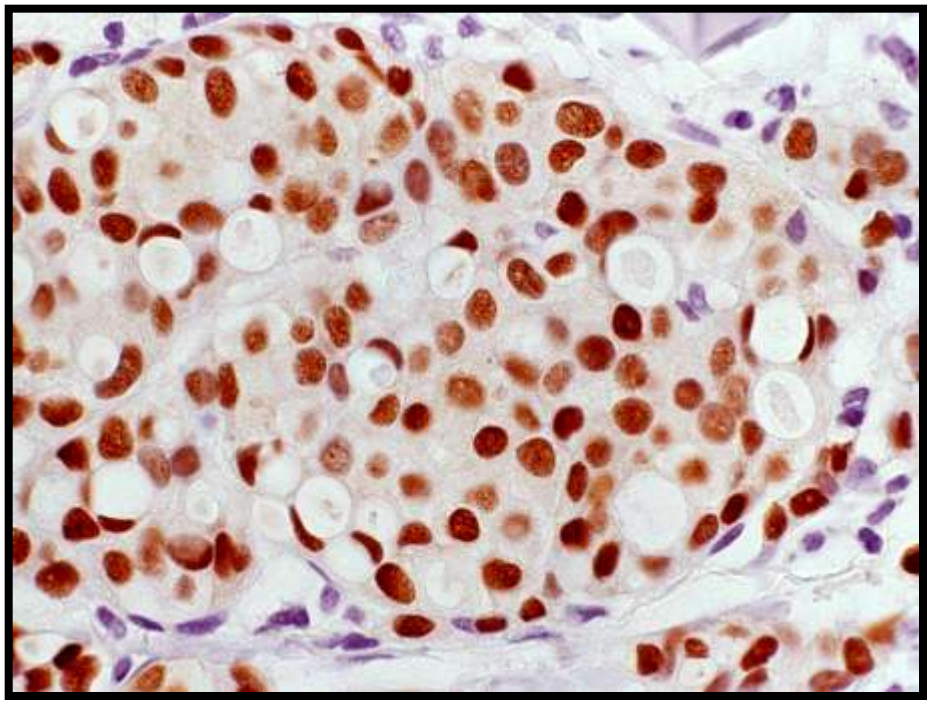
Citoplasmático



Nuclear

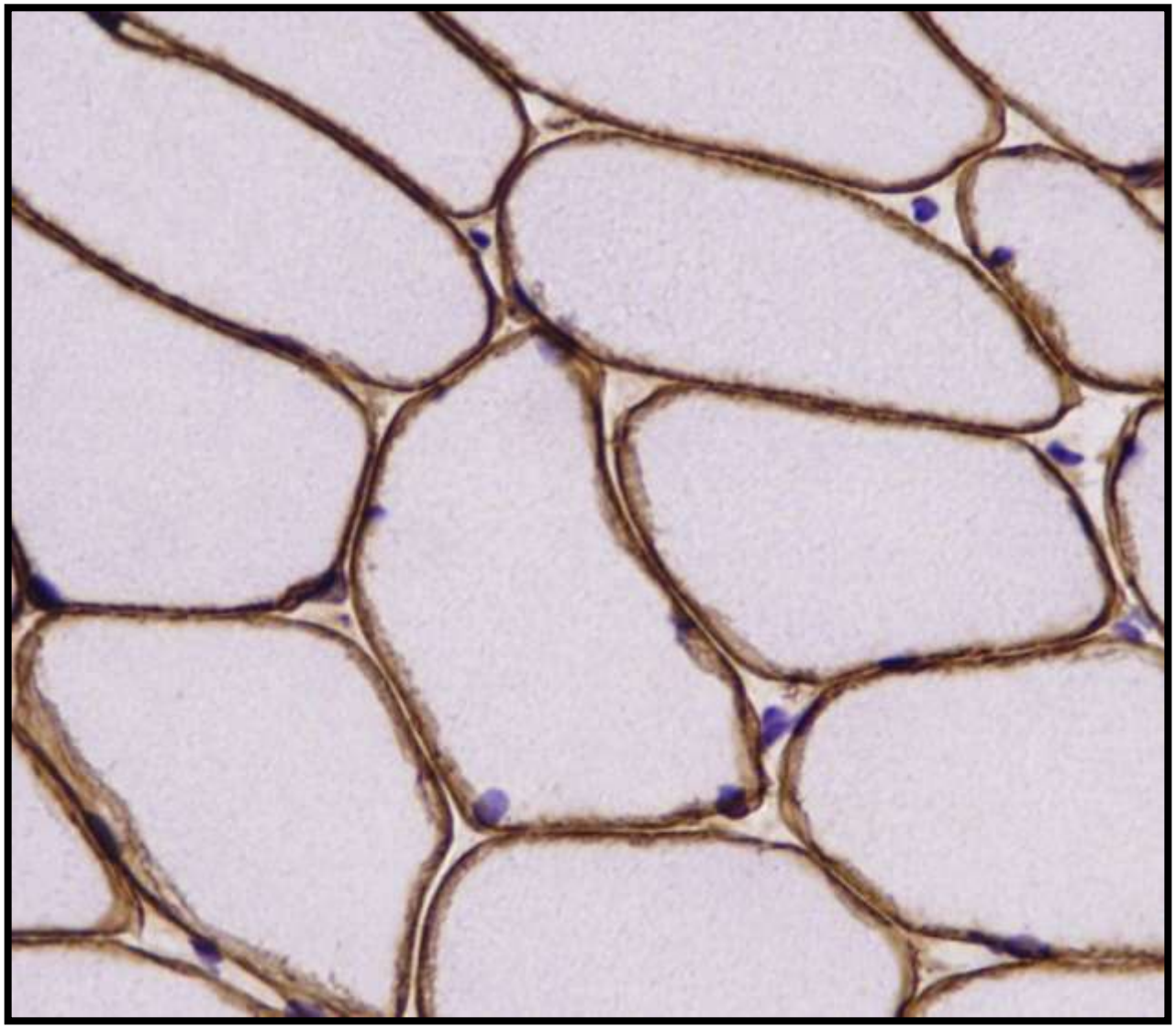


Ki-67/MIB-1



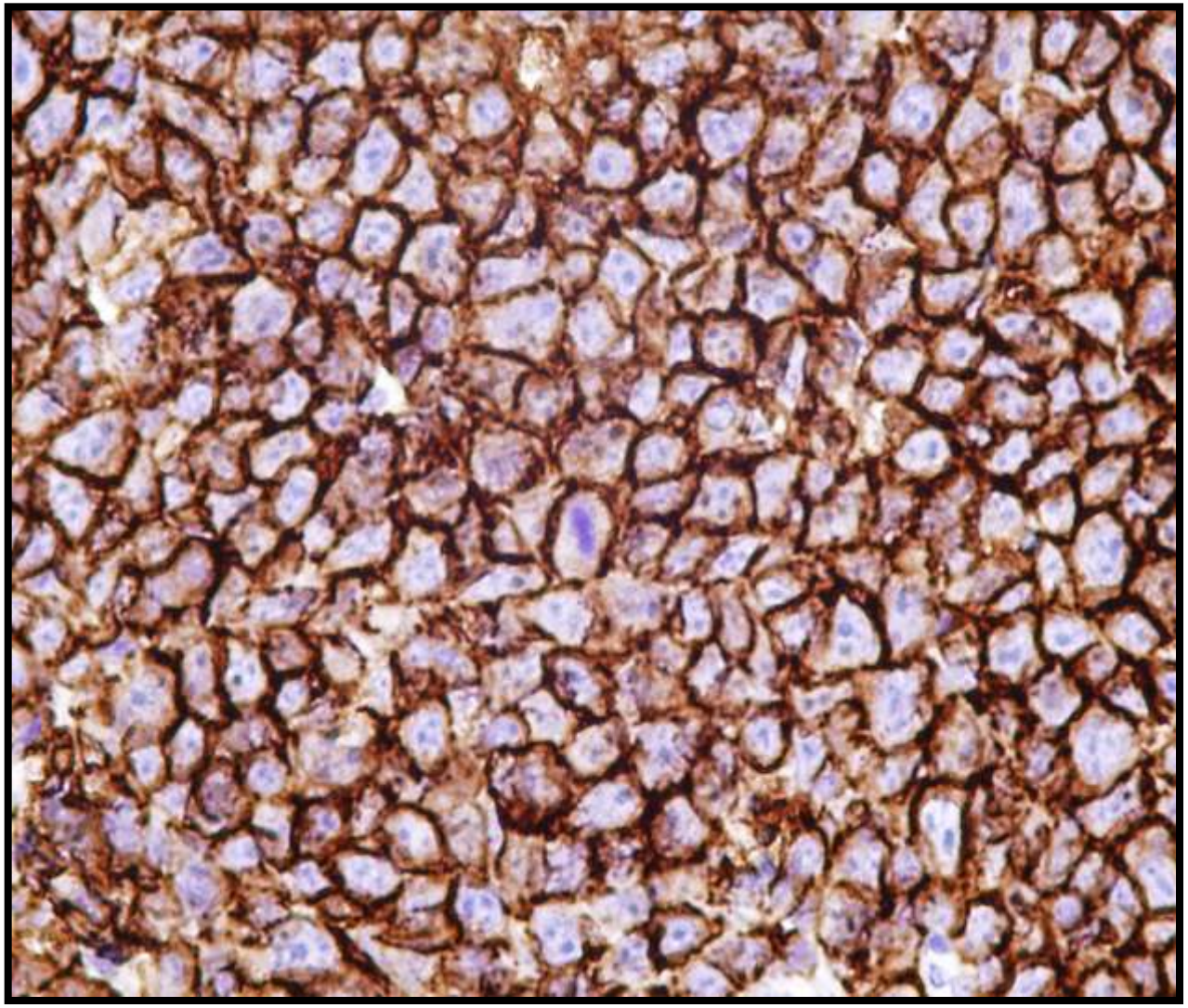
Rec. estrógenos

Membrana



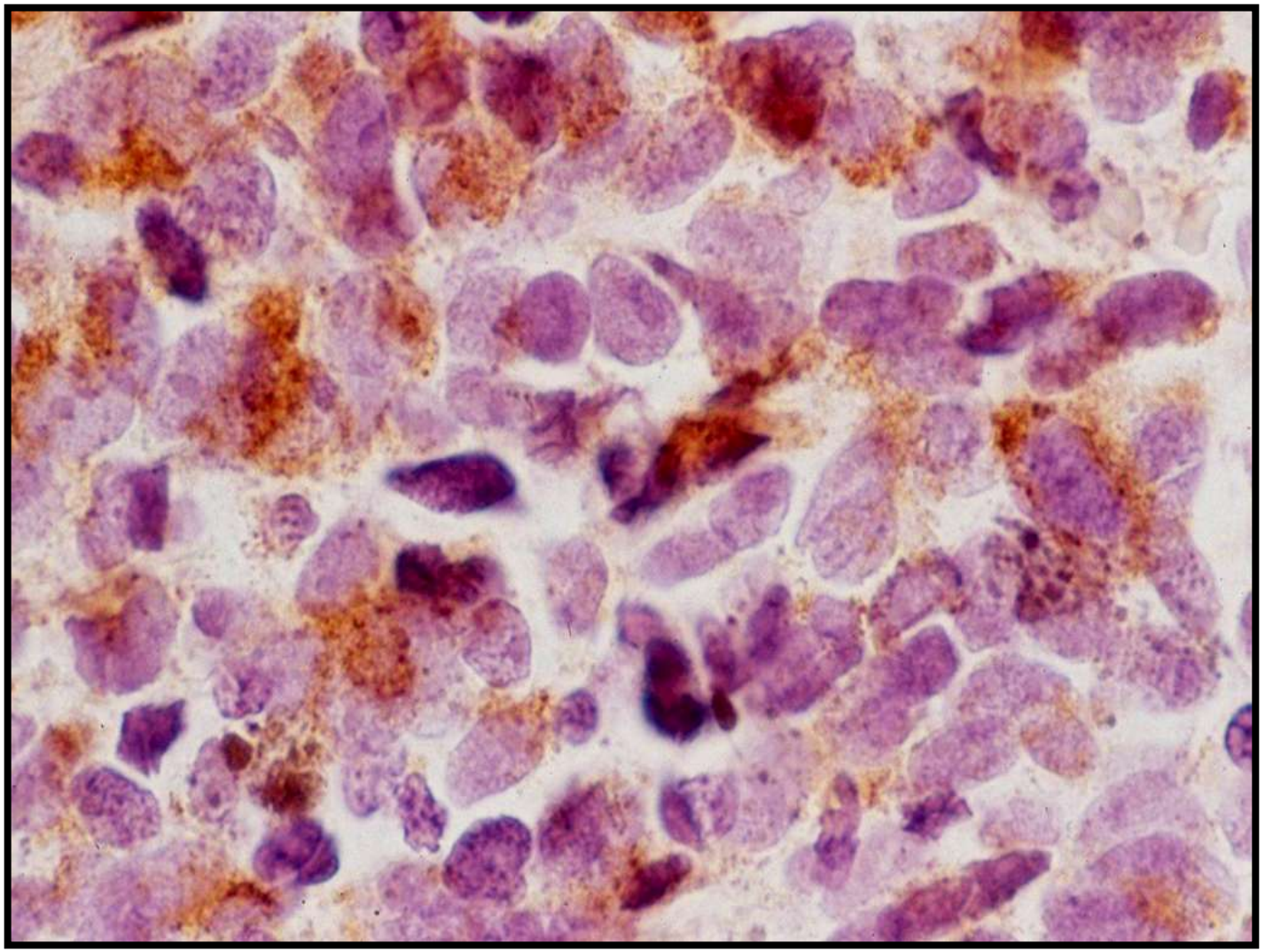
Espectrina

Membrana



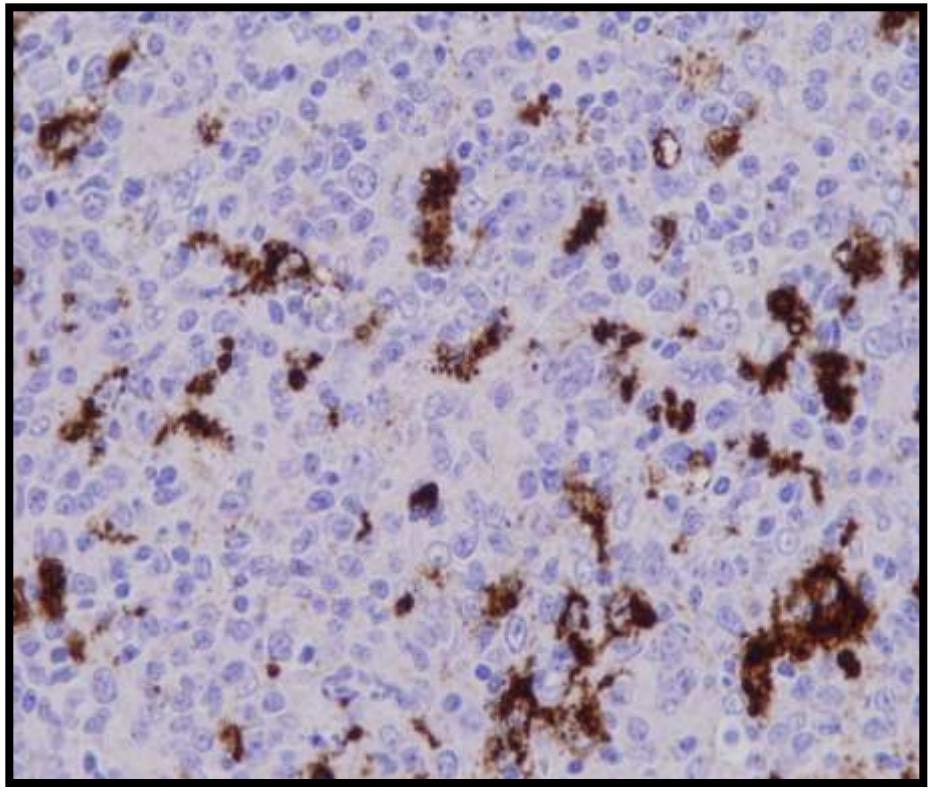
LCA

Patrones especiales

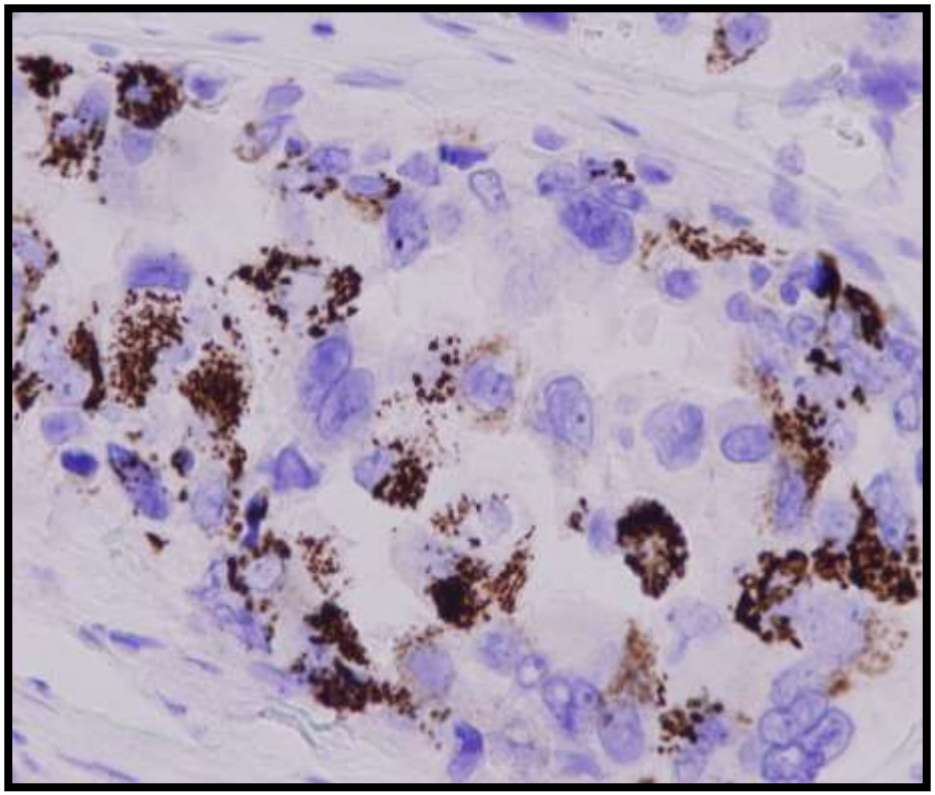


HMB-45

Patrones especiales

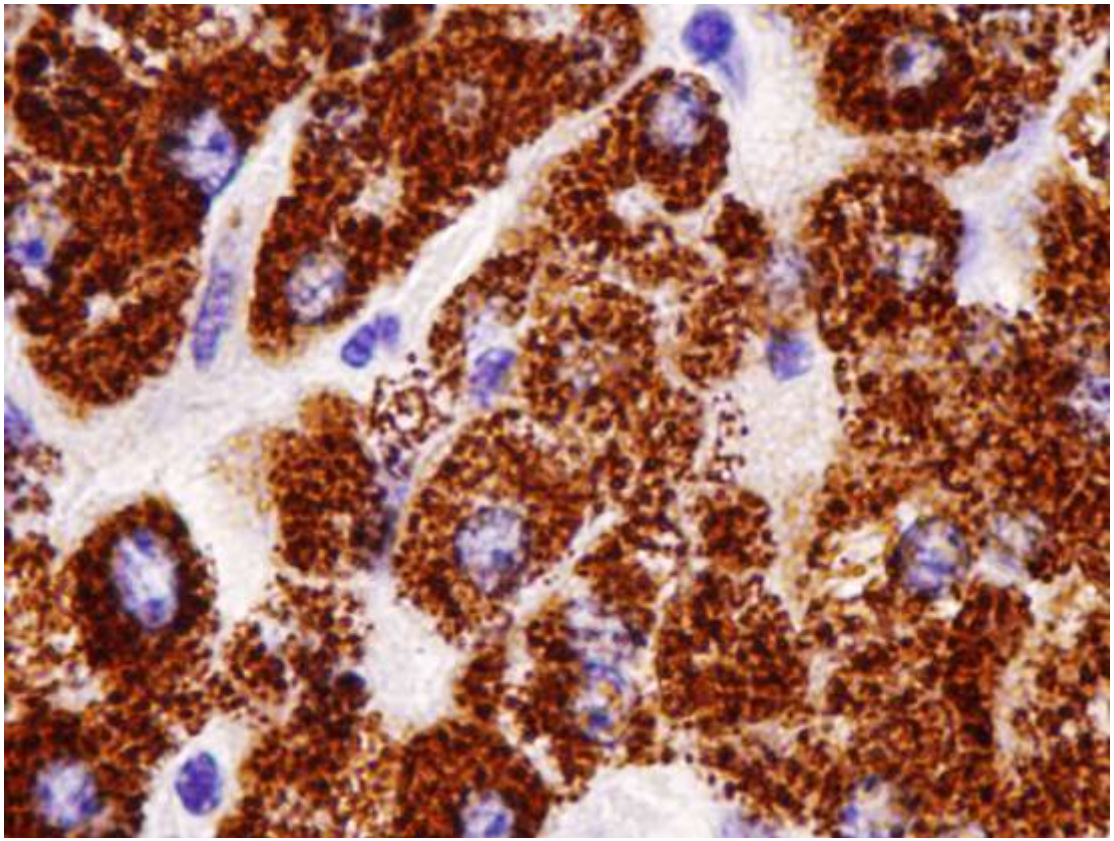


CD 68



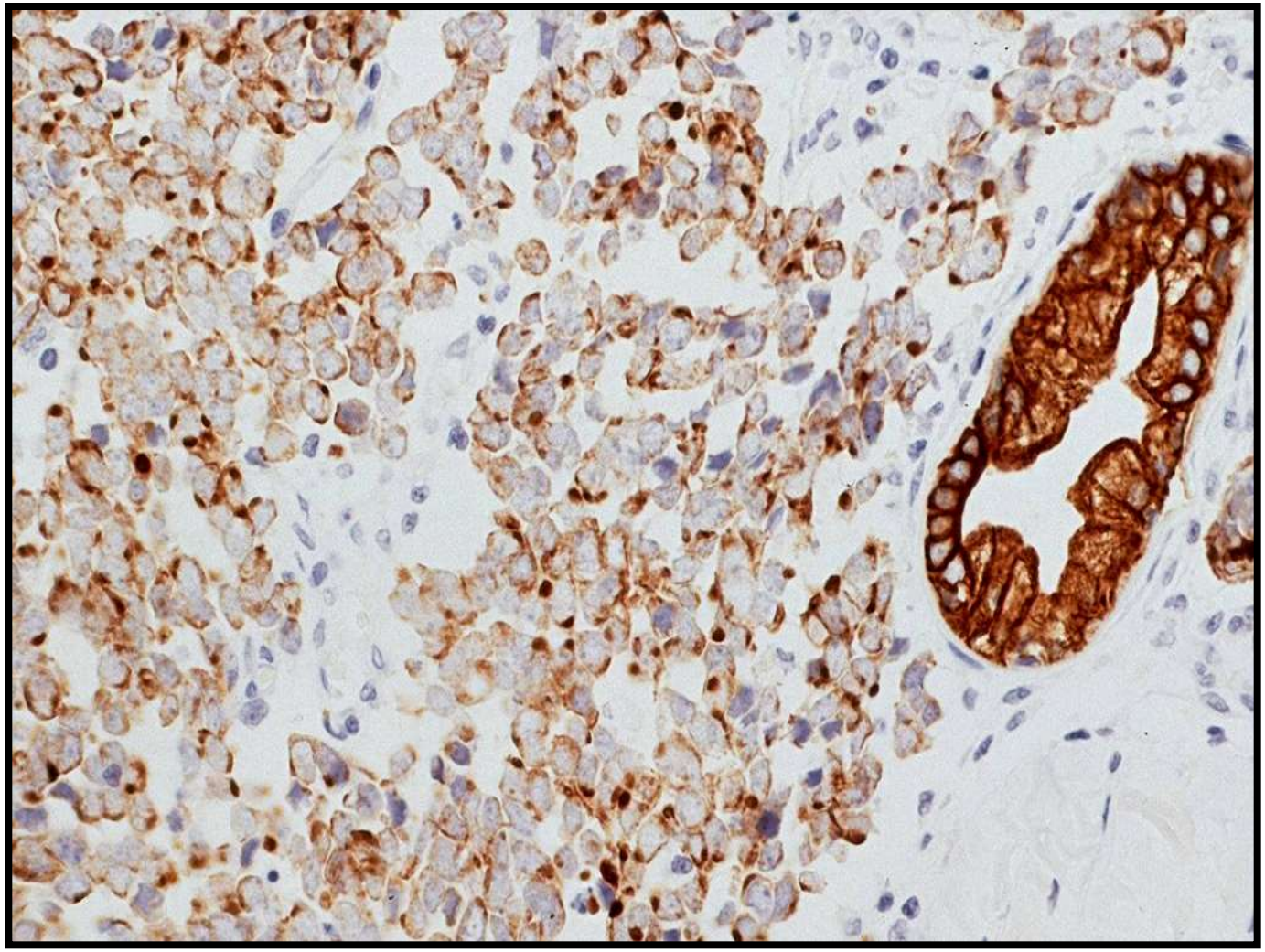
Racemasa

Patrones especiales



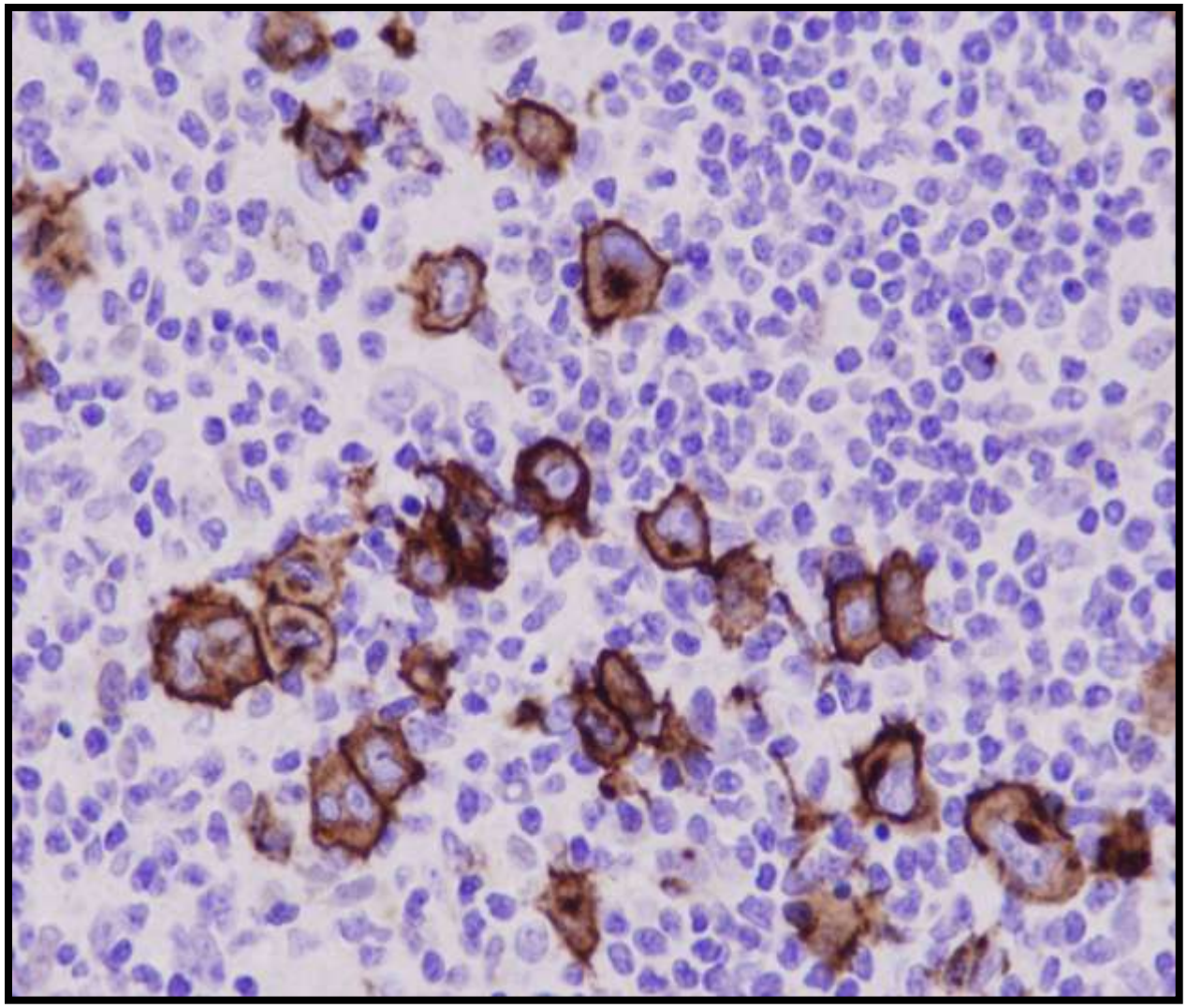
Hepatocito

Patrones especiales



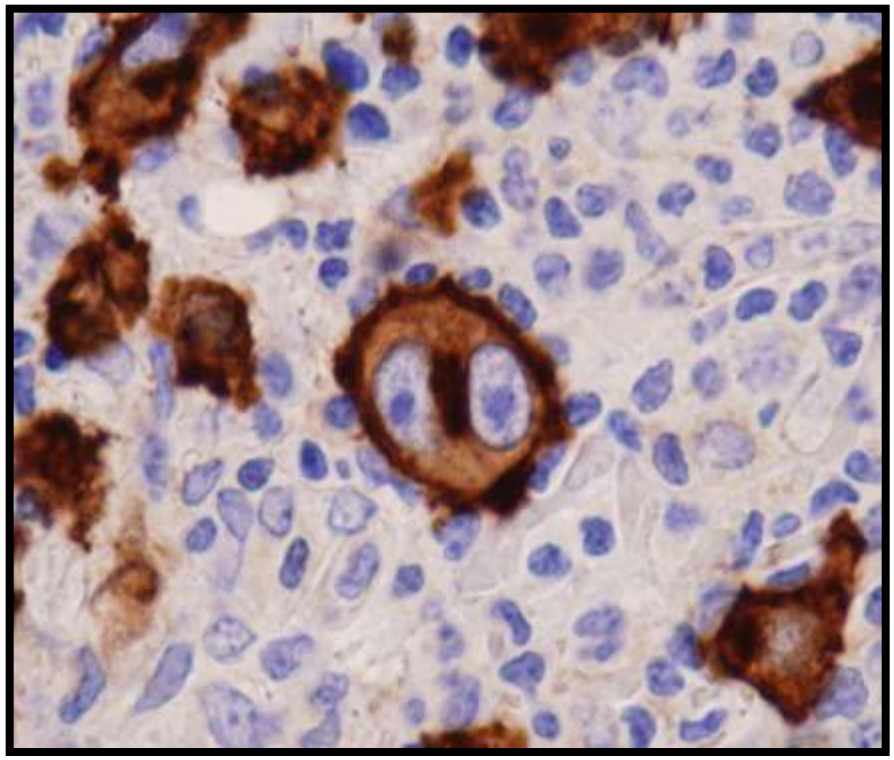
CK-CAM 5.2

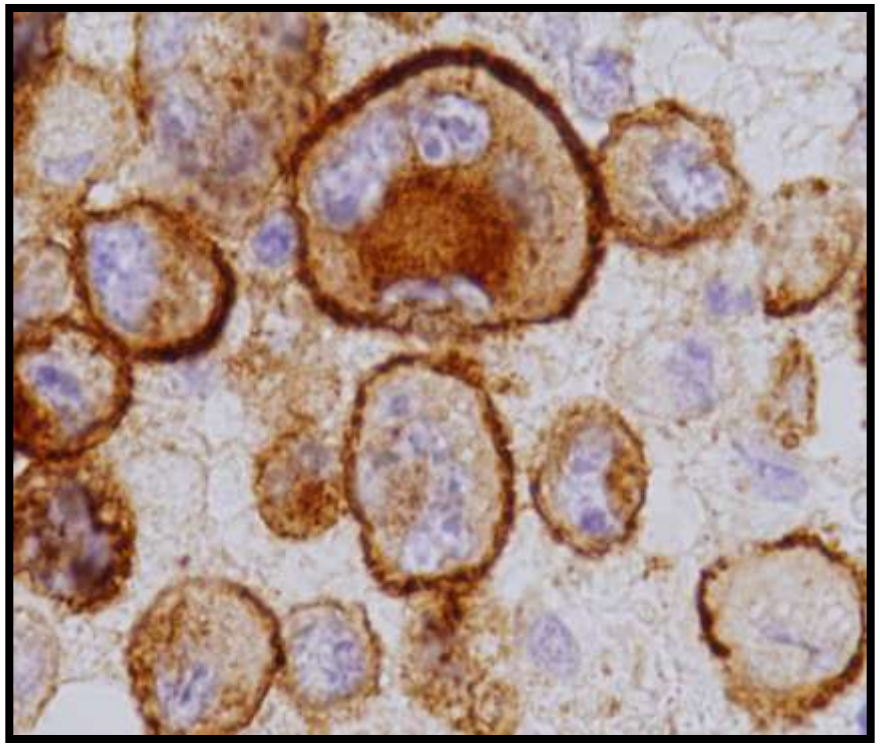
Patrones especiales



CD 57

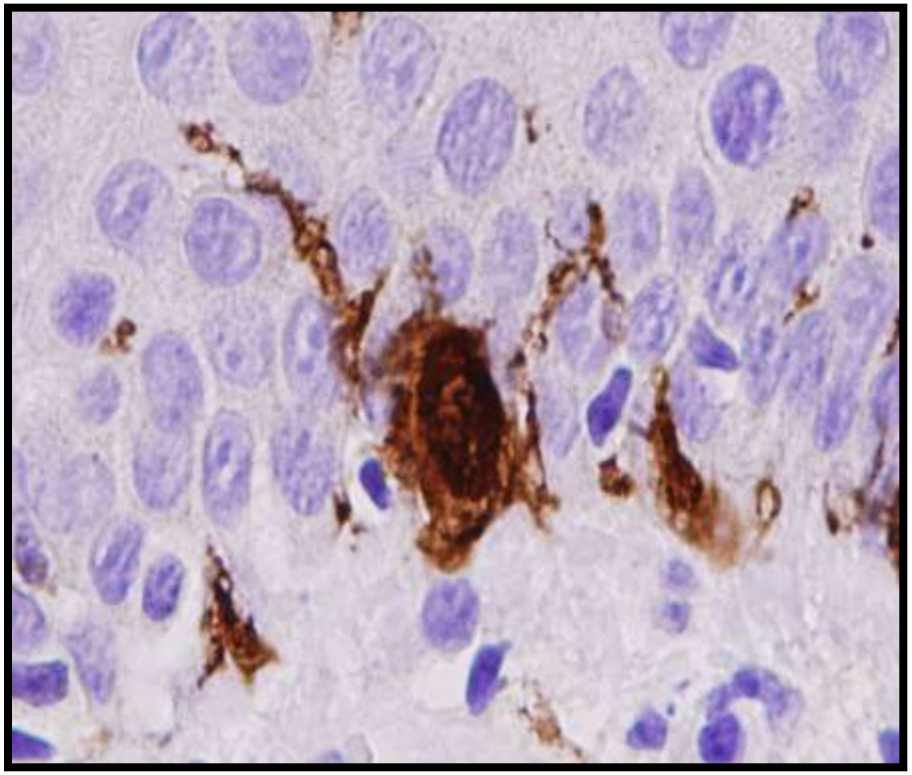
Patrones especiales

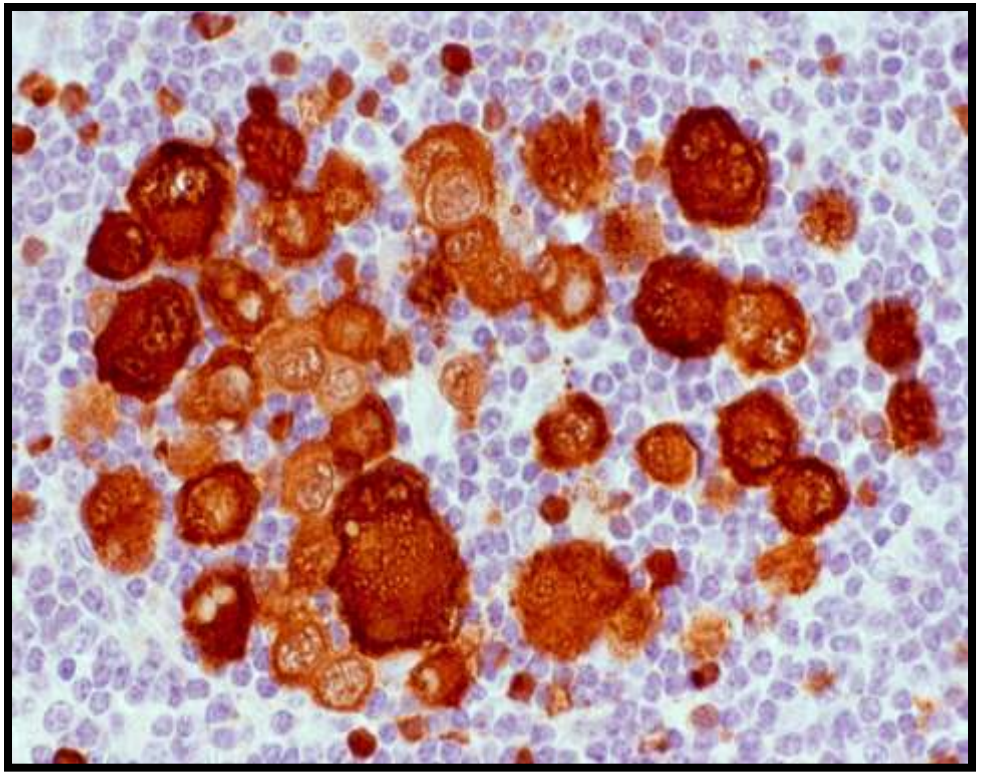




CD 30

Patrones especiales

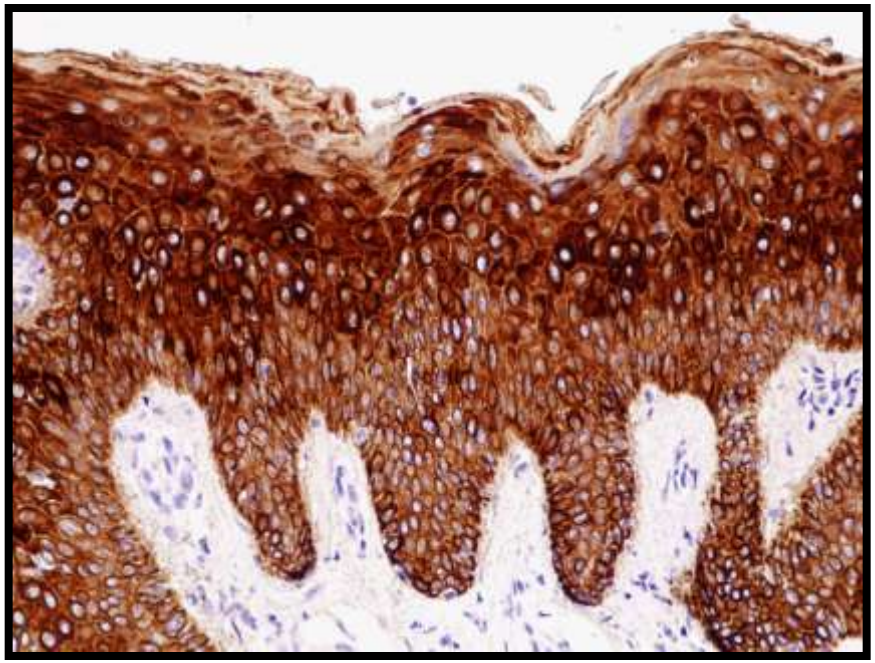


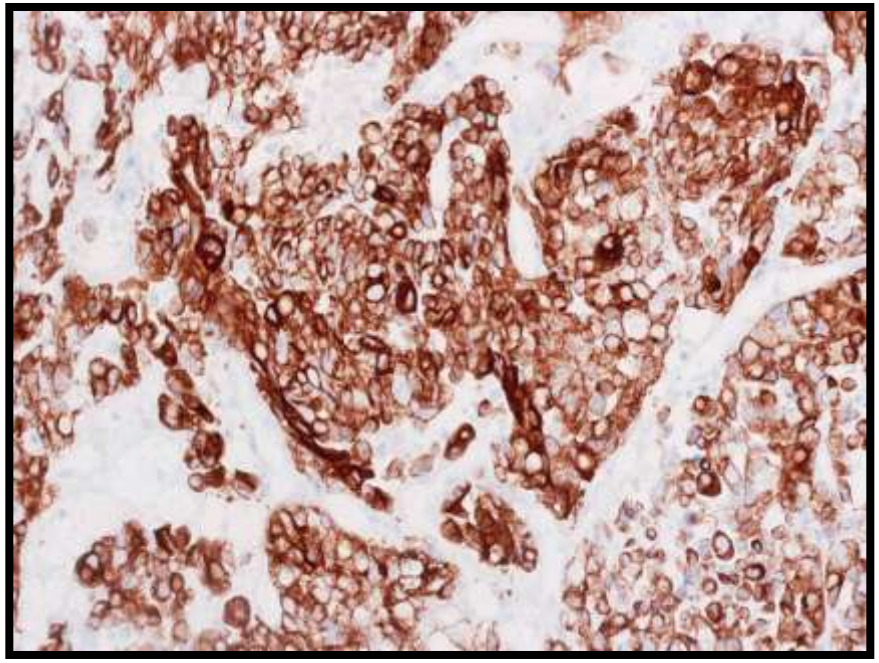


S-100

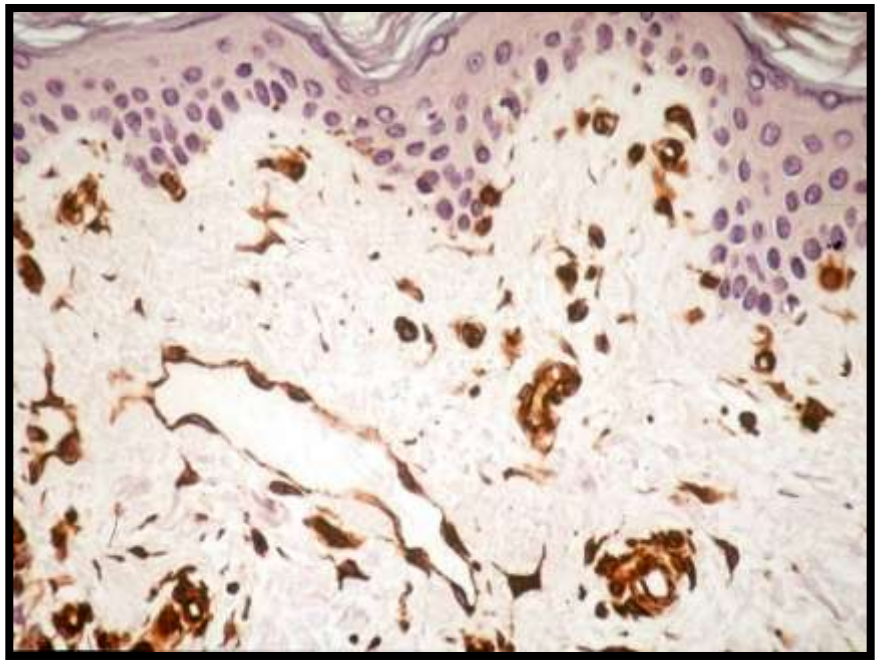
Aplicaciones

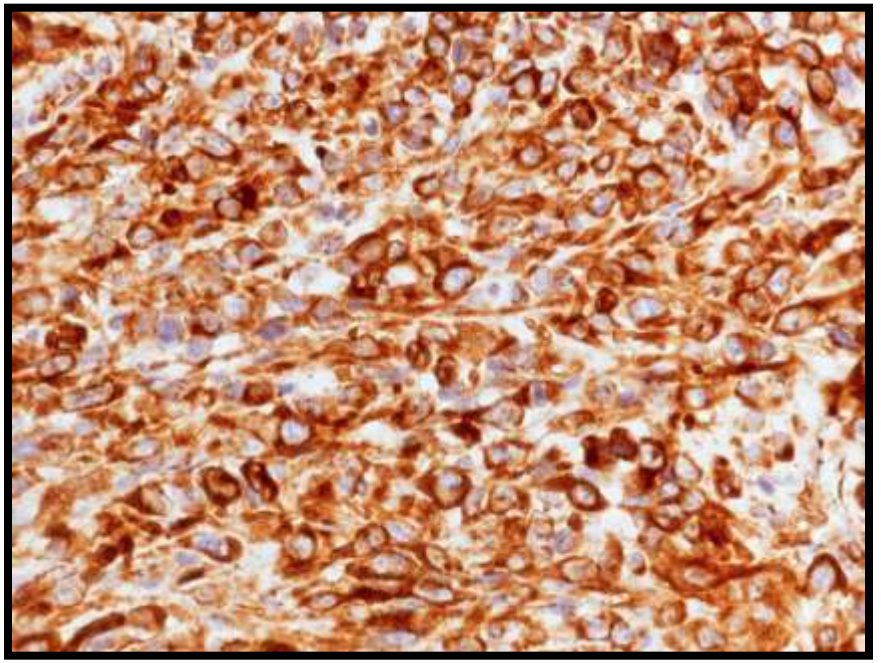
Citoqueratinas



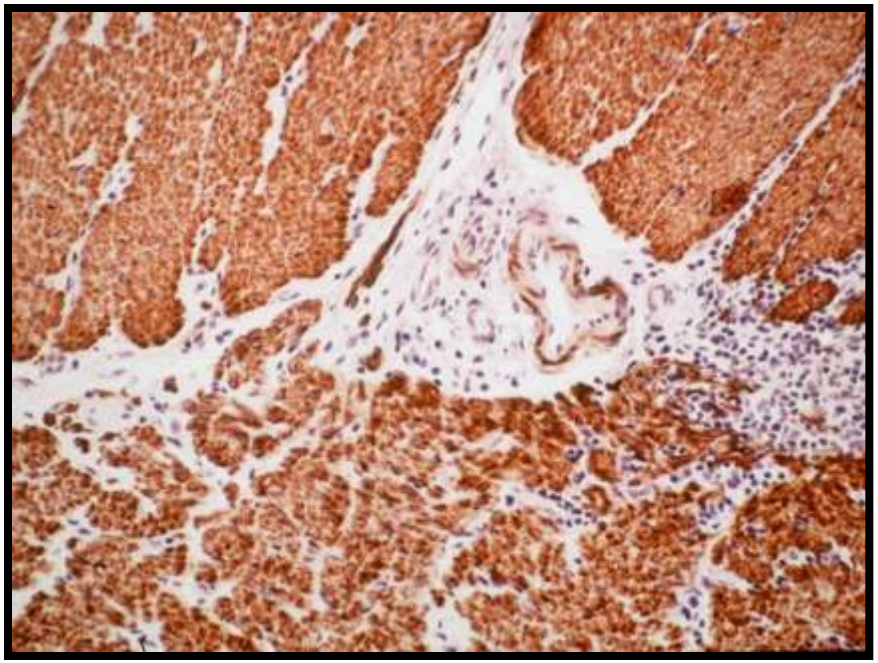


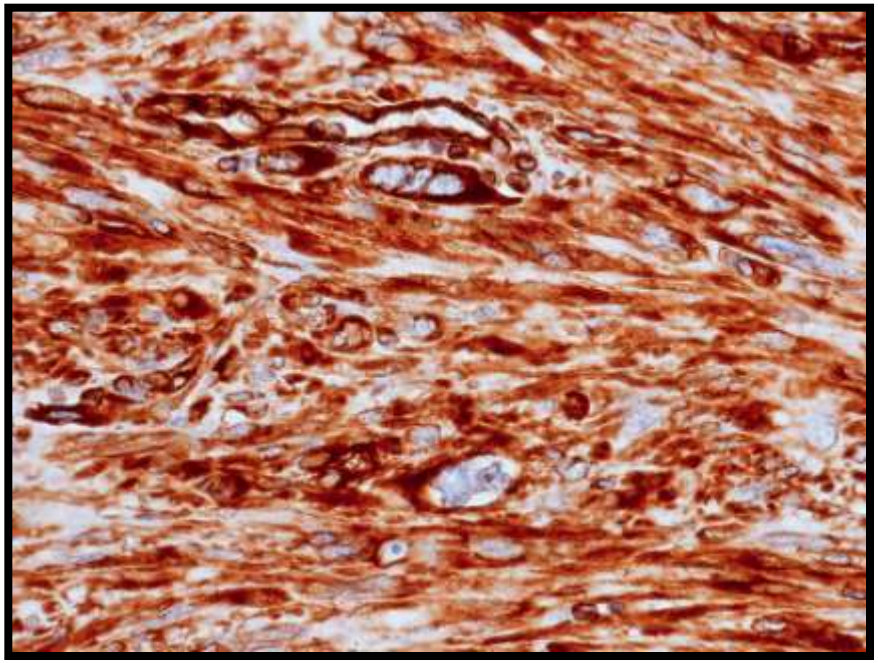
Vimentina



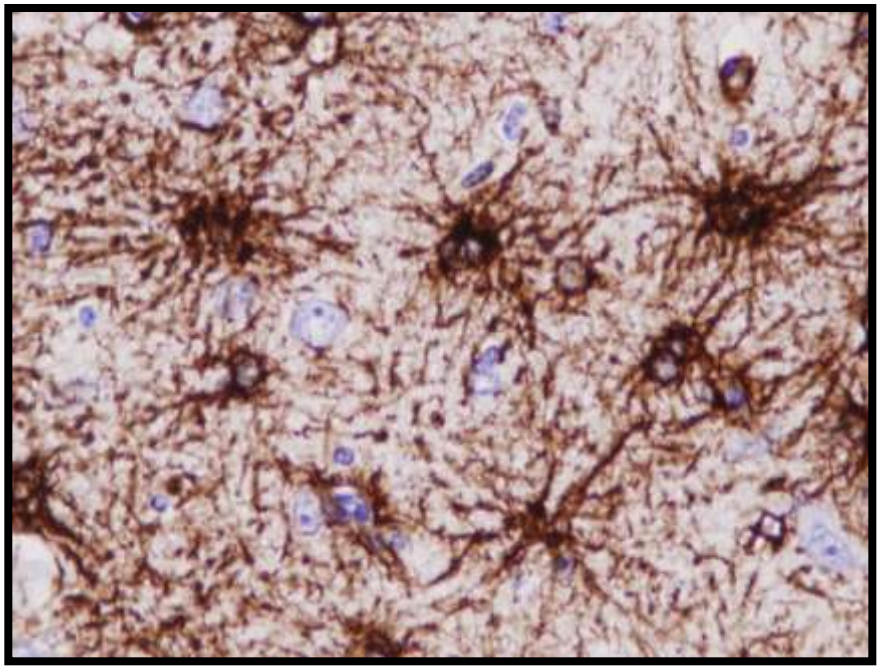


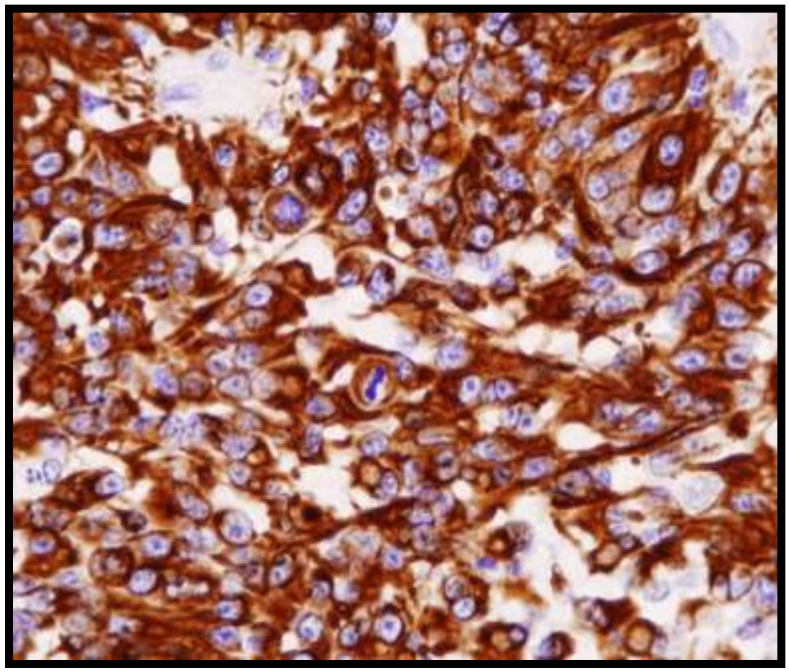
Desmina



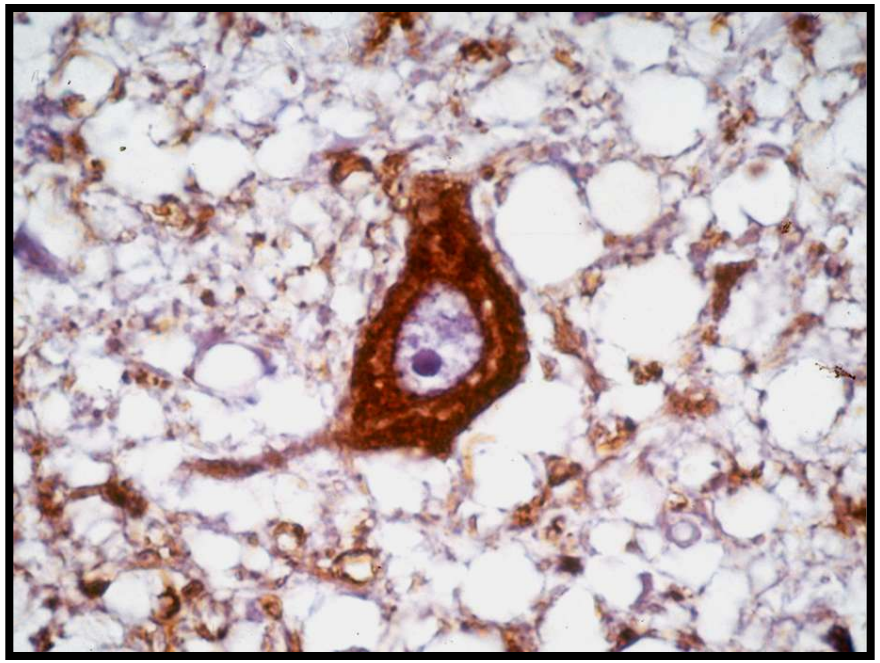


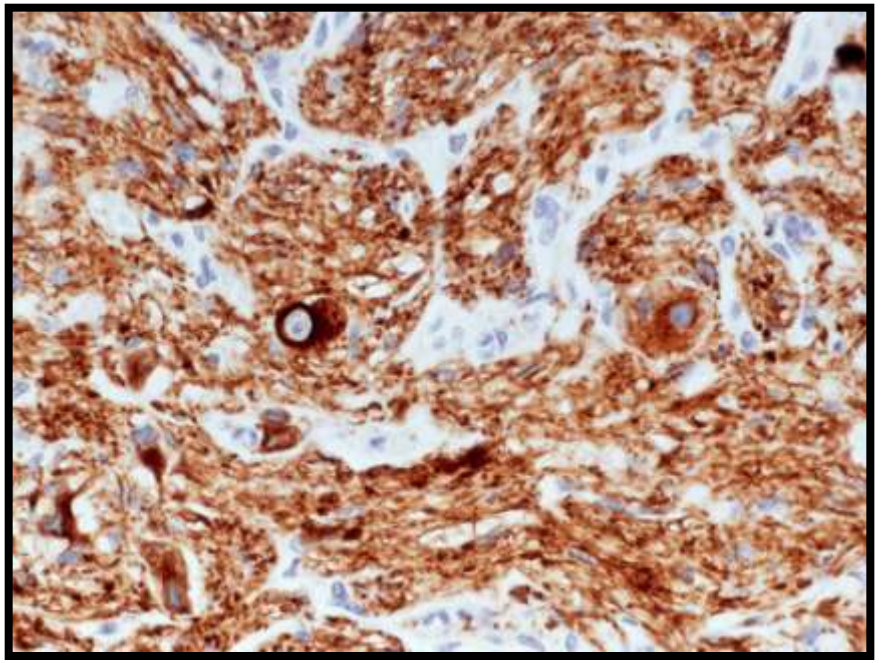
GFAP



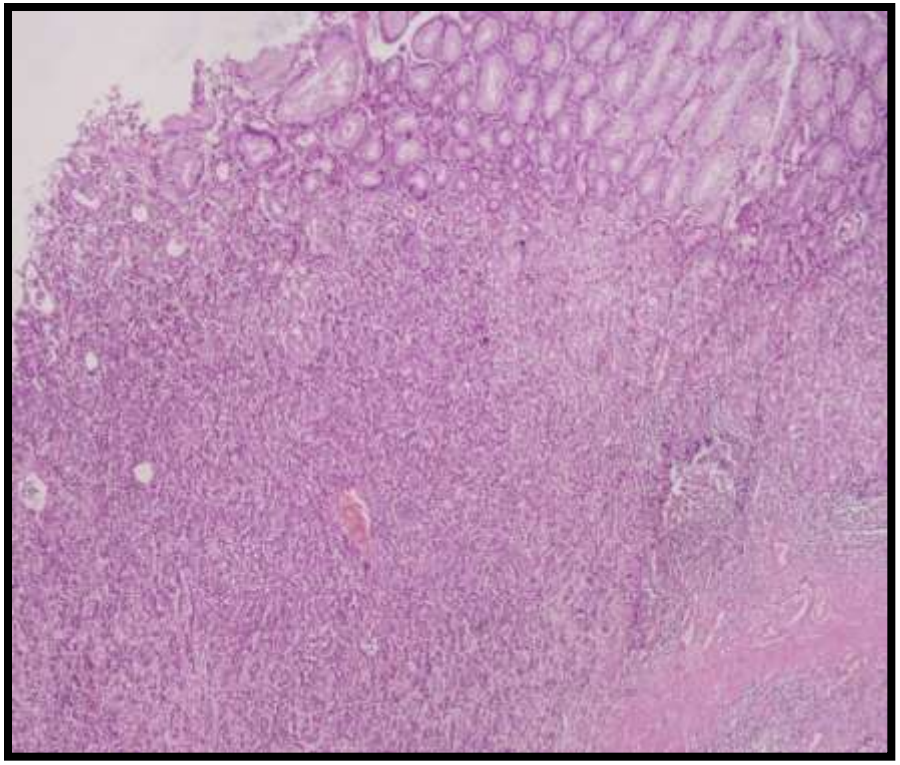


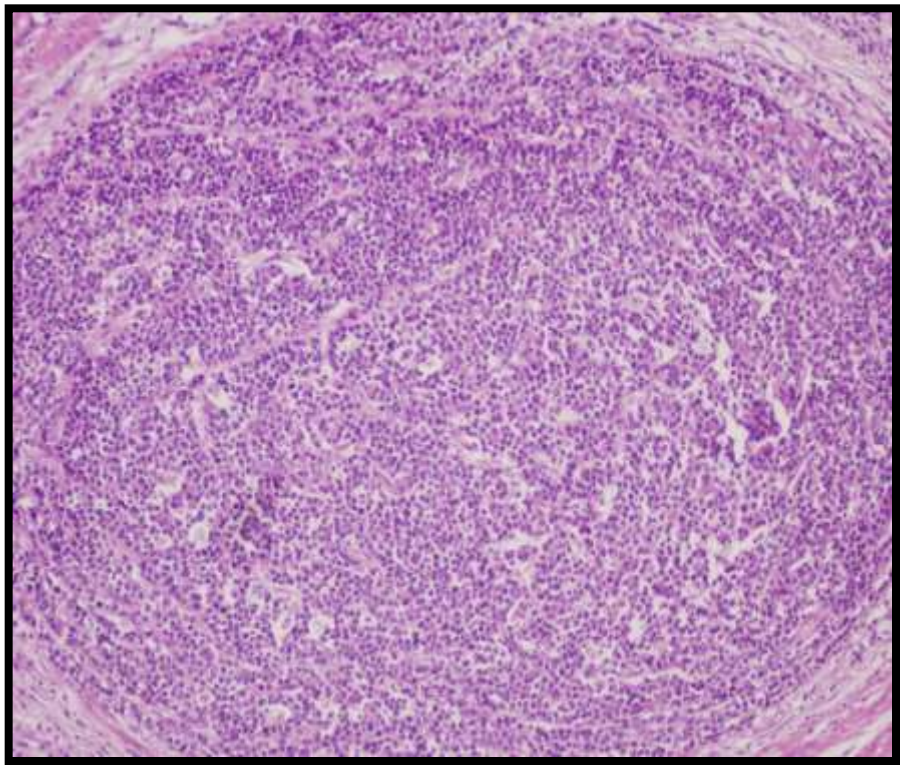
Neurofilamentos



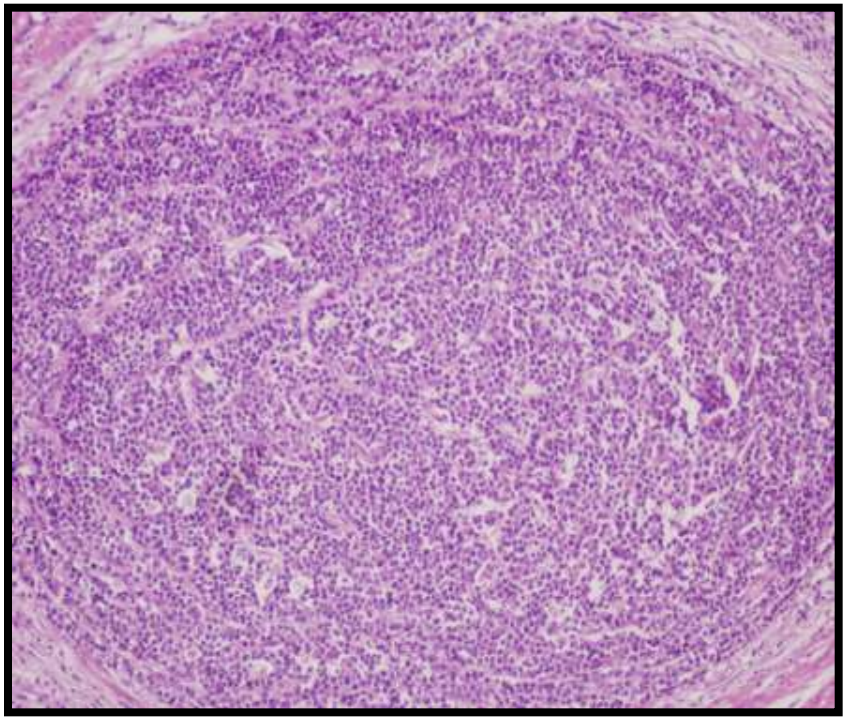


Diagnóstico diferencial

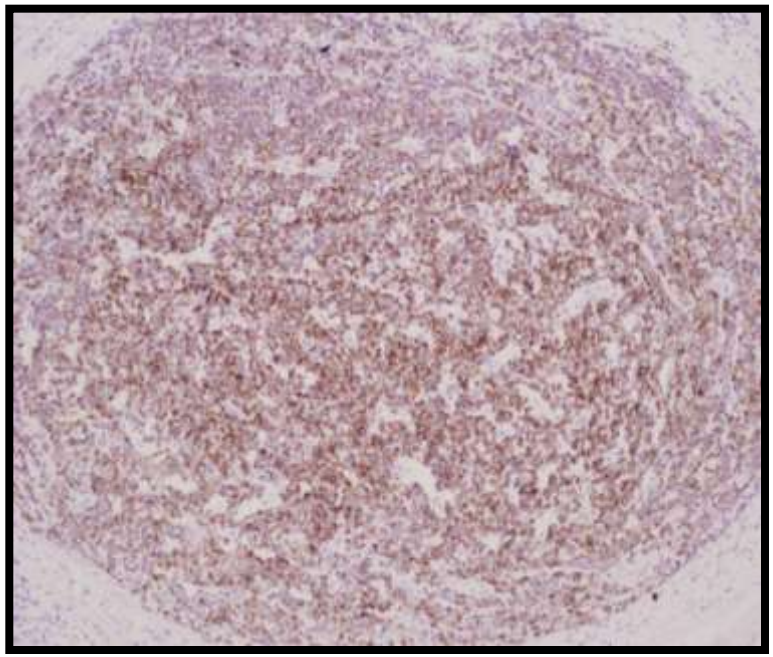


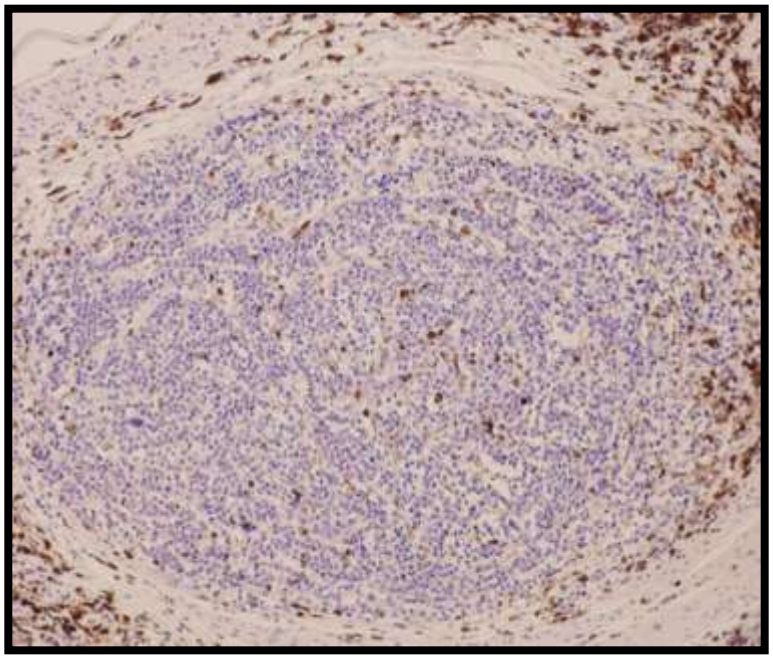


Tumoración gástrica



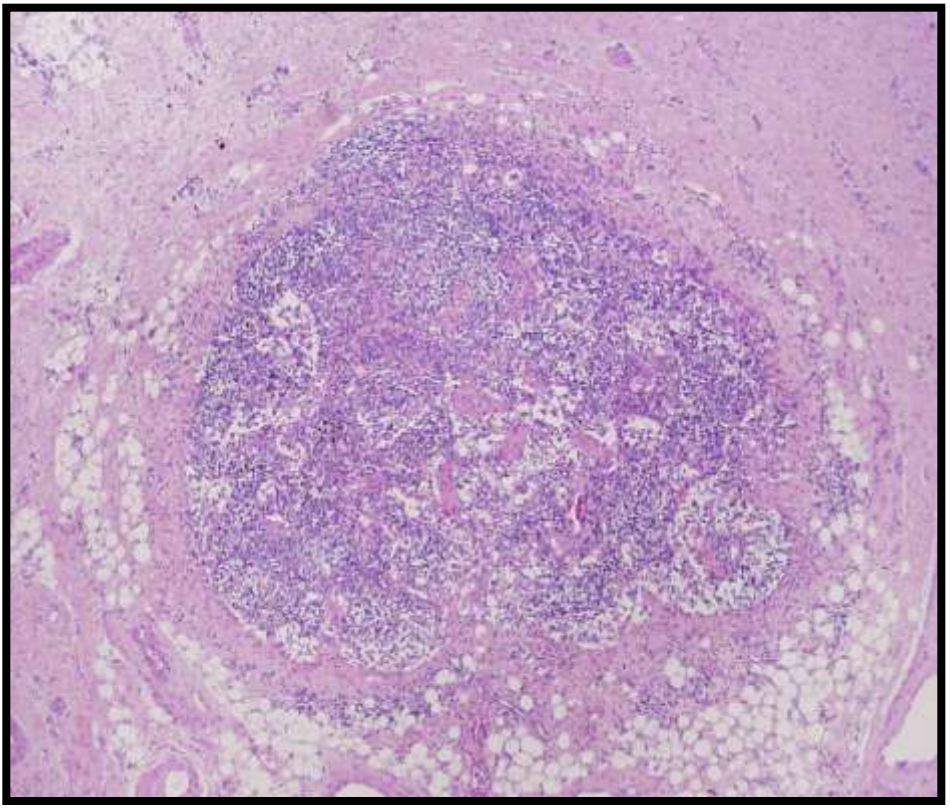
CK

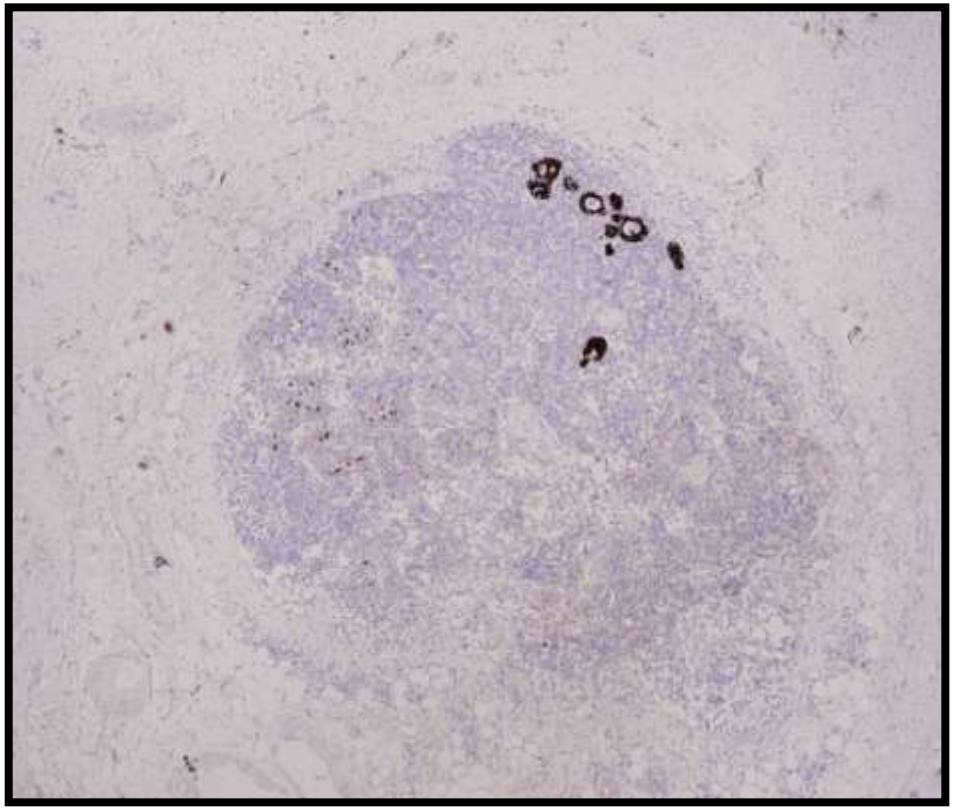




LCA

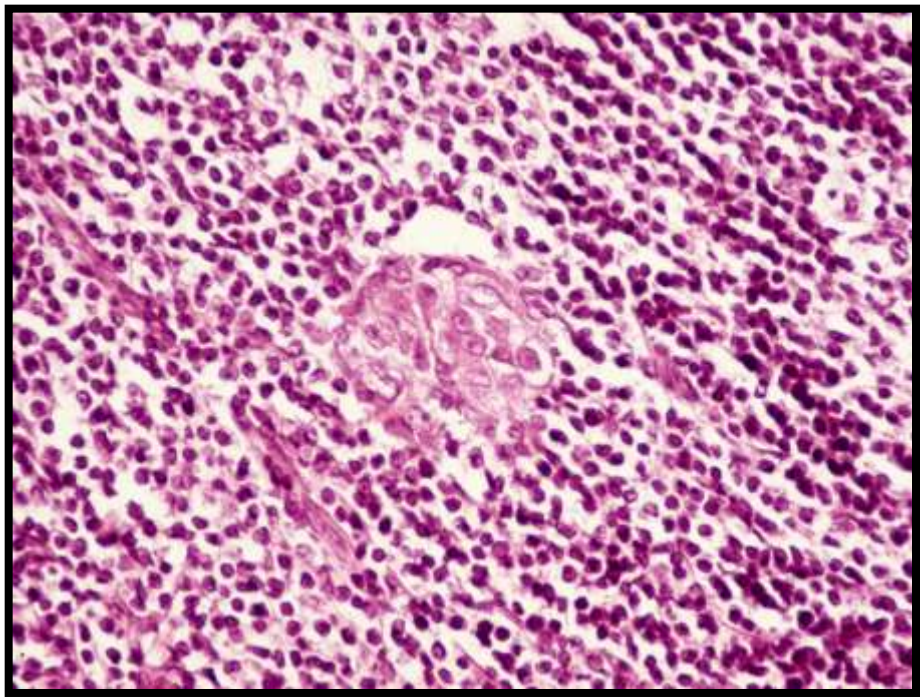
Micrometástasis

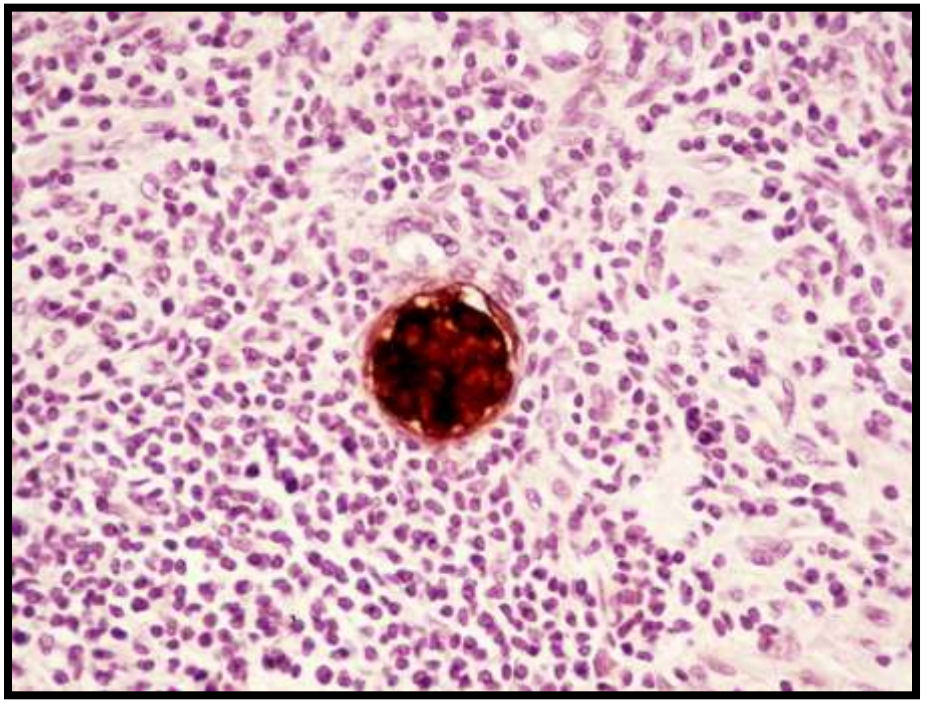




HE **CK**

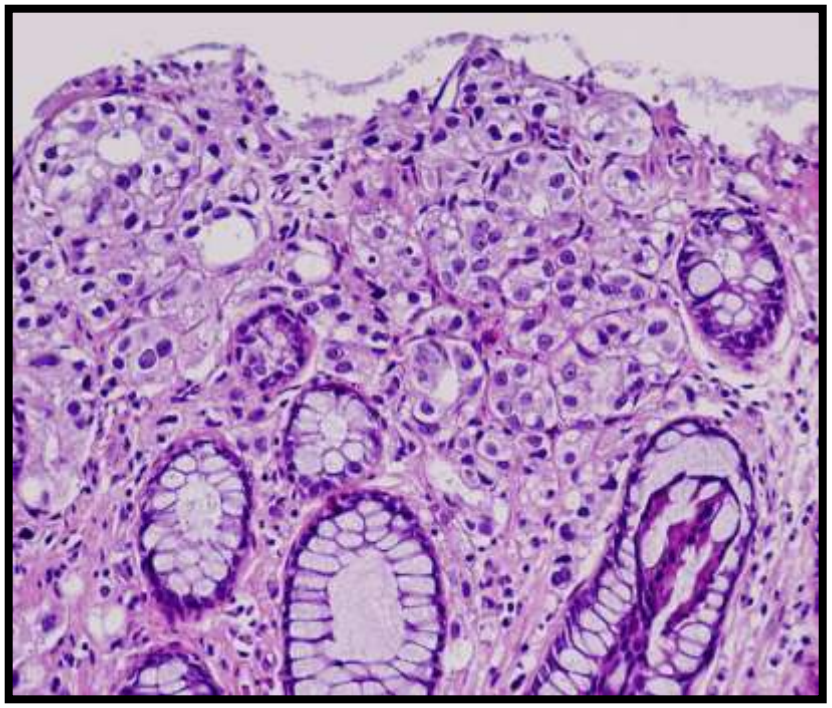
Origen metástasis





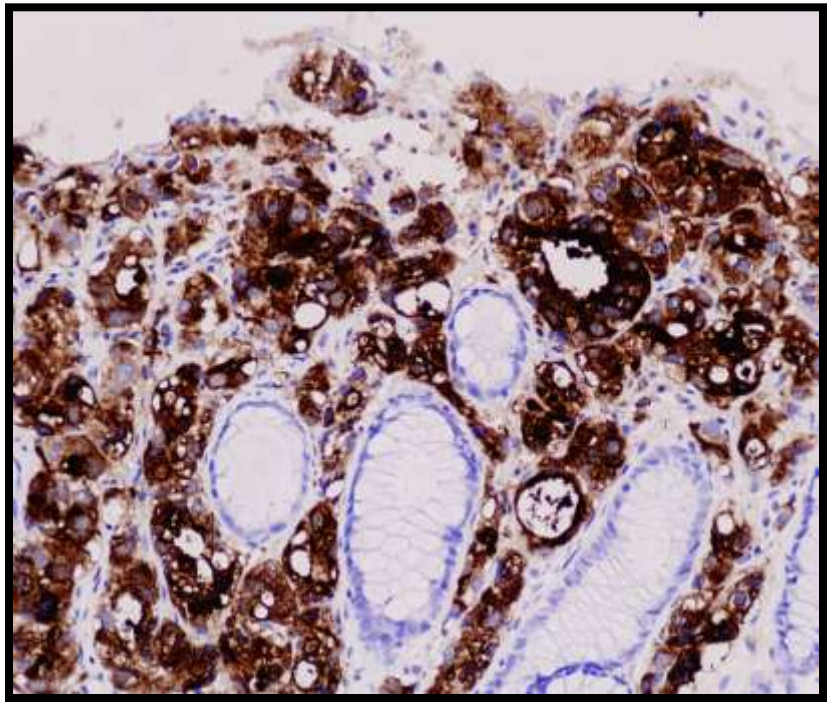
HE

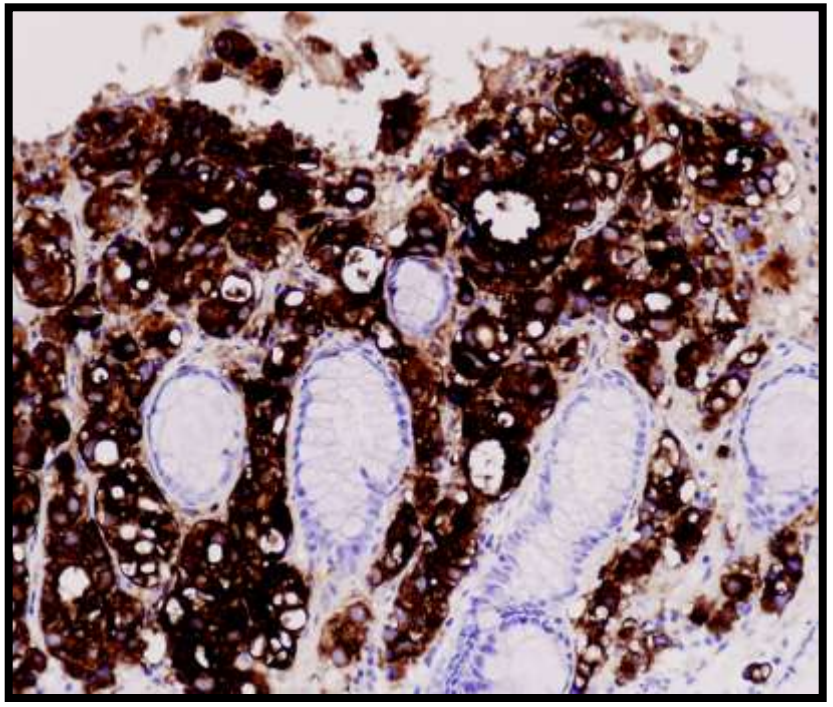
PSA

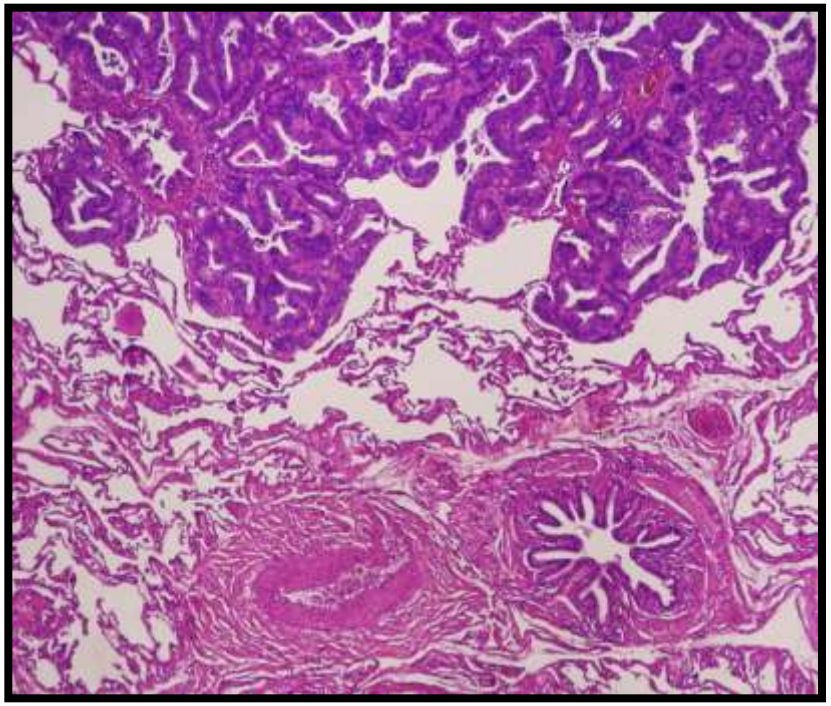


PSA

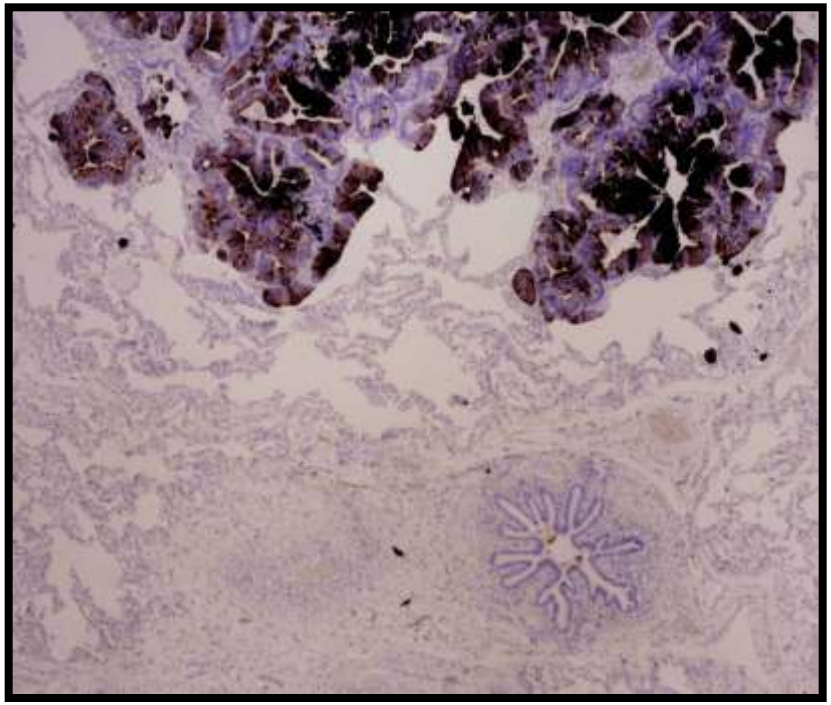
PAP



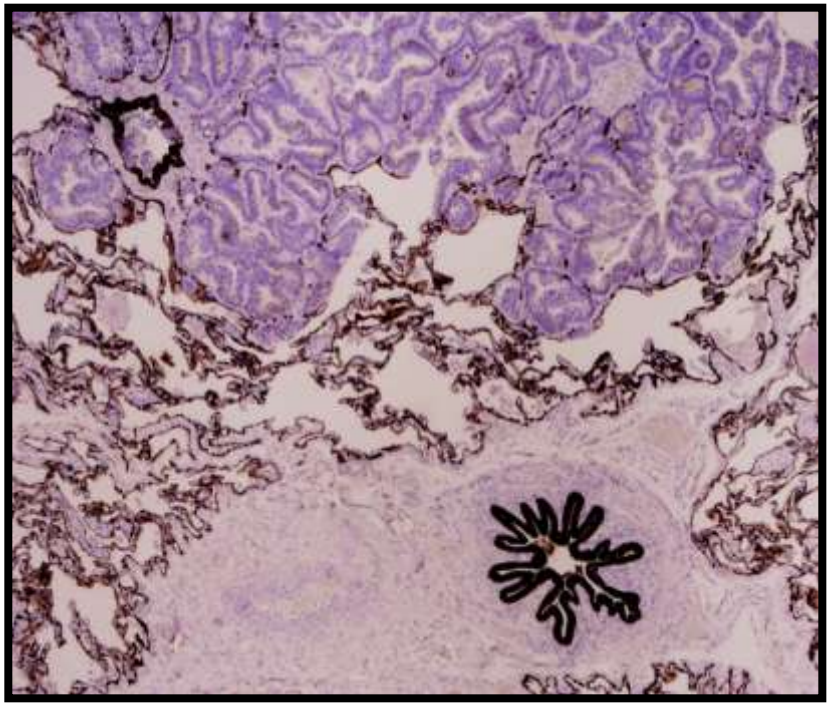




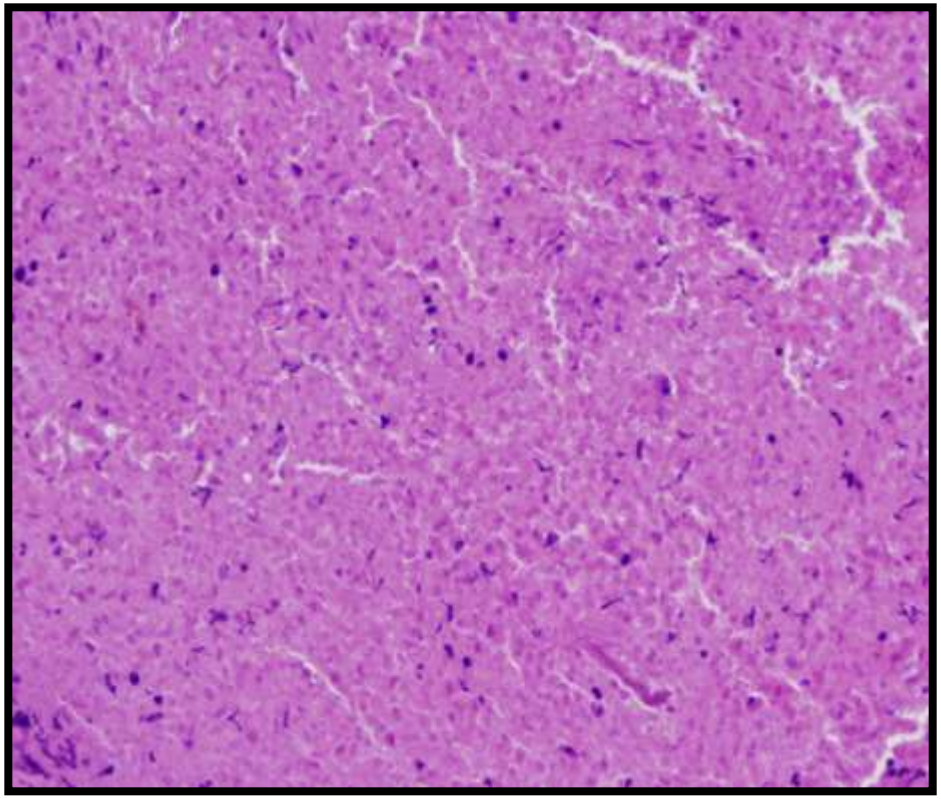
CK20+

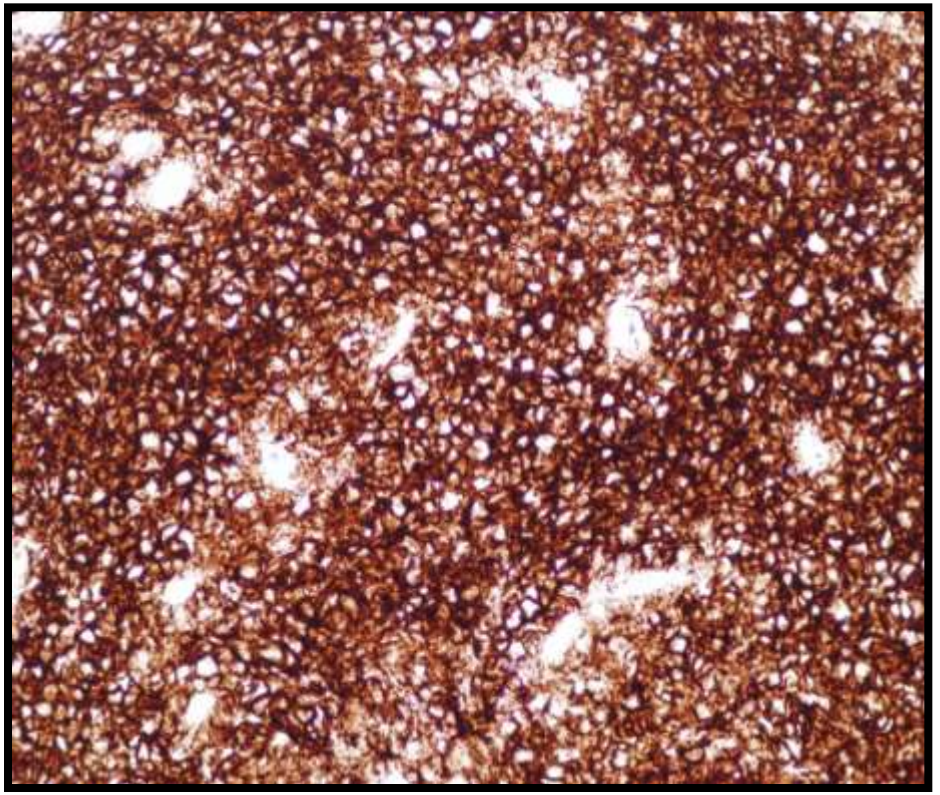


CK7-



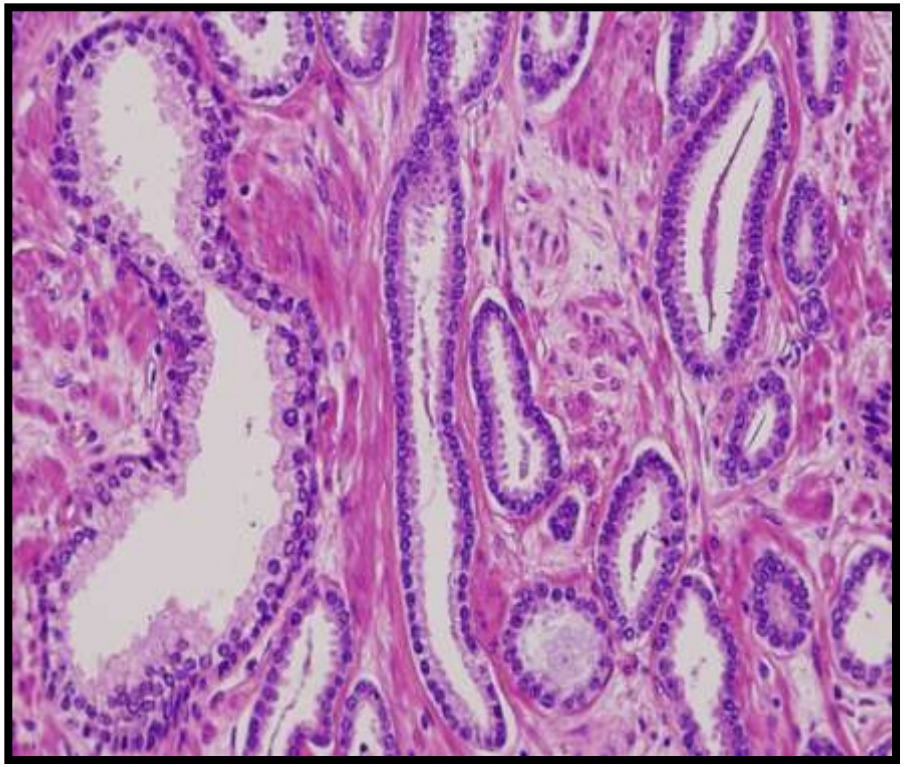
Tumores necrosados



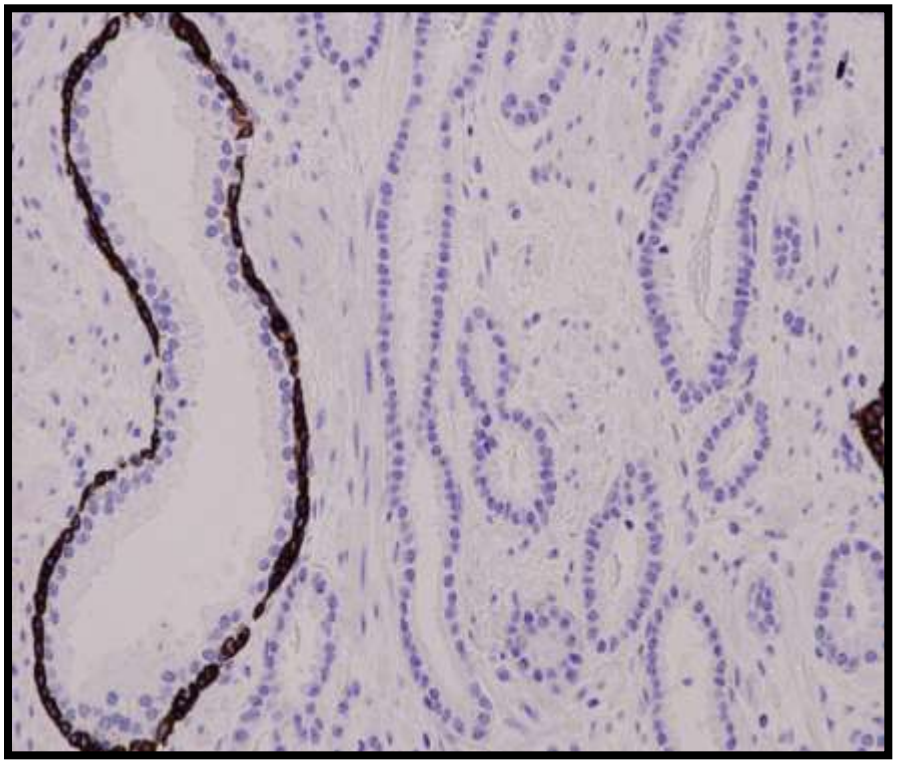


HE **CD20**

Benignidad / Malignidad

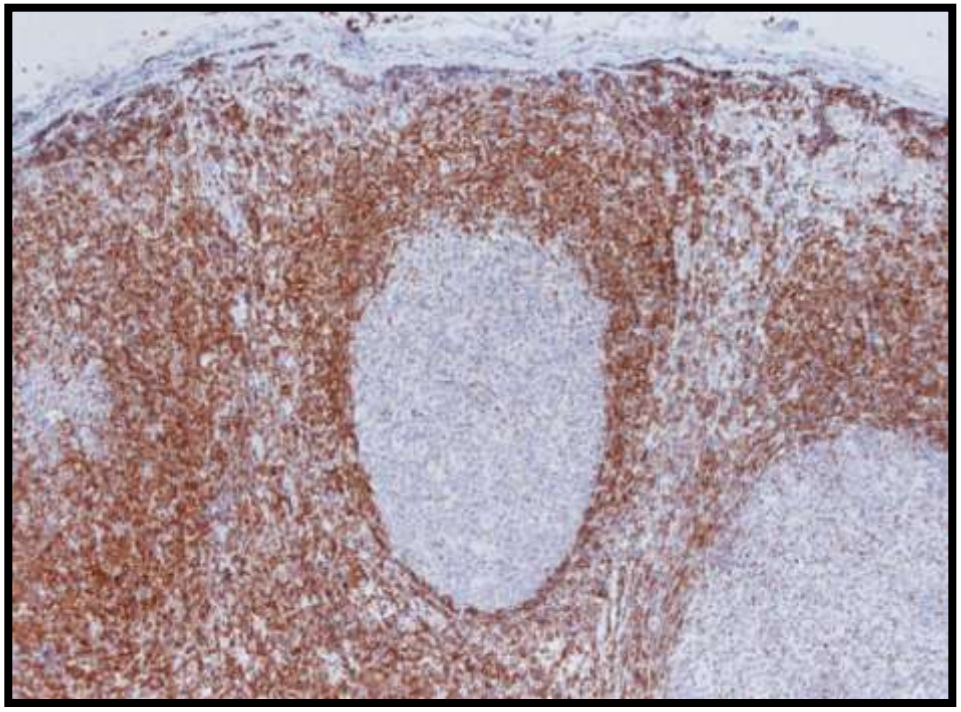


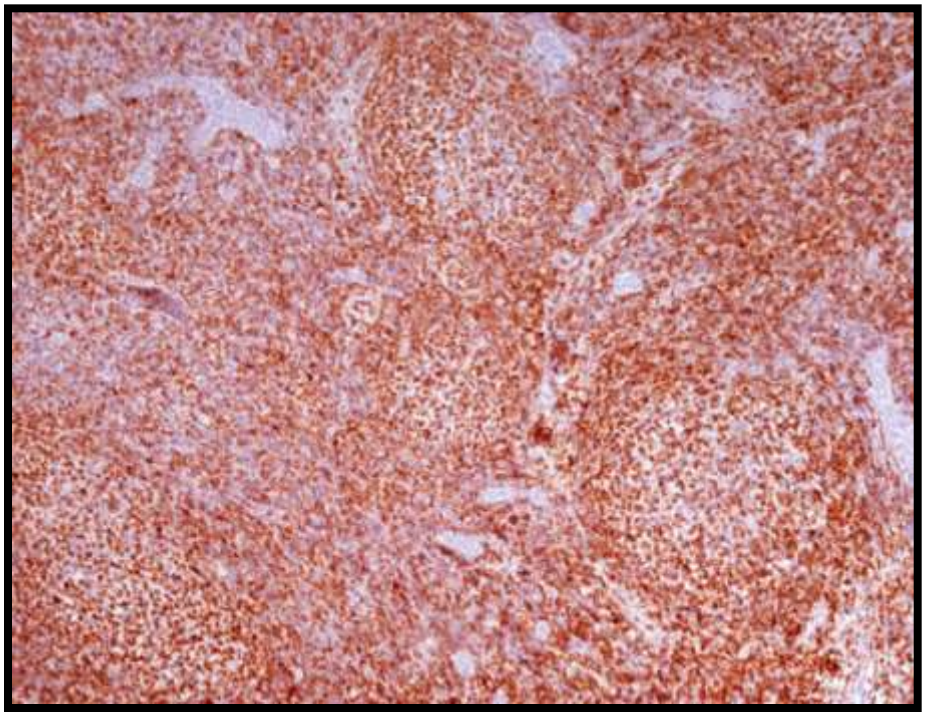
HE



CK 34βE12

Benignidad / Malignidad

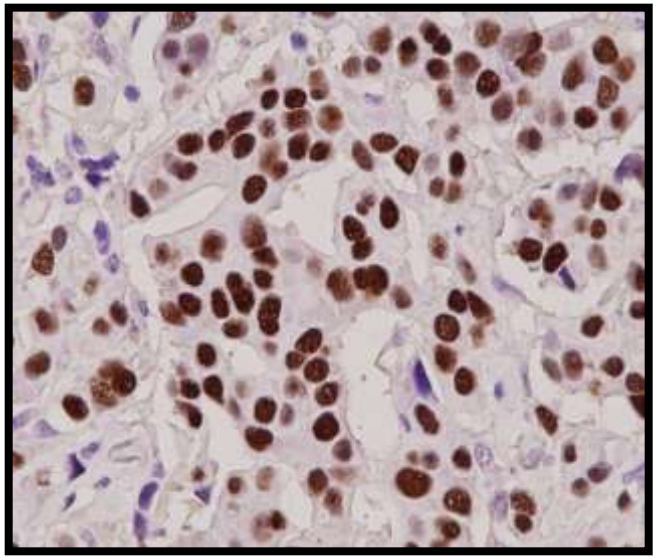


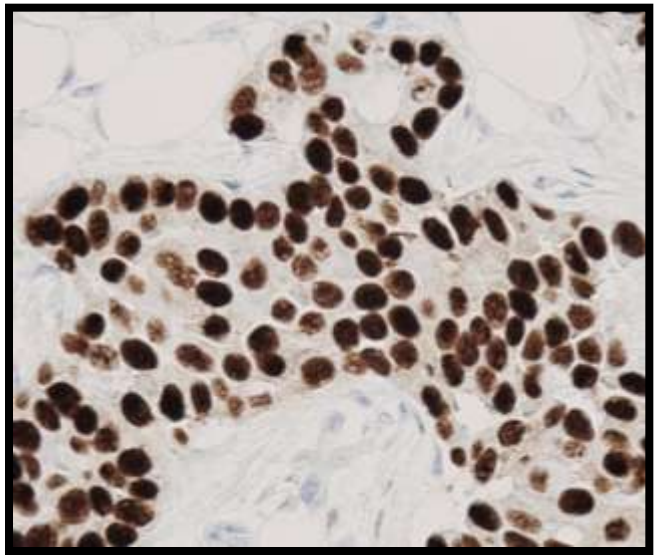


Bcl 2

Factores pronóstico / predictivos

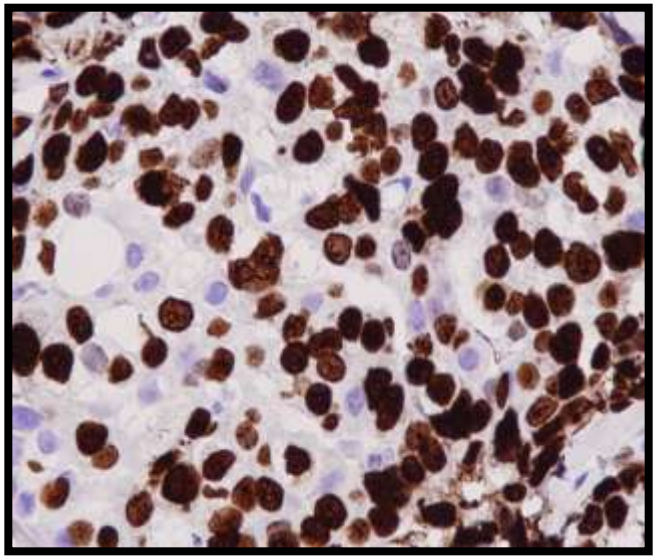
RE

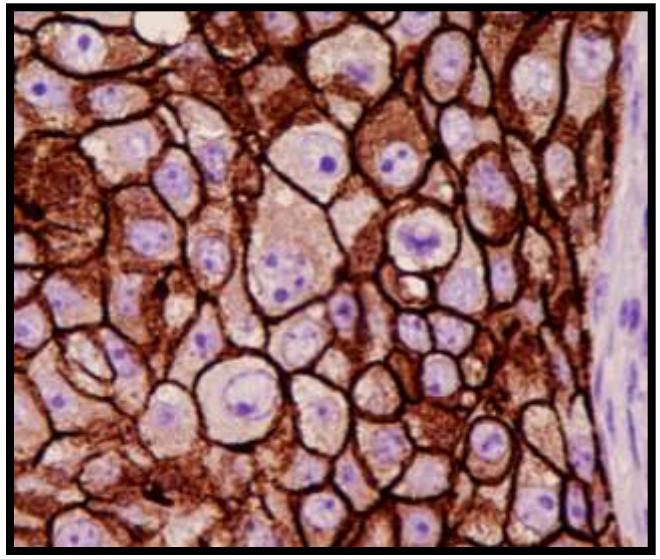




RP

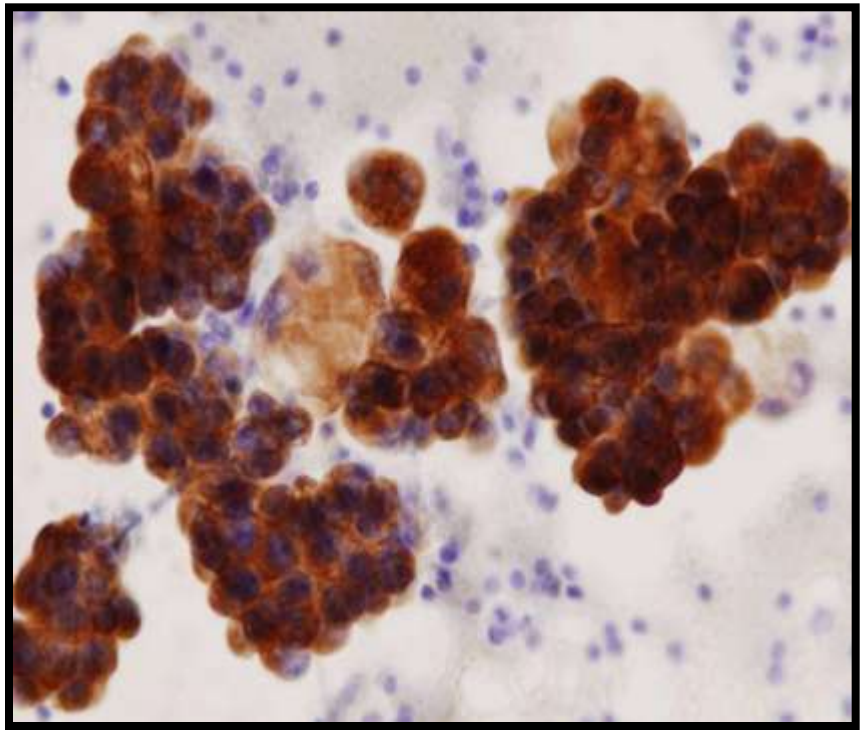
MIB-1



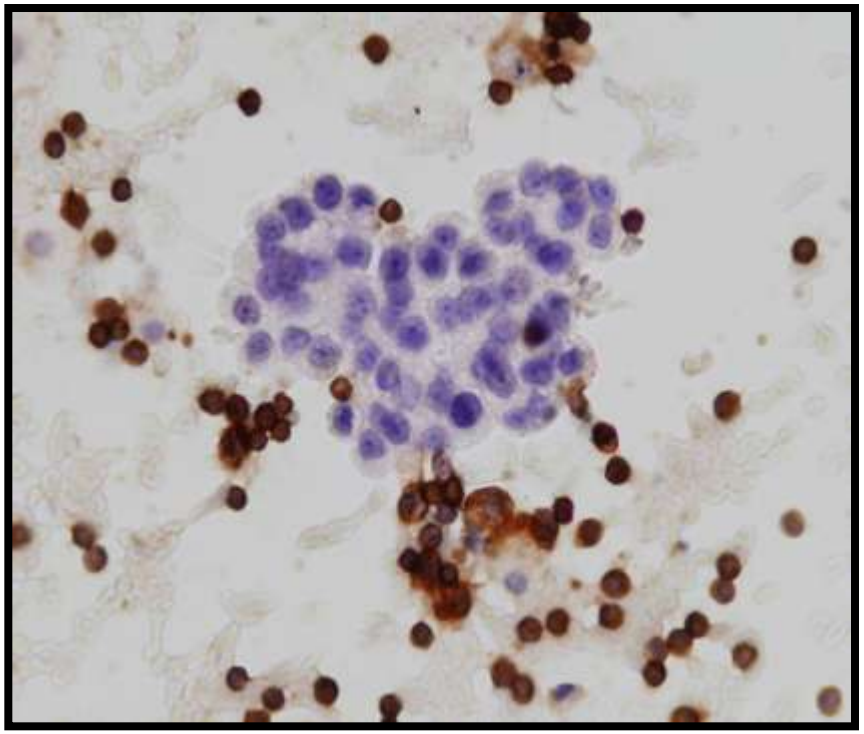


HER2

Citologías



CK

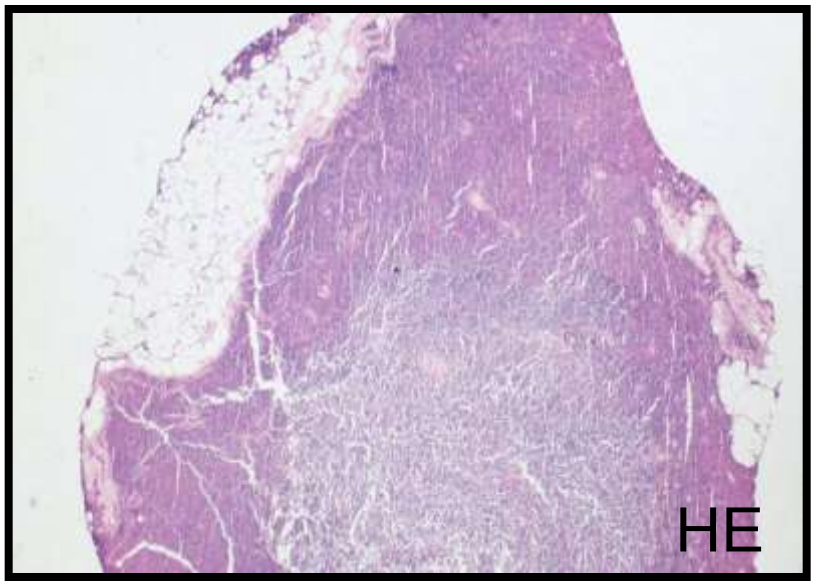


LCA

- Fijación / Enmascaramiento de epitopos
- Concentración del anticuerpo
- Almacenamiento secciones / anticuerpo
- Calidad del anticuerpo
- Deterioro reactivos
- **Controles positivos**

IR débil o ausente

1. Fijación



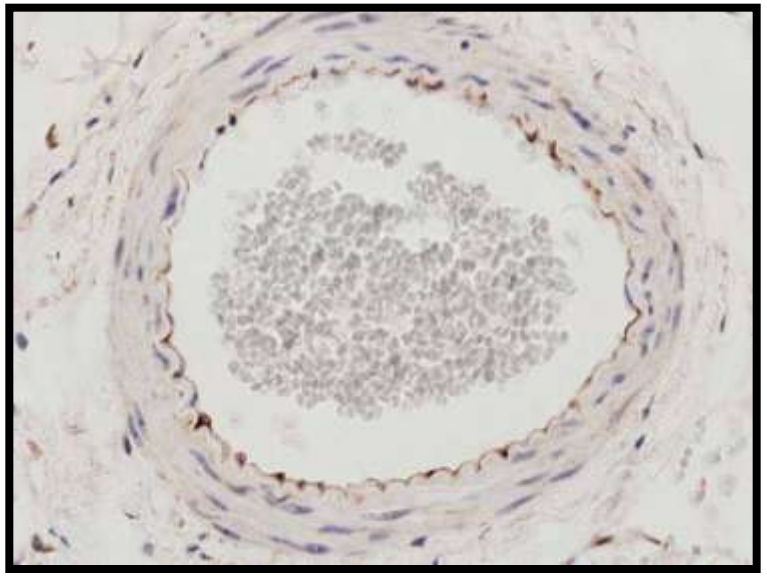
Fijación óptima: 24 h
Fijación adecuada: 6-72 h

Penetración formol (1 mm/h) ≠ Fijación
Fijación: Reacción del reloj (Burnett MG, 1982)
Subfijación peor que sobrefijación

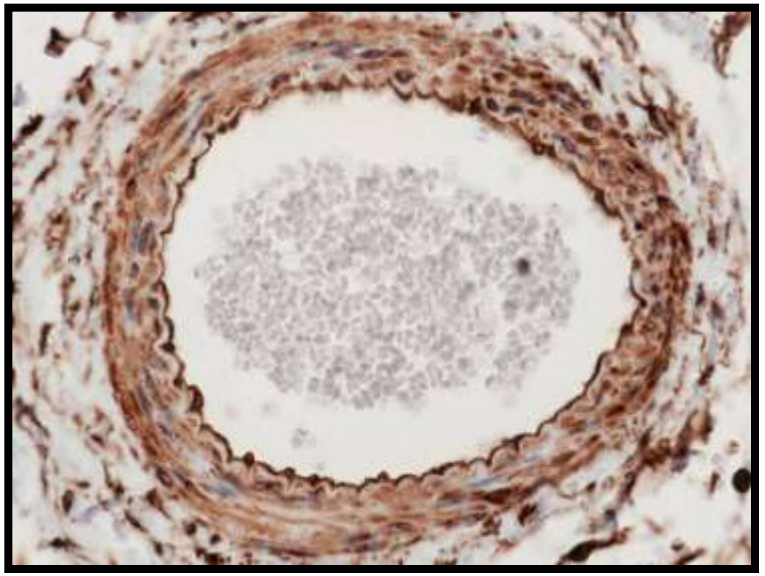
Control fijación

VIMENTINA

¡sin recuperación antigénica!



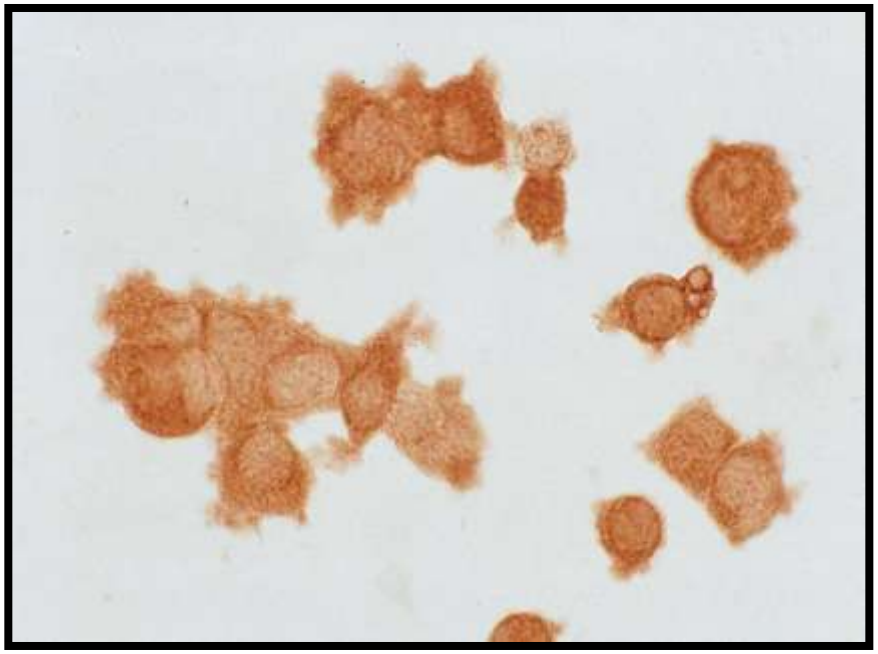
VIM / sin RA



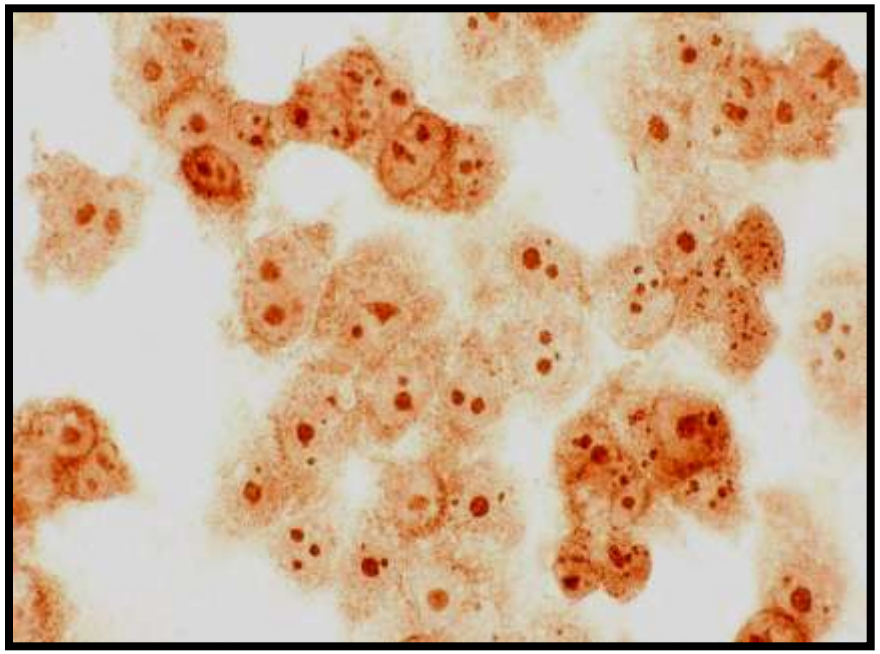
VIM / μo

Battifora H, *Am J Clin Pathol* 96:669-71,1991

Enmascaramiento de epitopos



Sin RA

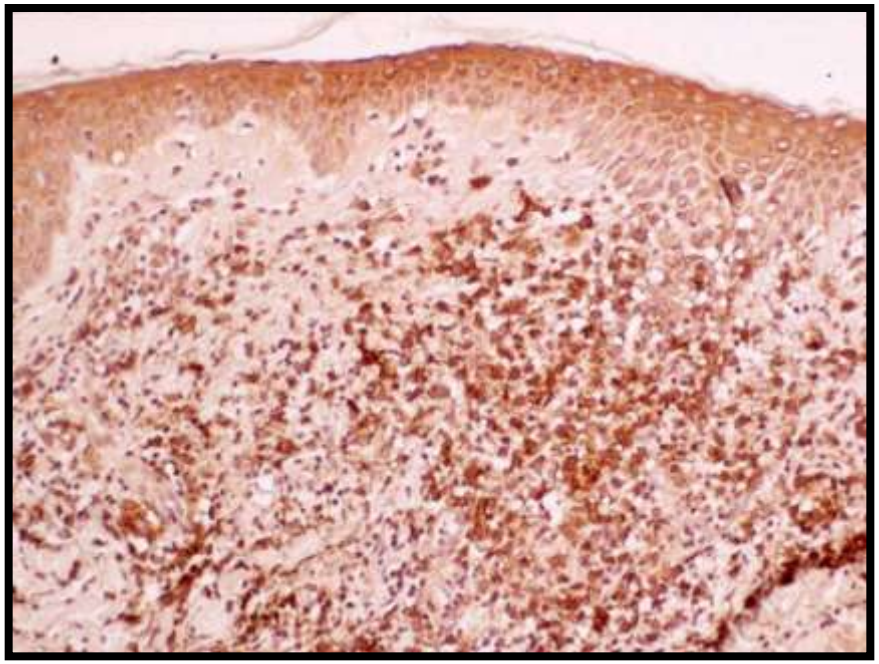


Microondas

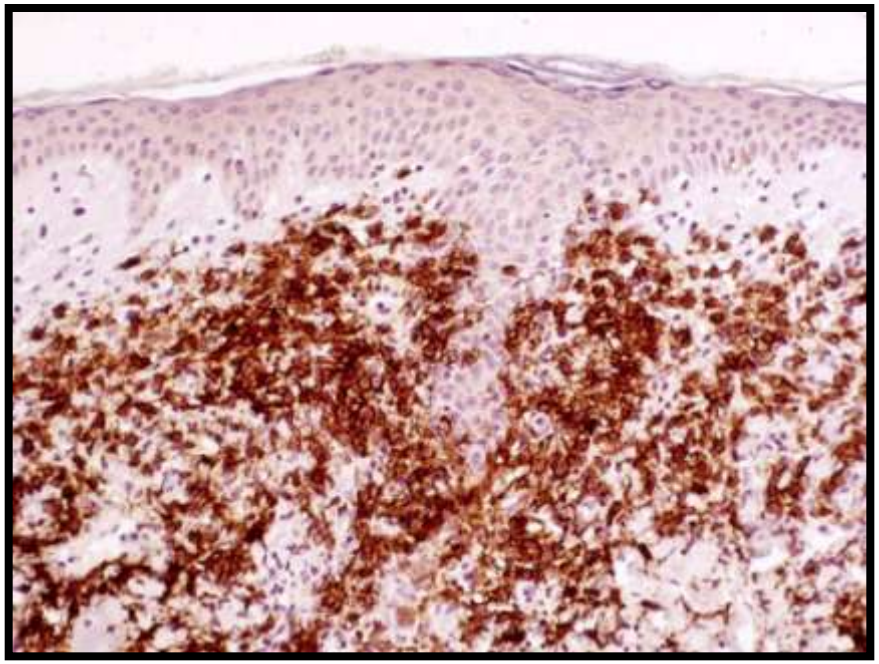
Pit1

Recuperación antigénica

↑ Señal ↓ Fondo



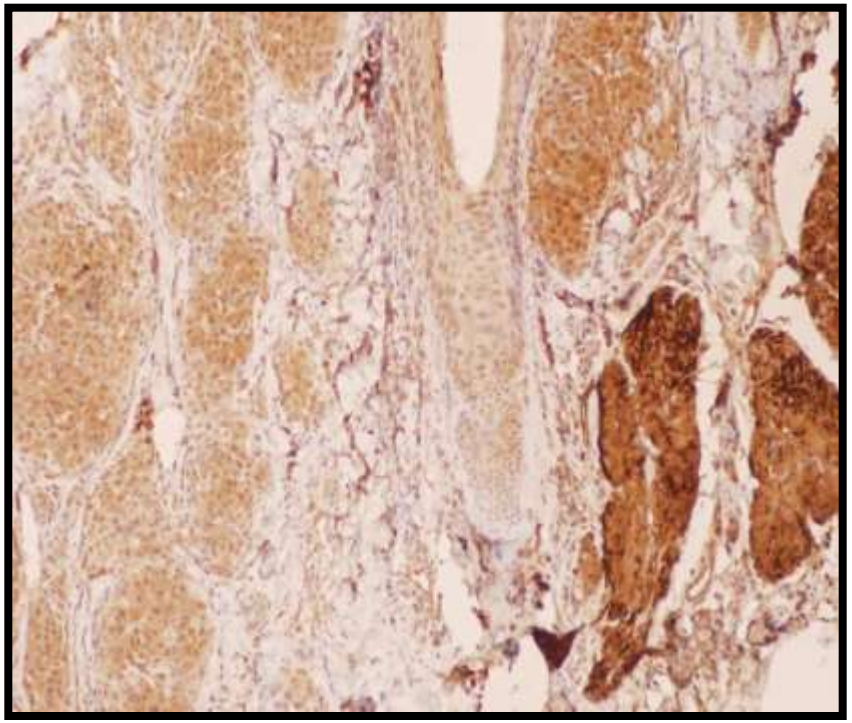
Sin RA

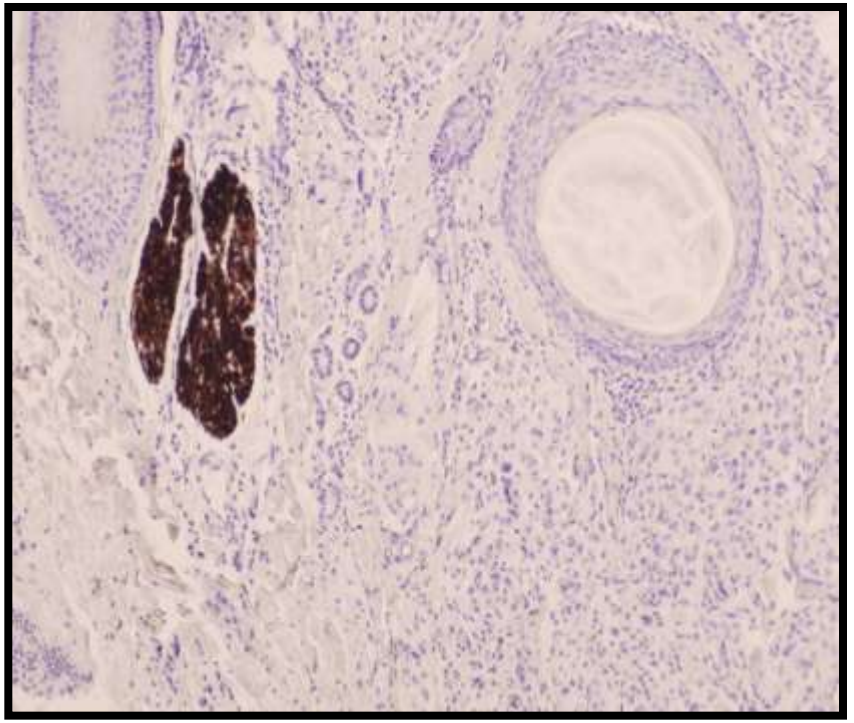


Microondas

CD20

Recuperación antigénica





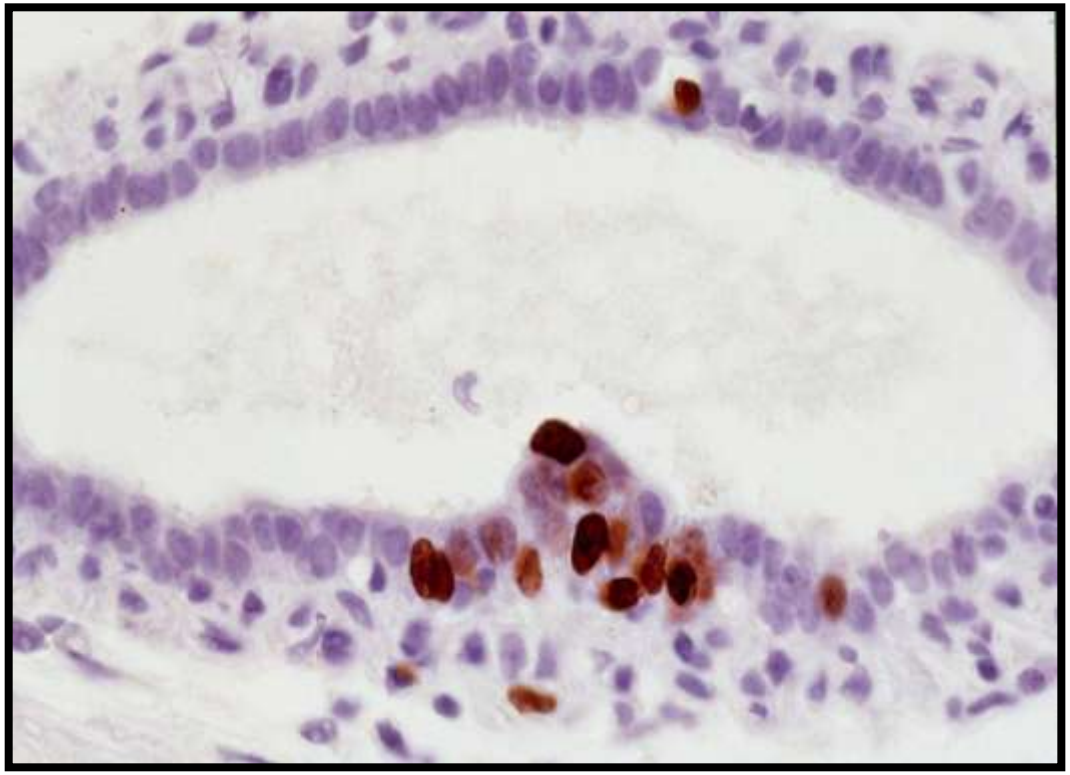
1/10 (μO)

1/50 (sin RA)

DESMINA

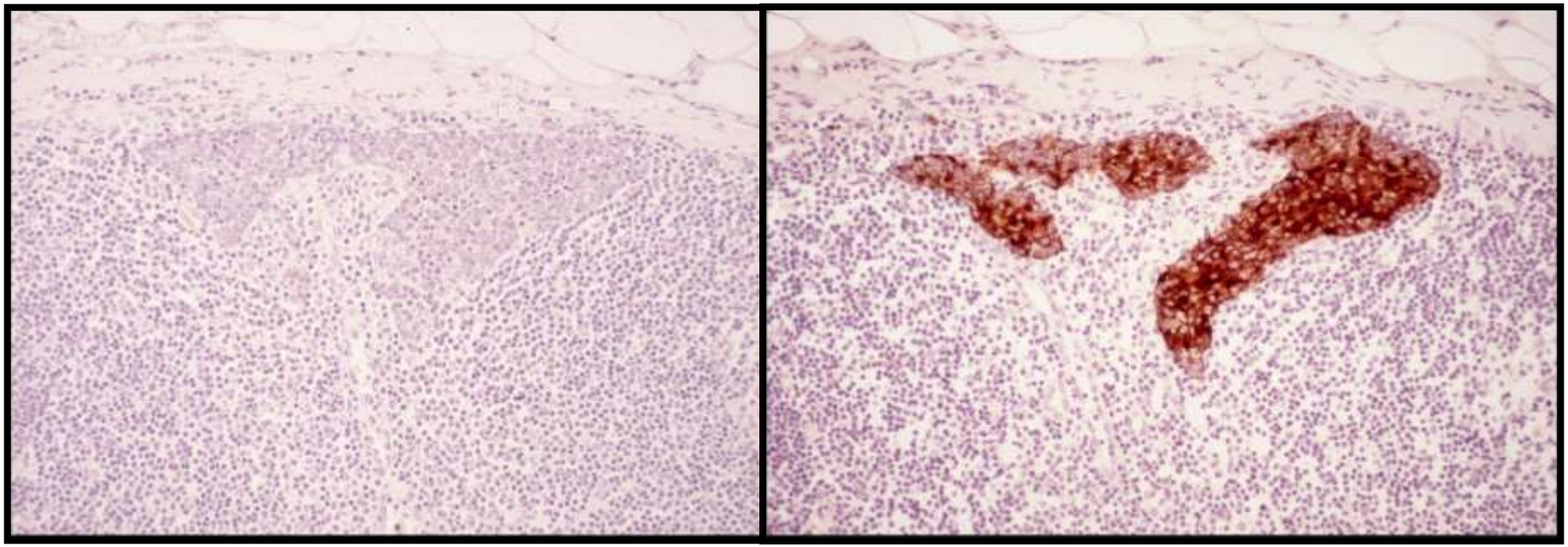
Control recuperación Ag

MIB1



Chan JKC, *Adv Anat Pathol* 5:314-25,1998

2. Concentración

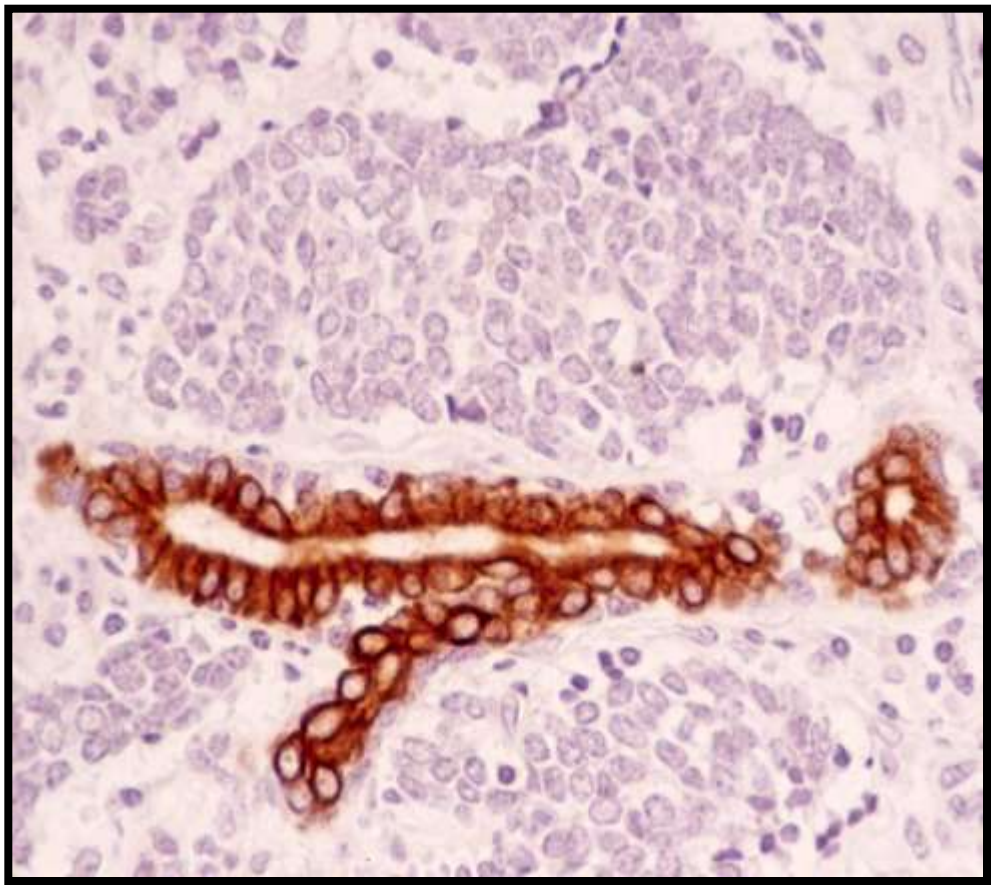


1/500

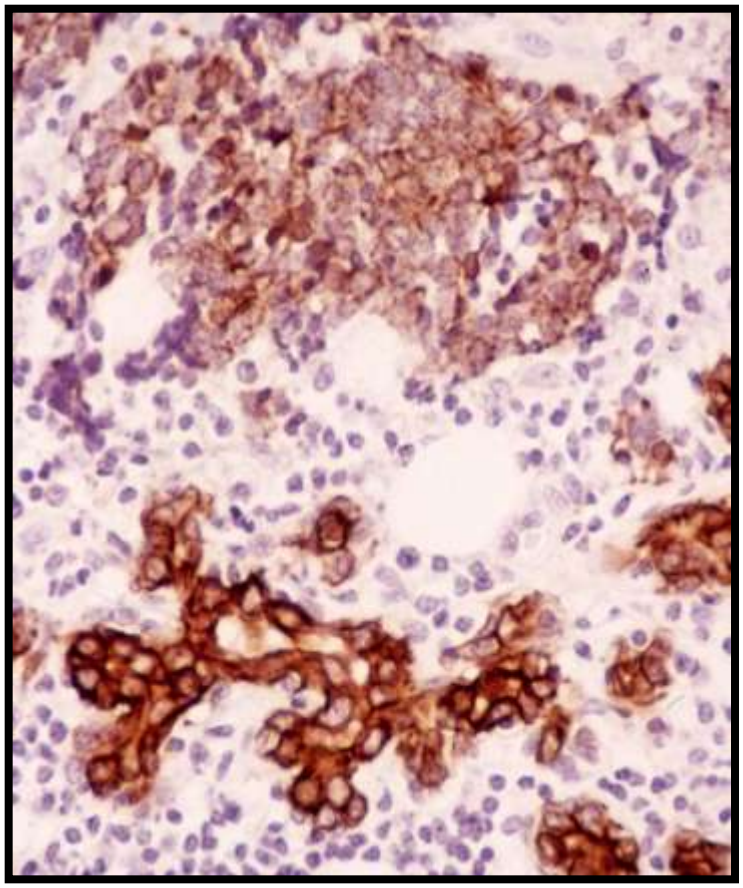
1/100

CK AE1-AE3

Concentración insuficiente



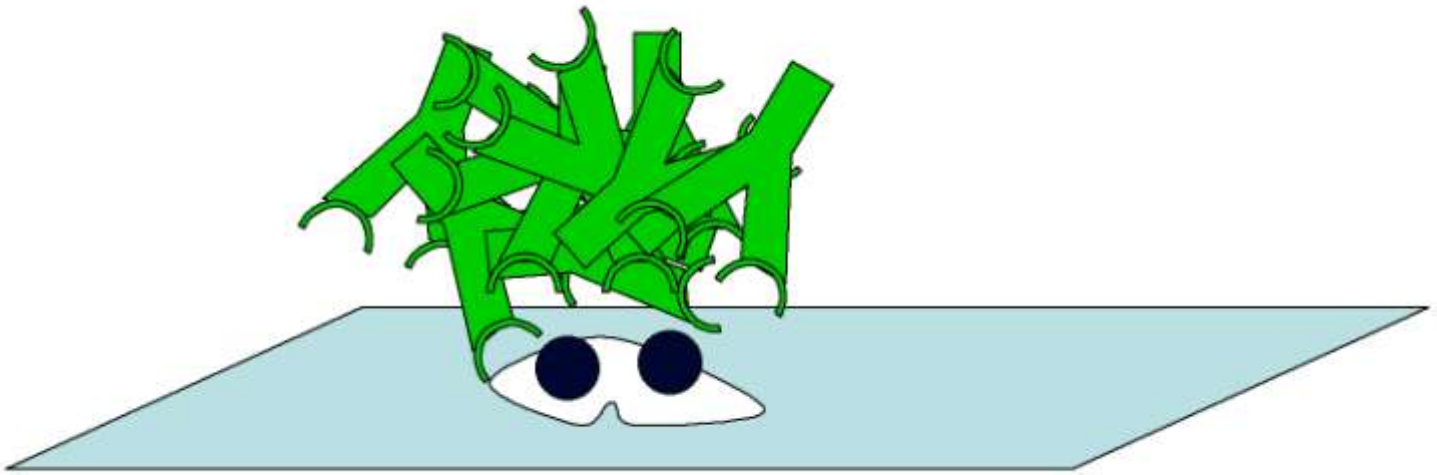
1/500



1/100

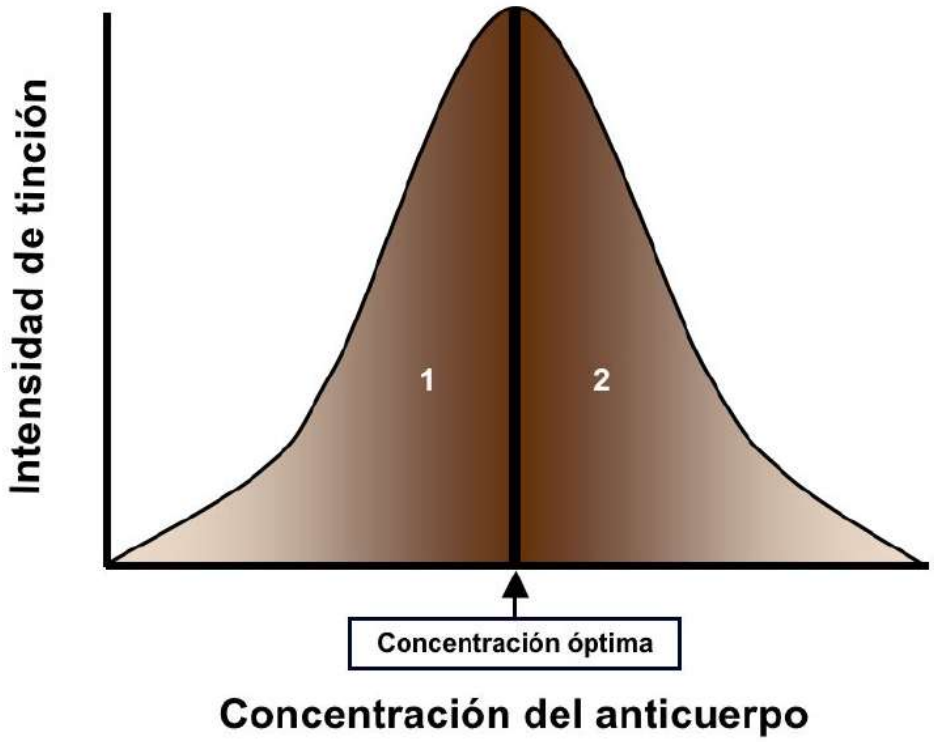
CK AE1-AE3

Concentración excesiva



Efecto prozona / Impedimento espacial

Concentración óptima



Titulación

1/100

1/50 – 1/100 – 1/200 – 1/500 – 1/1000

Máxima señal y mínimo fondo

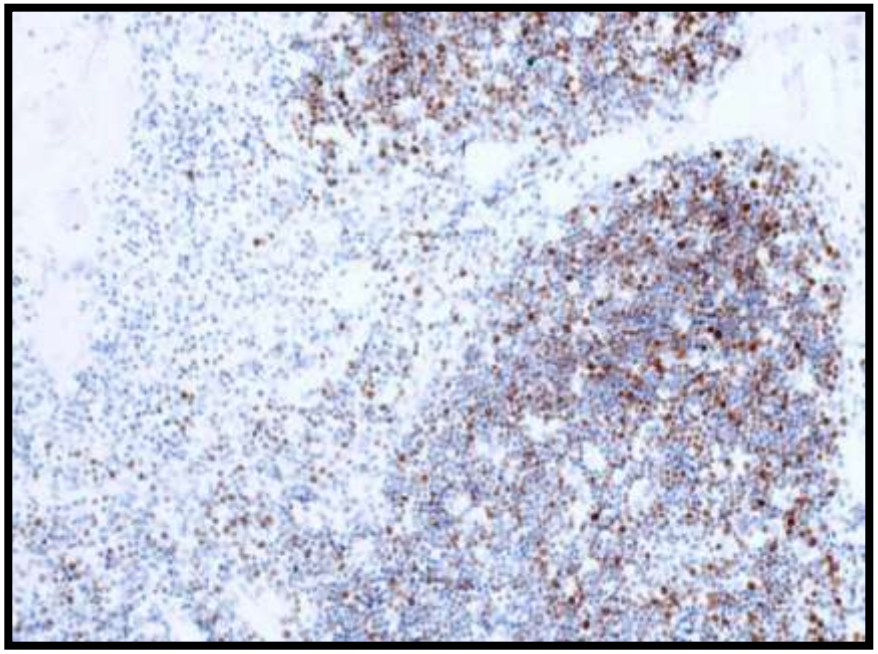
Incremento inmunotinción

Sistemas estandarizados (Ac prediluidos)

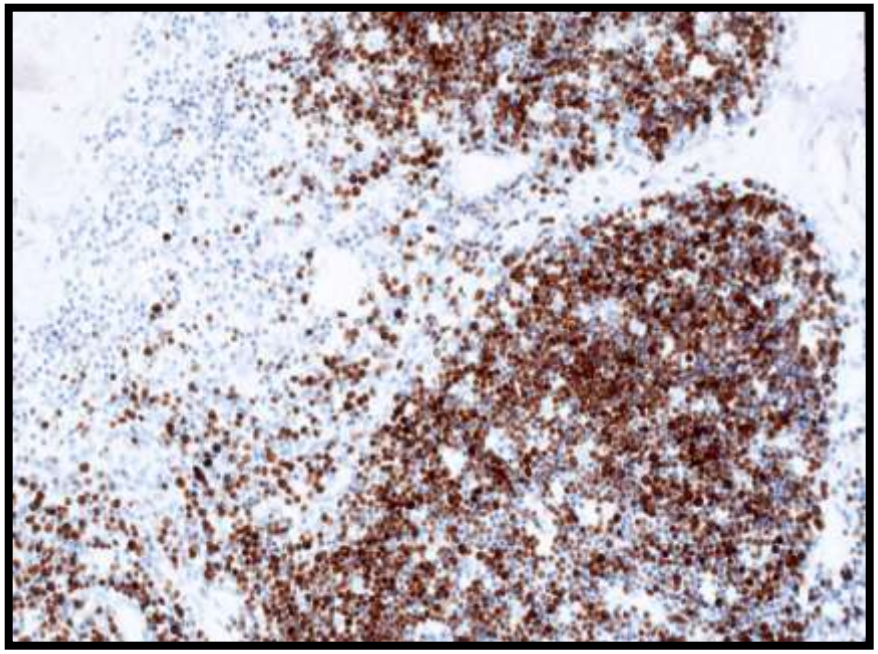
- **Tiempo de incubación del Ac primario: 20’ → 30’**
- **Recuperación antigénica: No → pH 6,1 → pH 9,0**
- **Sistema de amplificación de la señal *(Linker)***

3. Almacenamiento

Secciones



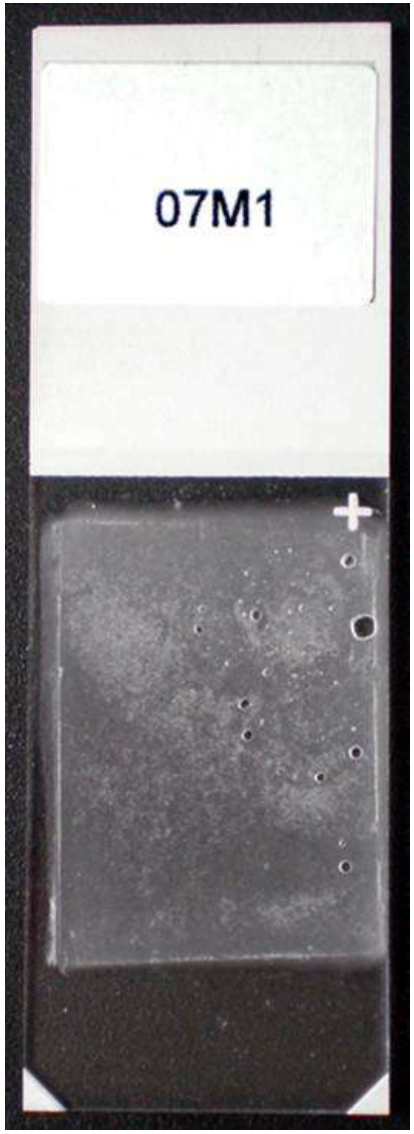
1 mes (TA)



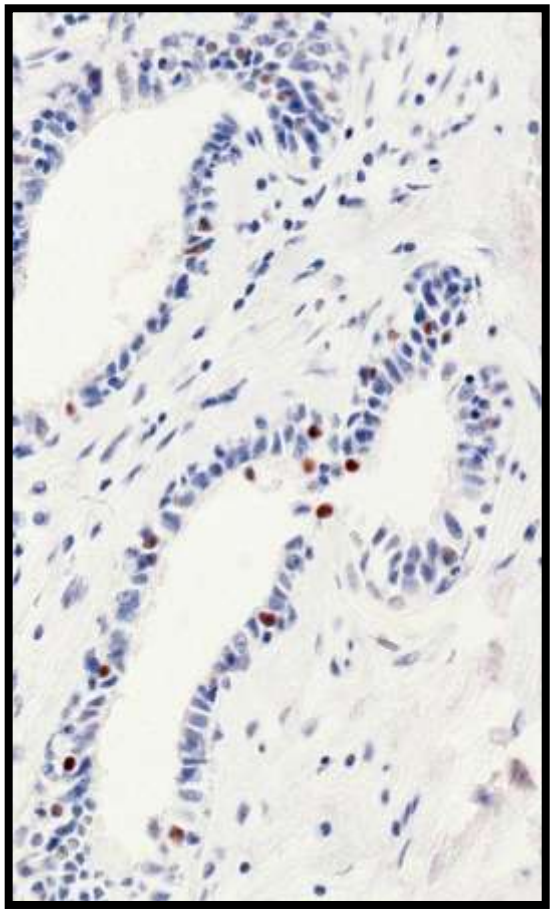
1 mes (–20ºC)

MIB1

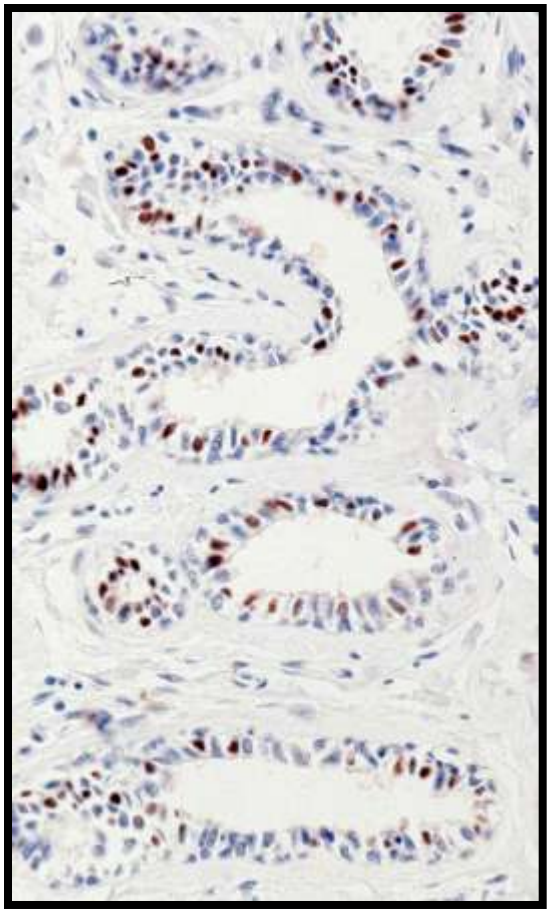
Almacenamiento de secciones



Almacenamiento de bloques



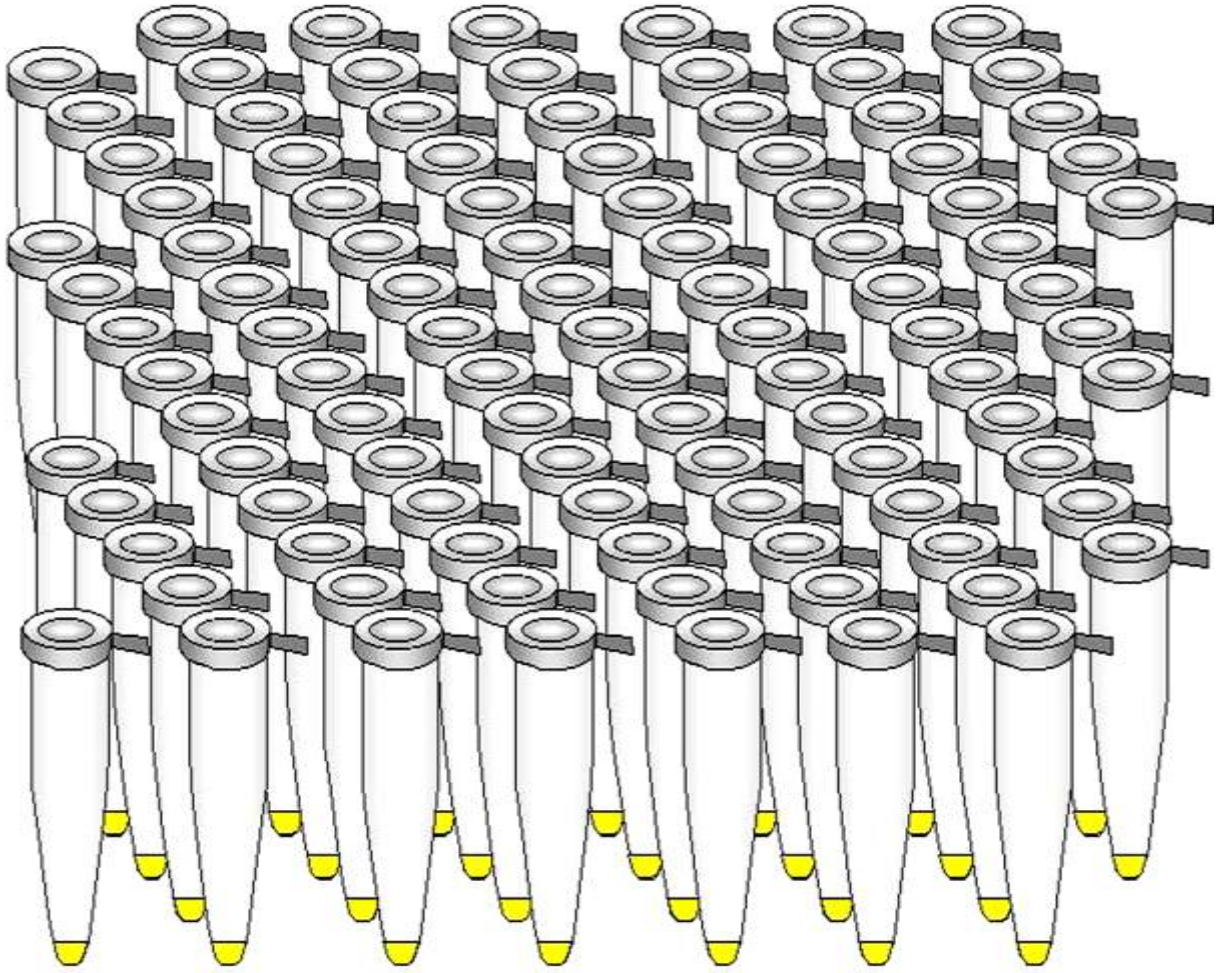
RE (6F11)



RP (1A6)

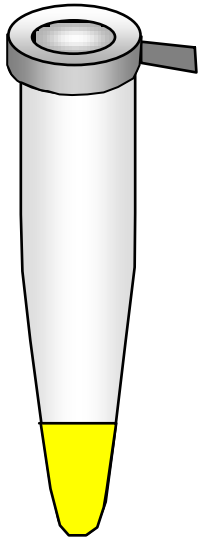
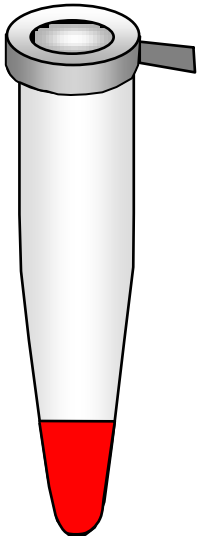
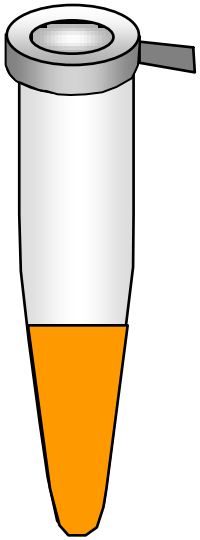
Bloques de 1962

Almacenamiento del anticuerpo

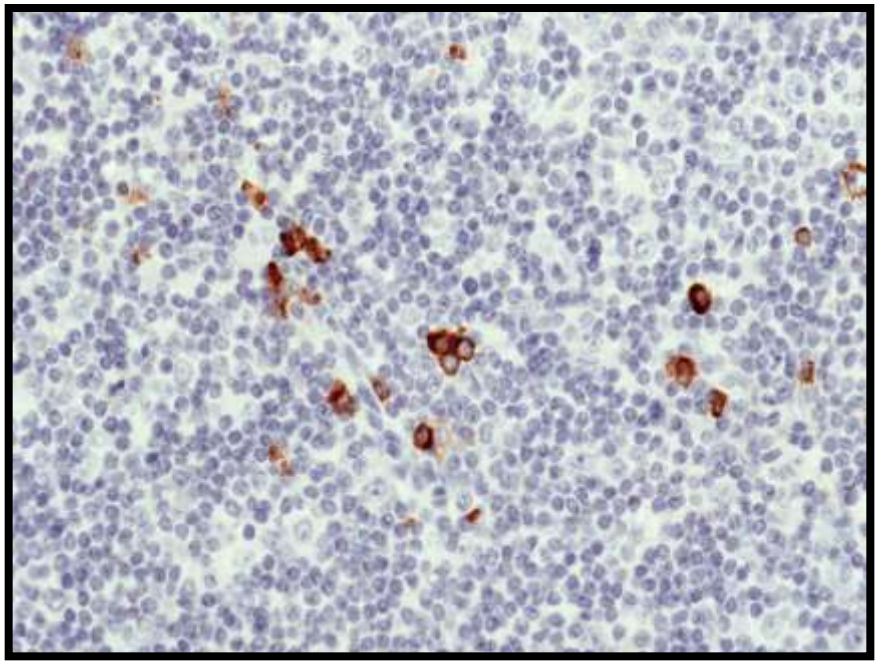


Alicuotas (-20 ºC)

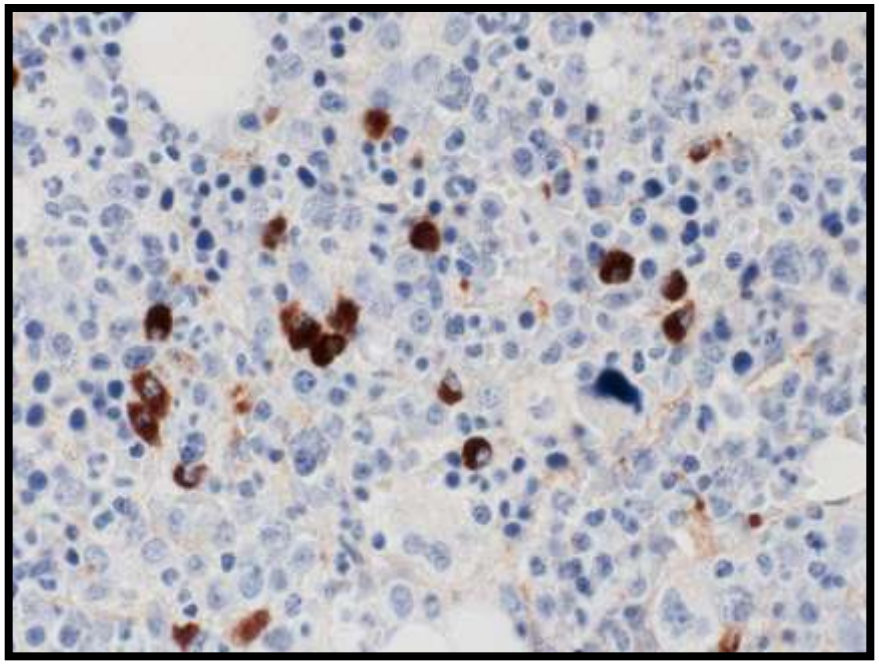
Almacenamiento del anticuerpo

Anticuerpo	+	Glicerol	=	Anticuerpo:Glicerol 1:1
				
				Punto de congelación < - 20 ºC

Almacenamiento del anticuerpo



IgA

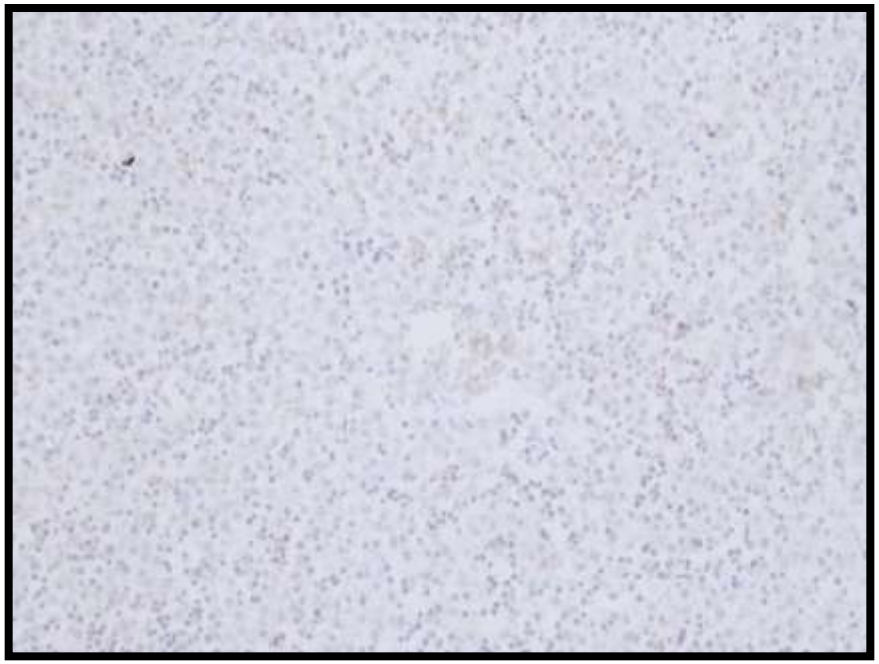


IgG

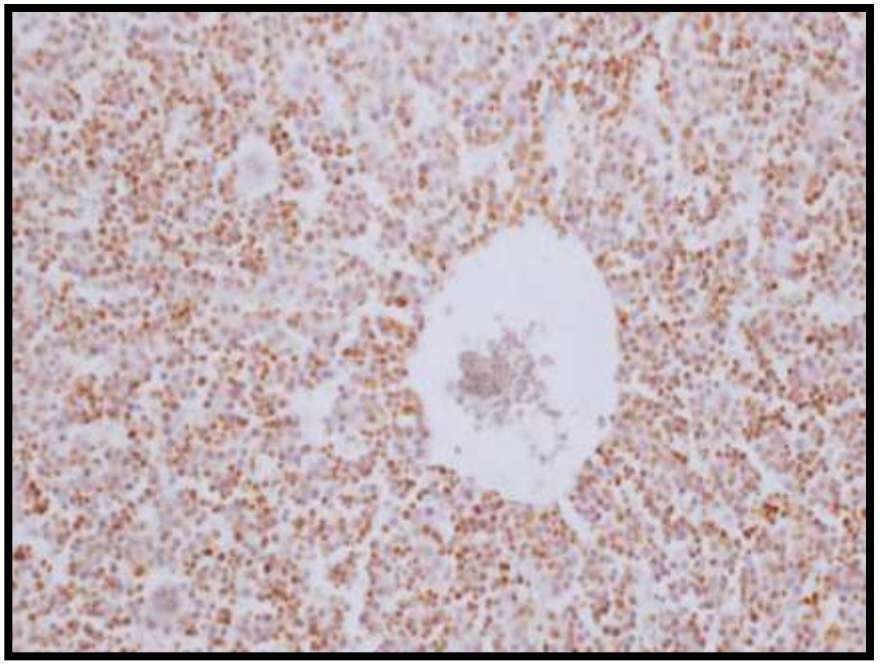
Anticuerpos: 1988 (glicerol)

4. Calidad del anticuerpo

Clon / Laboratorio comercial



9A7 / Laboratorio A



9A7 / Laboratorio B

Receptor vitamina D

5. Deterioro reactivos

¡¡¡No salió nada!!!

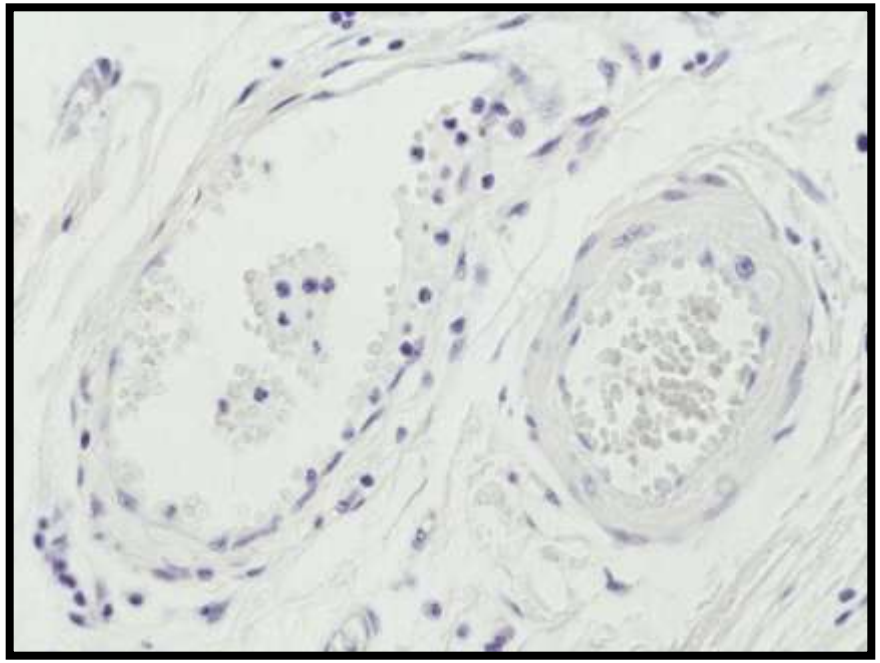
{ DAB / H_2O_2
Fallo humano

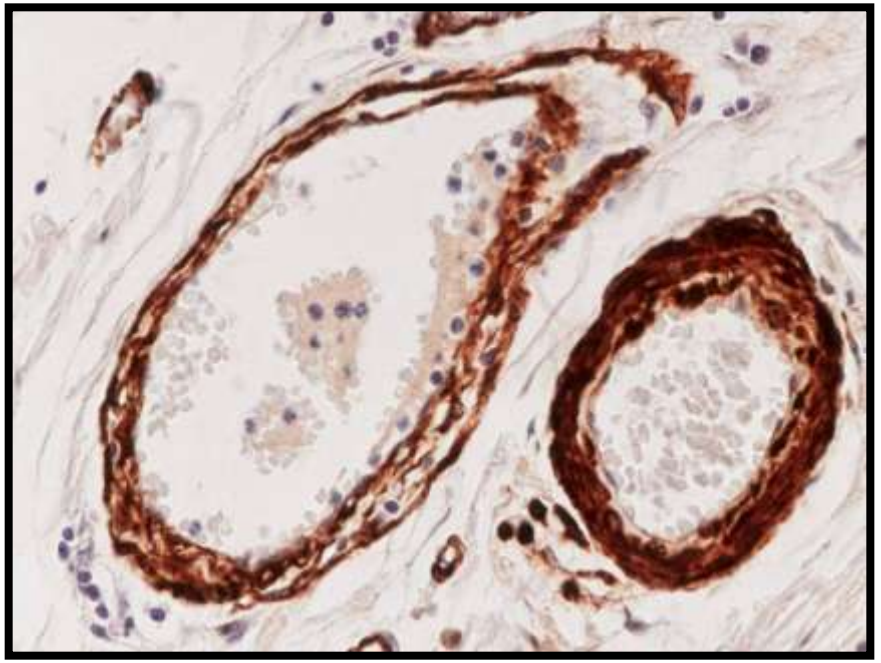
Sist. detección

Sist. detección + Cromógeno

¿Qué hacer?

Repetir encima

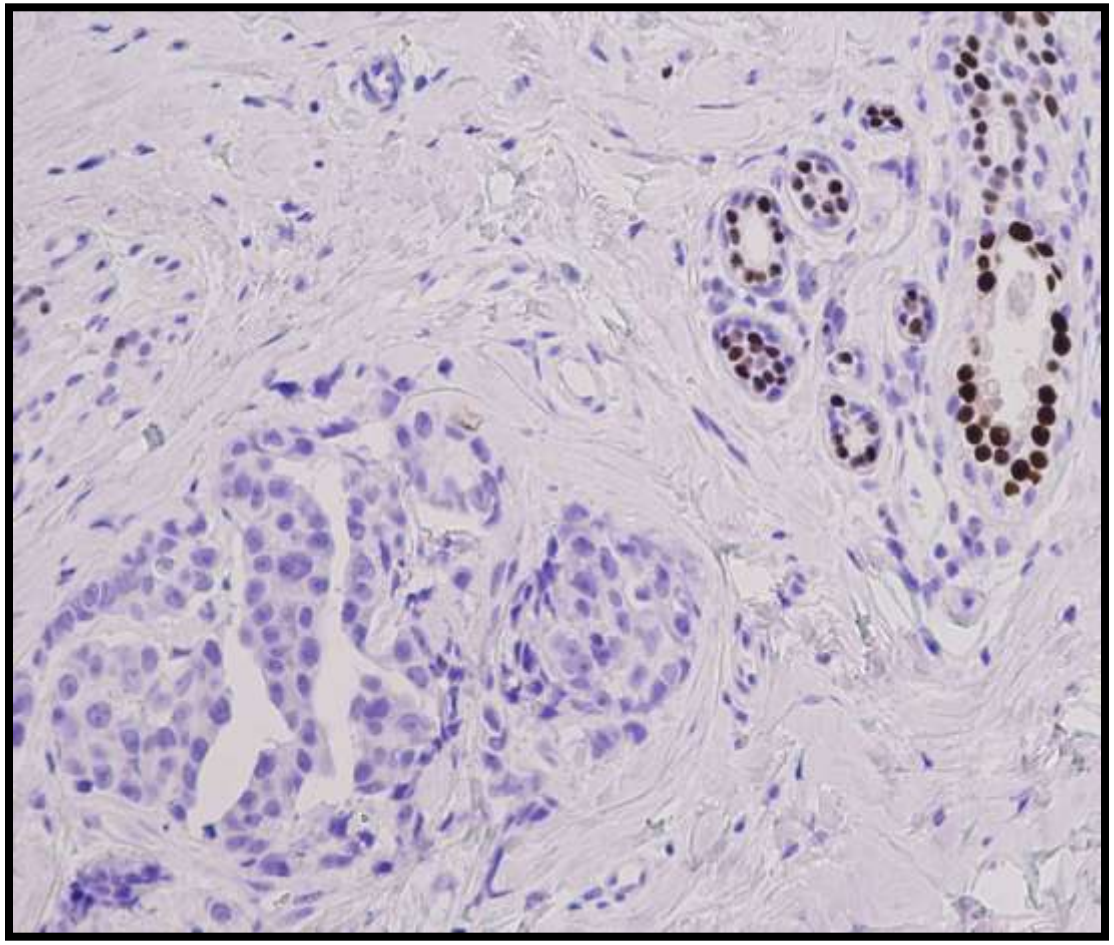




1ª IHQ **2ª IHQ**

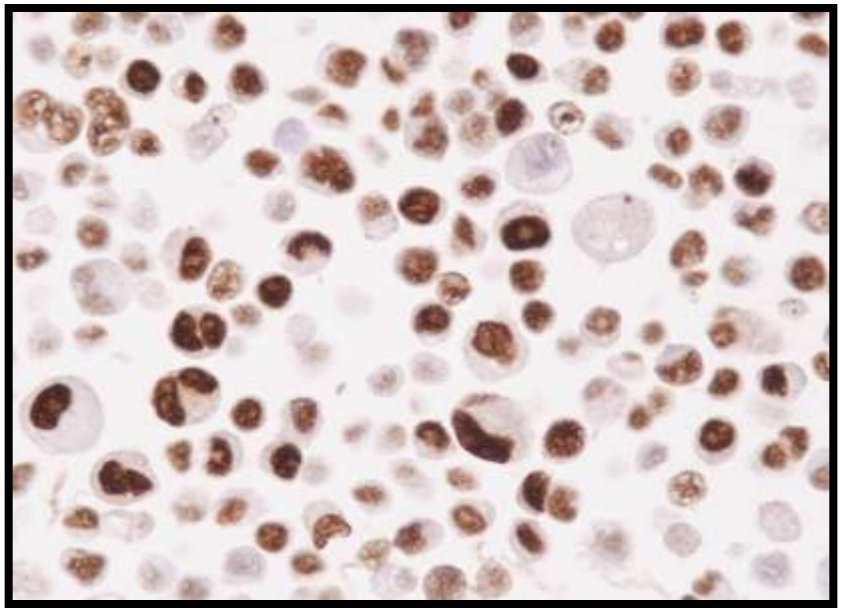
Actina músculo liso

6. Controles positivos

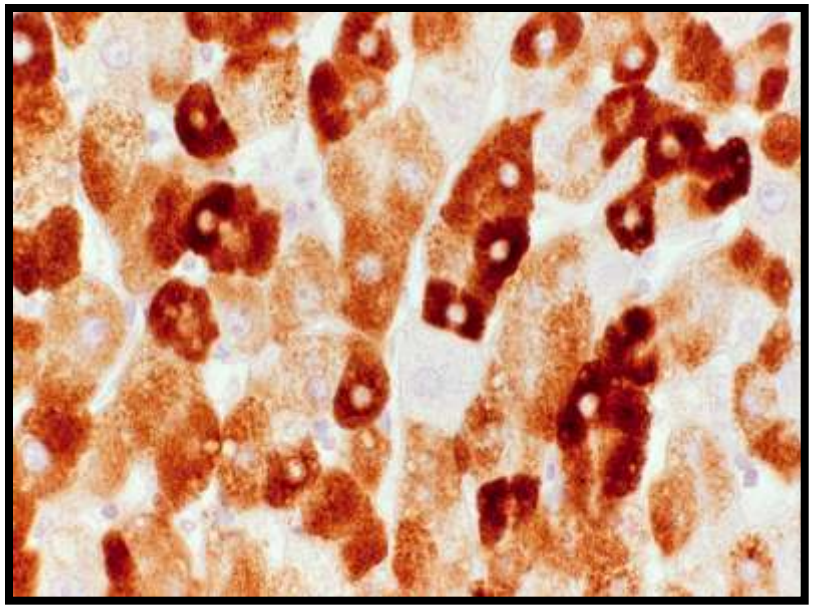


Control interno

Controles positivos



RE



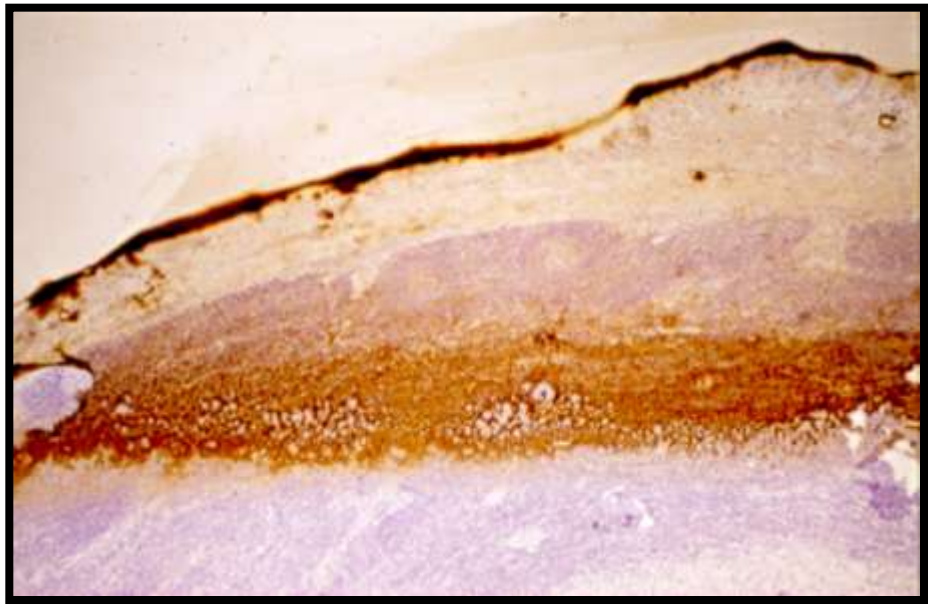
HBsAg

Controles externos

¡Esto tiene que salir positivo!: Control

- Artefactos
- Difusión
- Pigmentos
- Peroxidasa / biotina endógenas
- Recuperación antigénica
- Reactividad cruzada
- **Controles negativos**

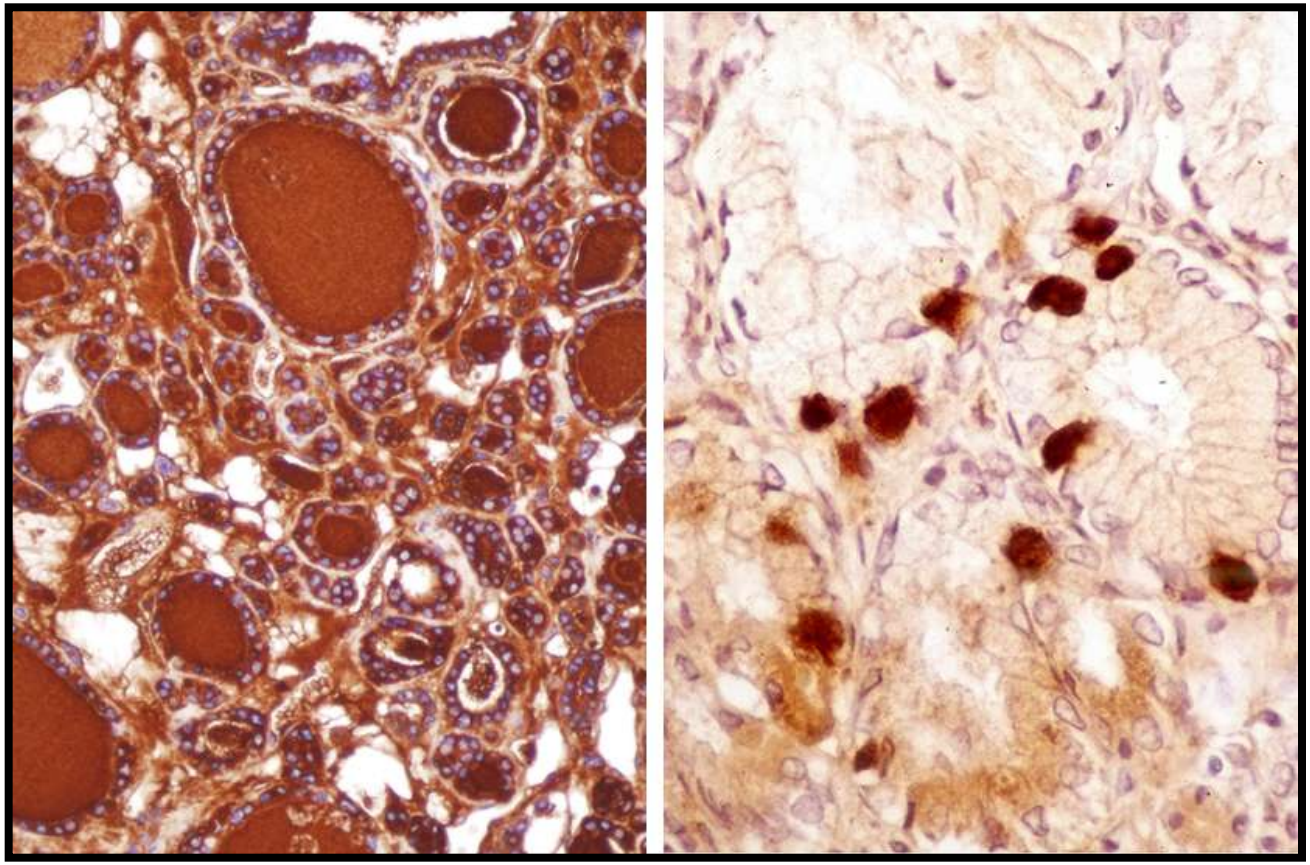
1. Artefactos



Efecto borde

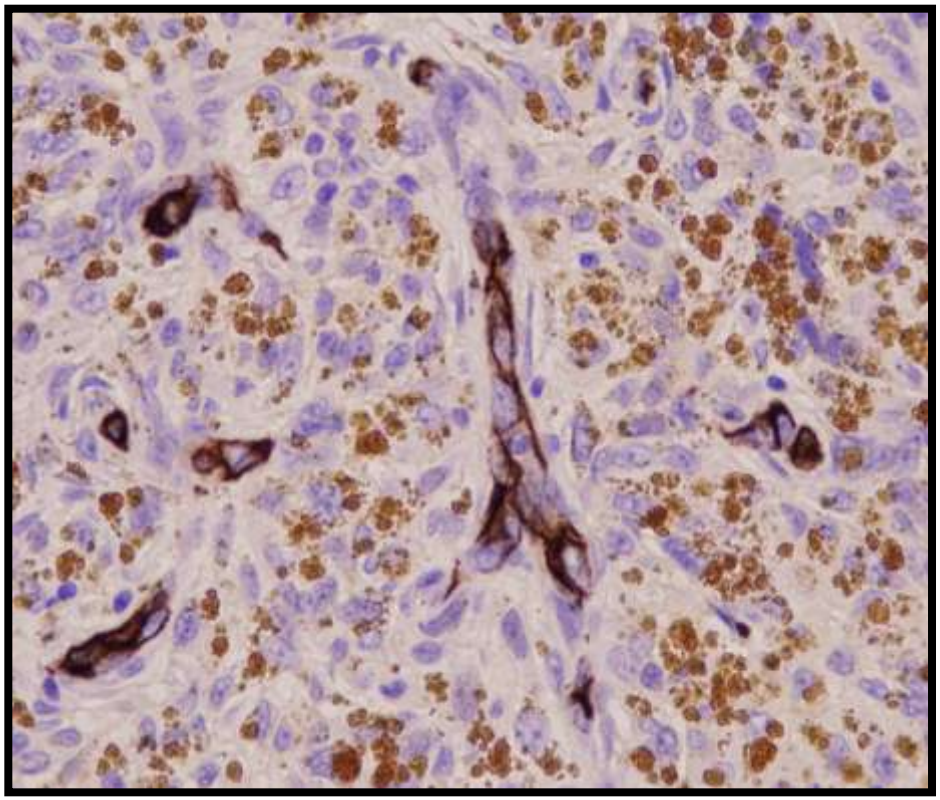
Pliegues

2. Difusión

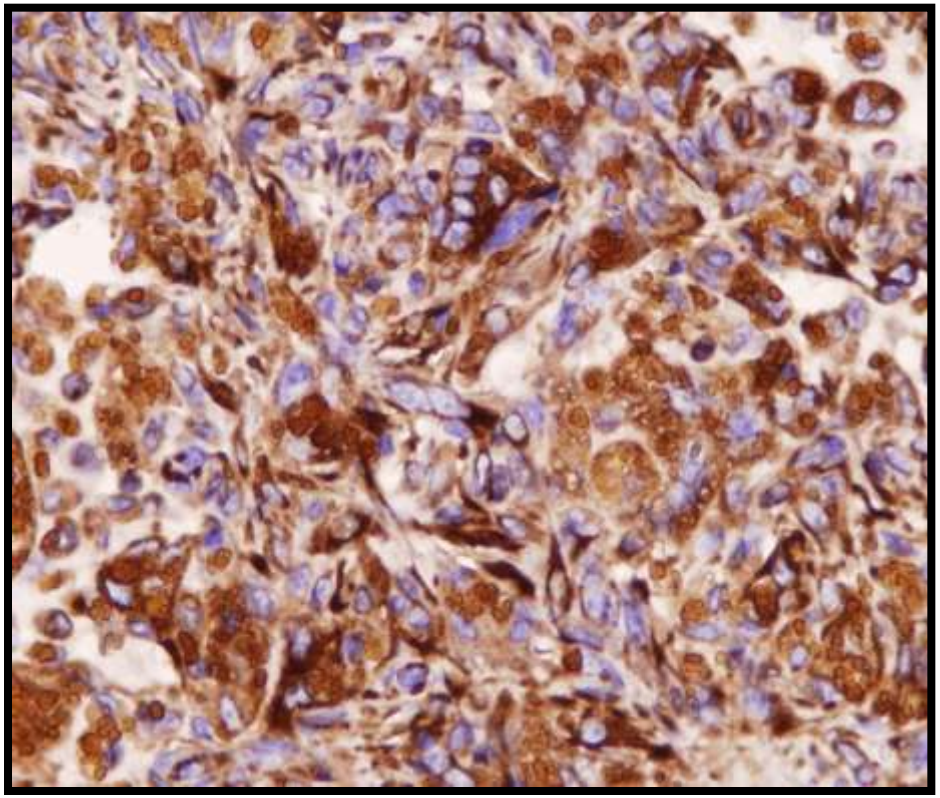


3. Pigmentos

Hemosiderina

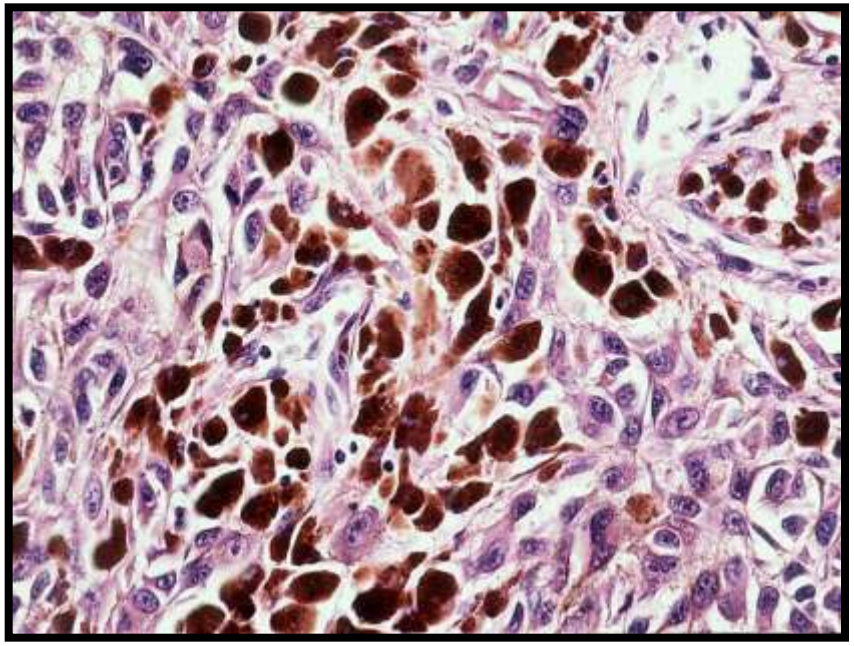


CD34



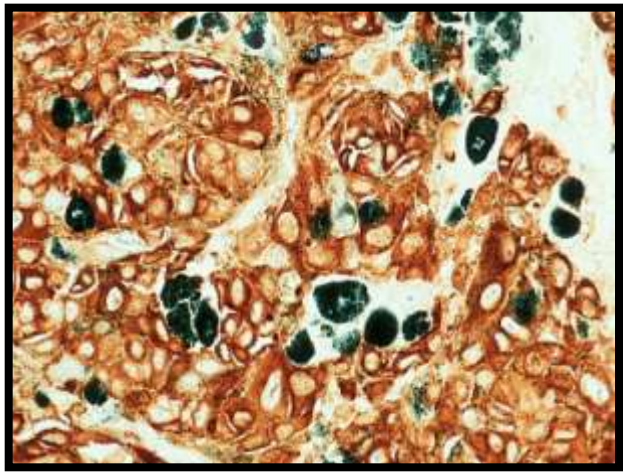
Vimentina

Melanina

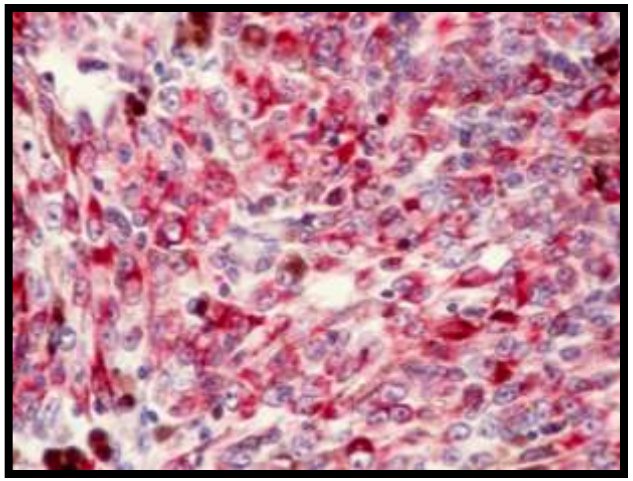


HE

Solución



HMB 45 + Giemsa

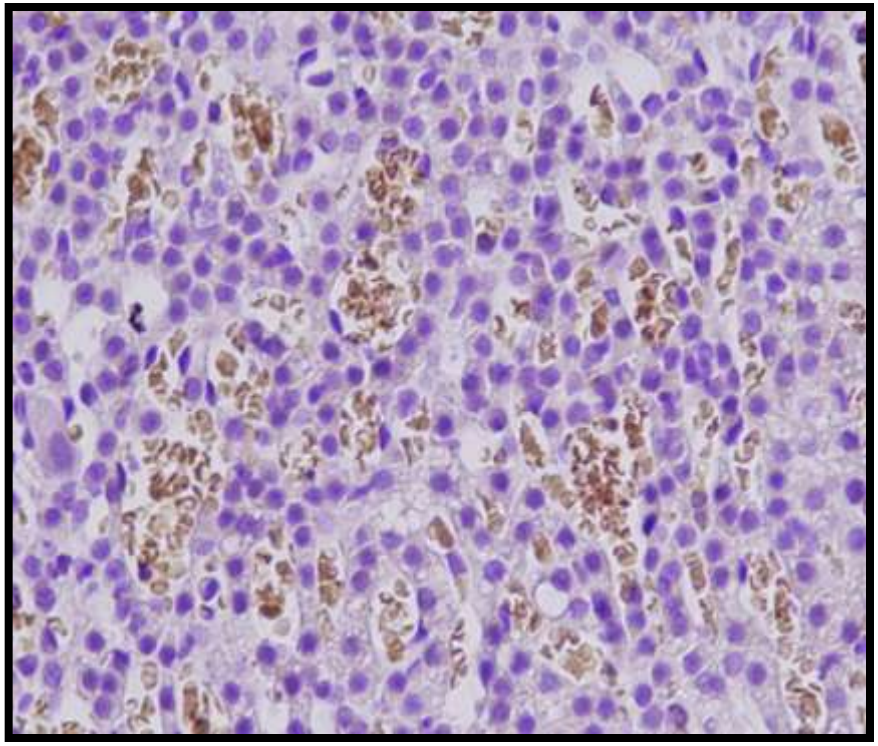


HMB 45: FA-Fast red

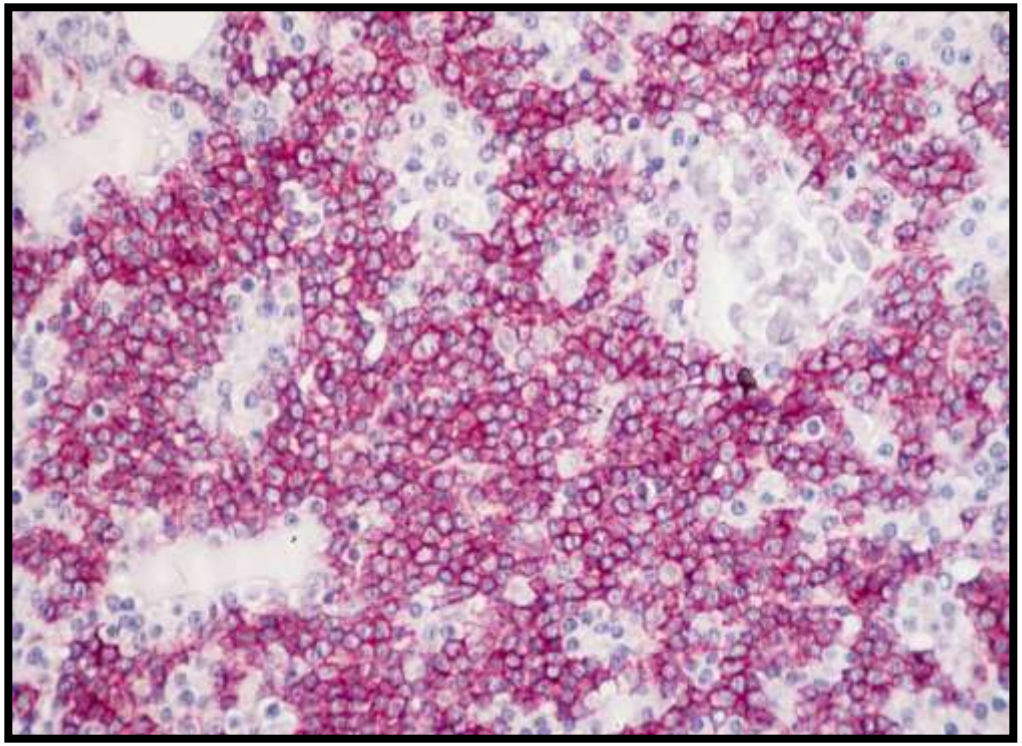
4. Peroxidasa / biotina endógenas

Peroxidasa

Solución

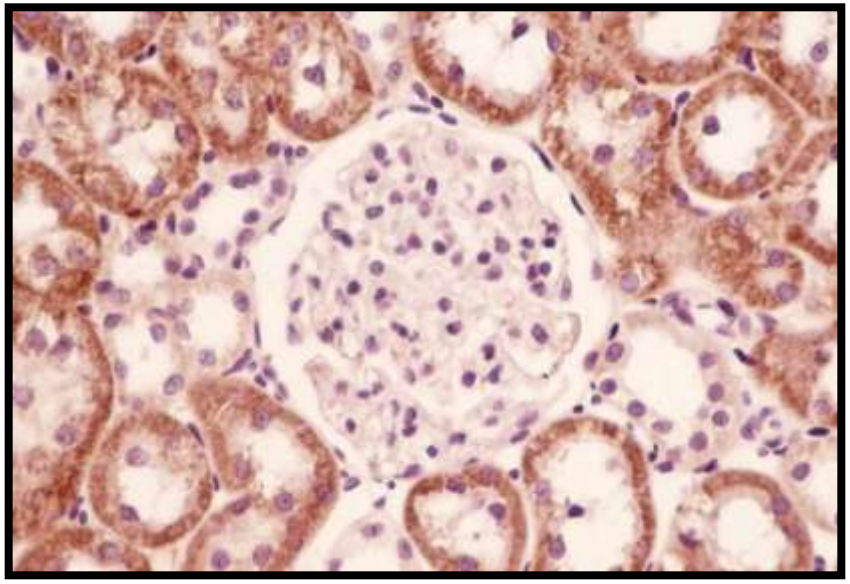


Px - DAB

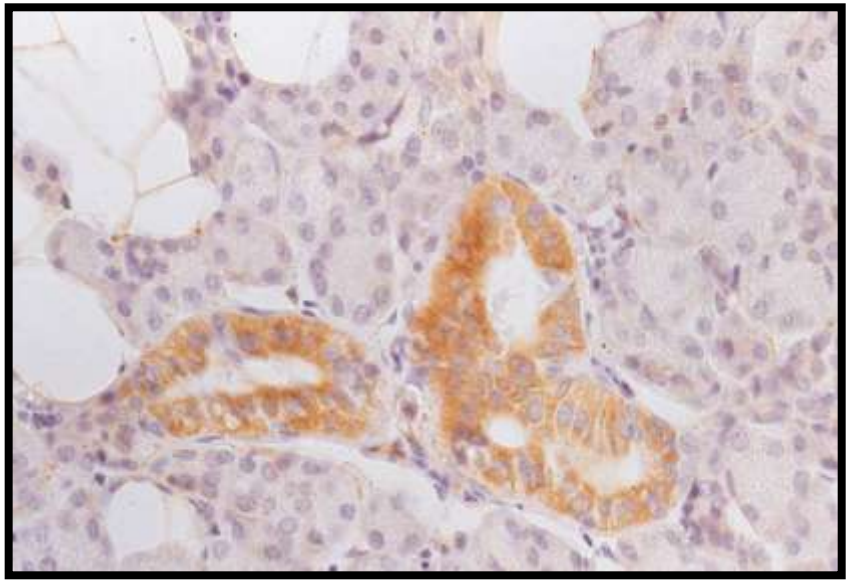


FA – Fast red

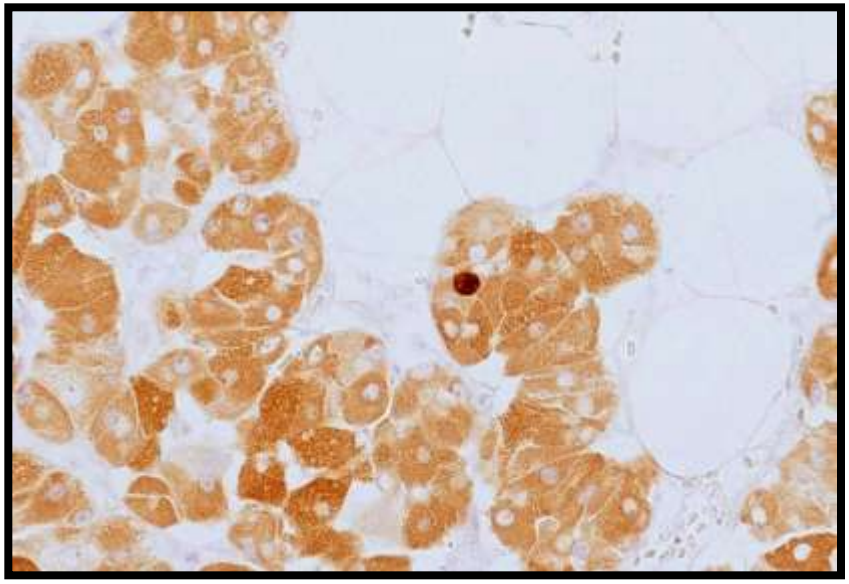
Biotina endógena



C. negativo

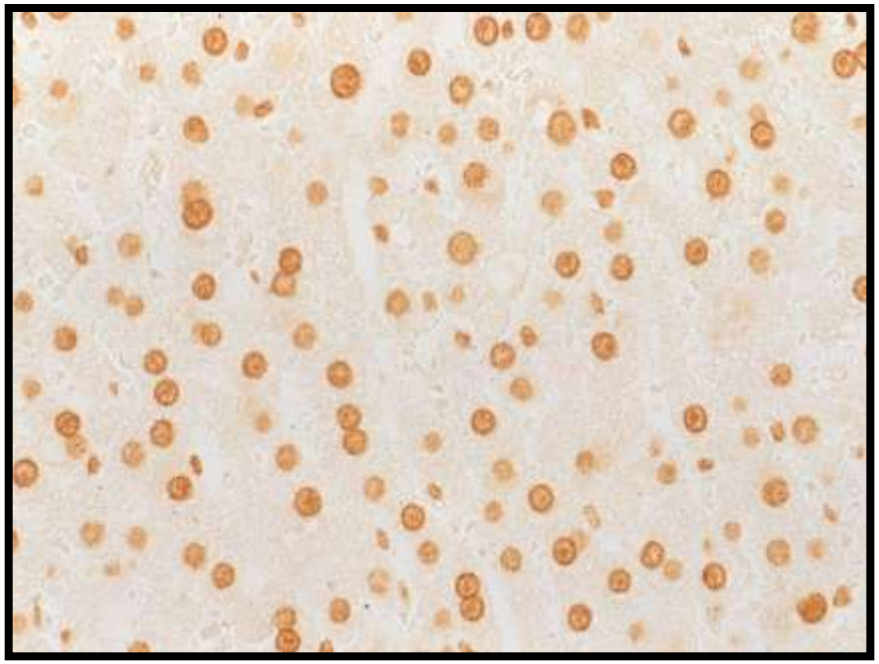


C. negativo

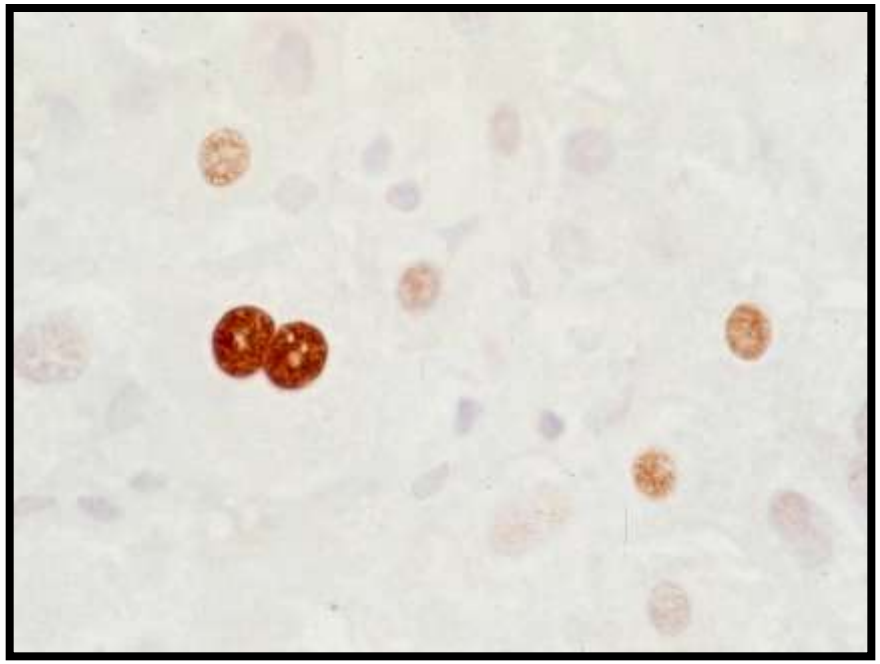


MIB1

Biotina endógena



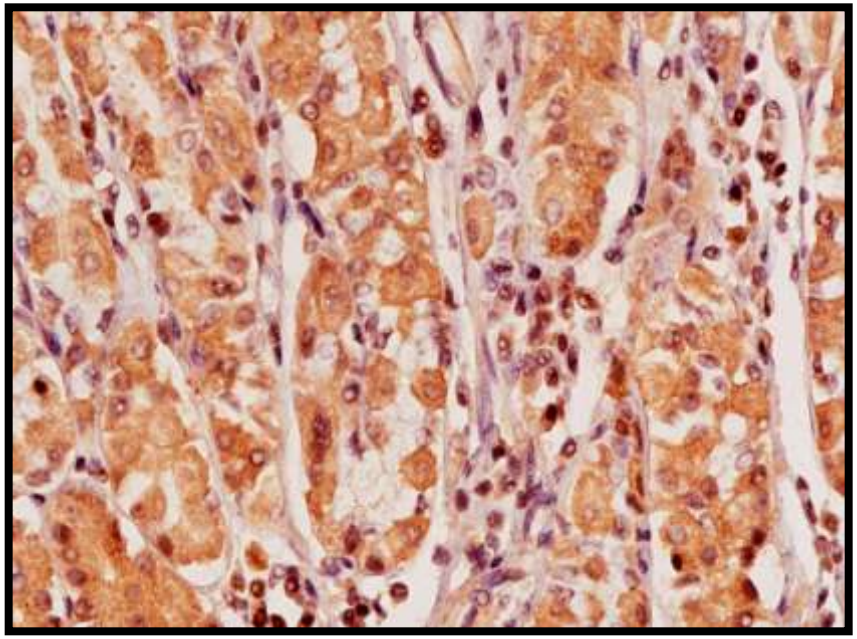
Receptor vitamina D

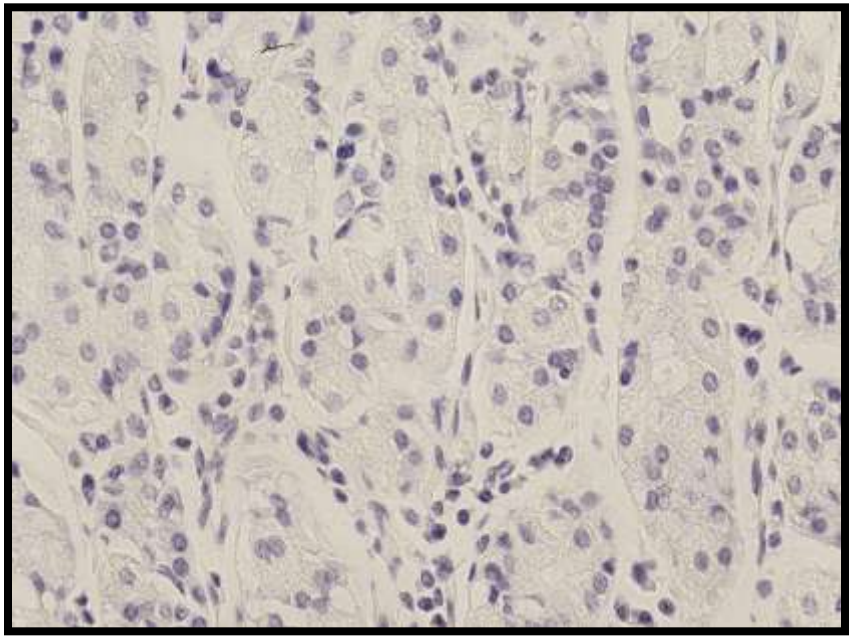


HBcAg

Biotina endógena

Solución



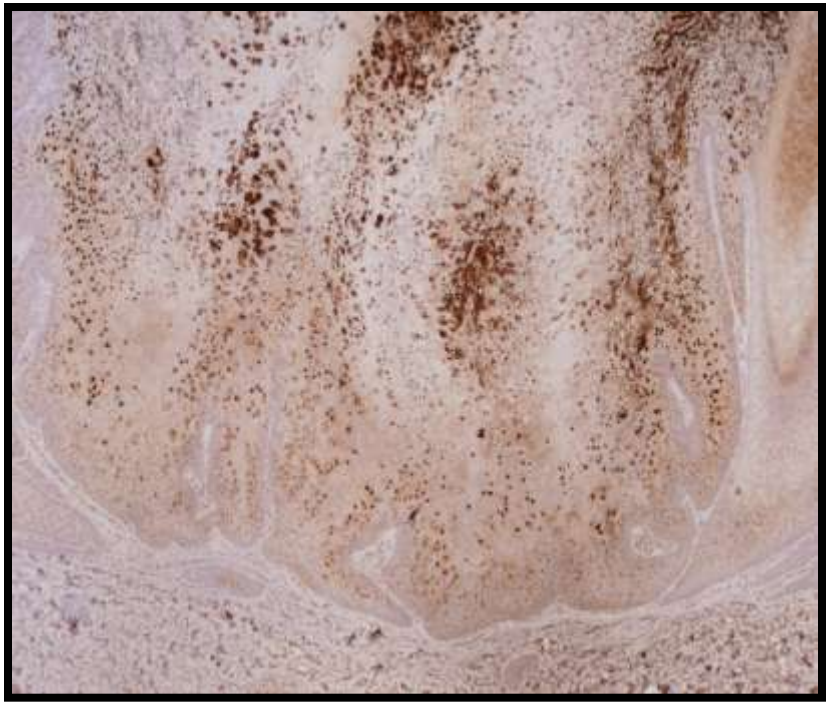


LSAB

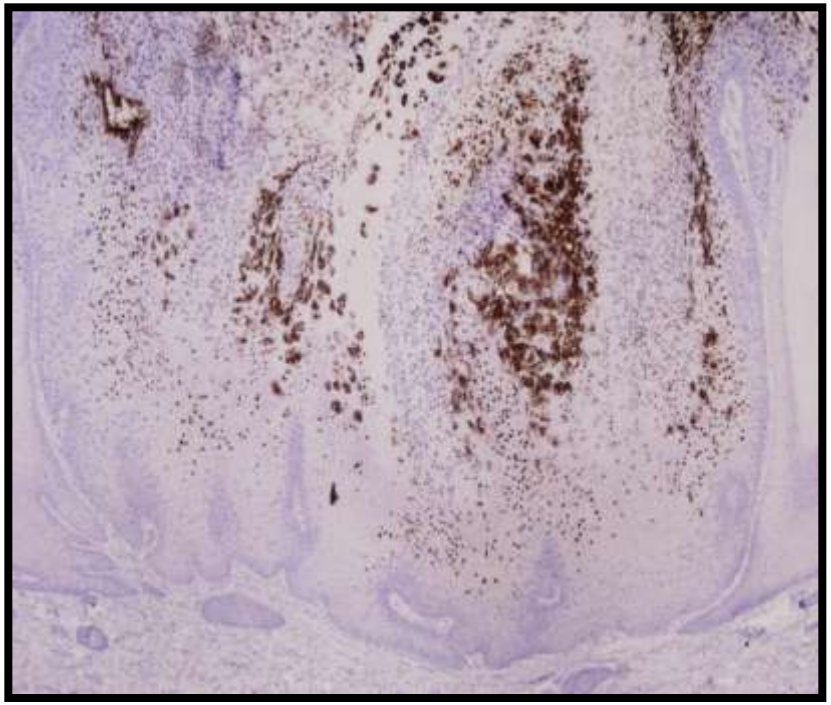
Polímero dextrano

5. Recuperación antigénica

Aparato



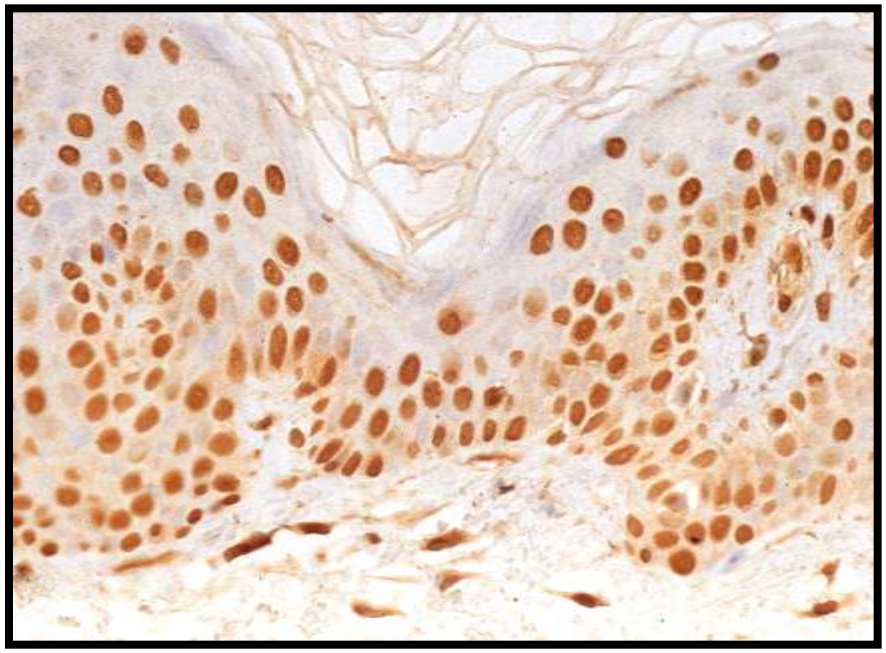
HPV – Baño termostático



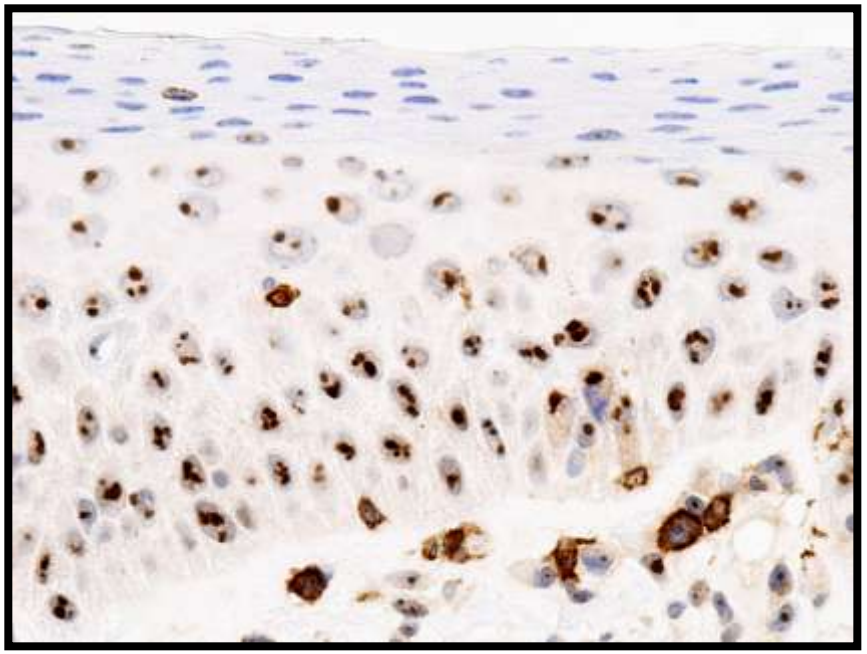
HPV - Microondas

Recuperación antigénica

Tampón

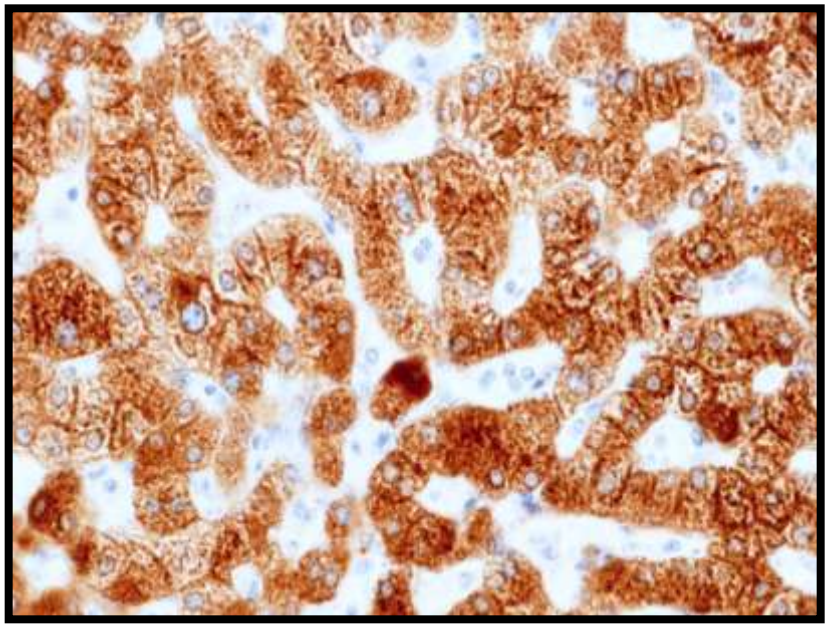


F XIIIa

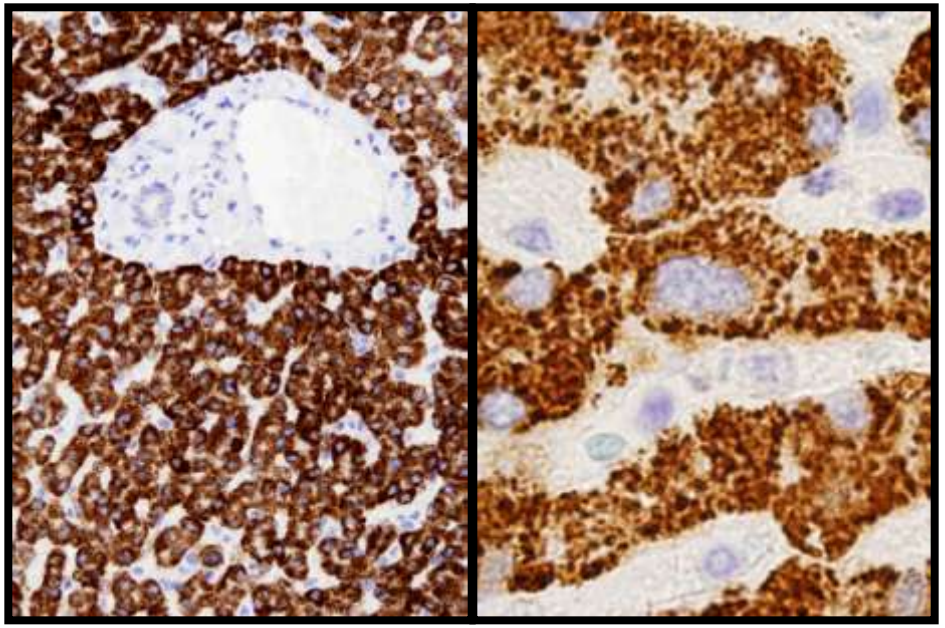


CD7

6. Reactividad cruzada

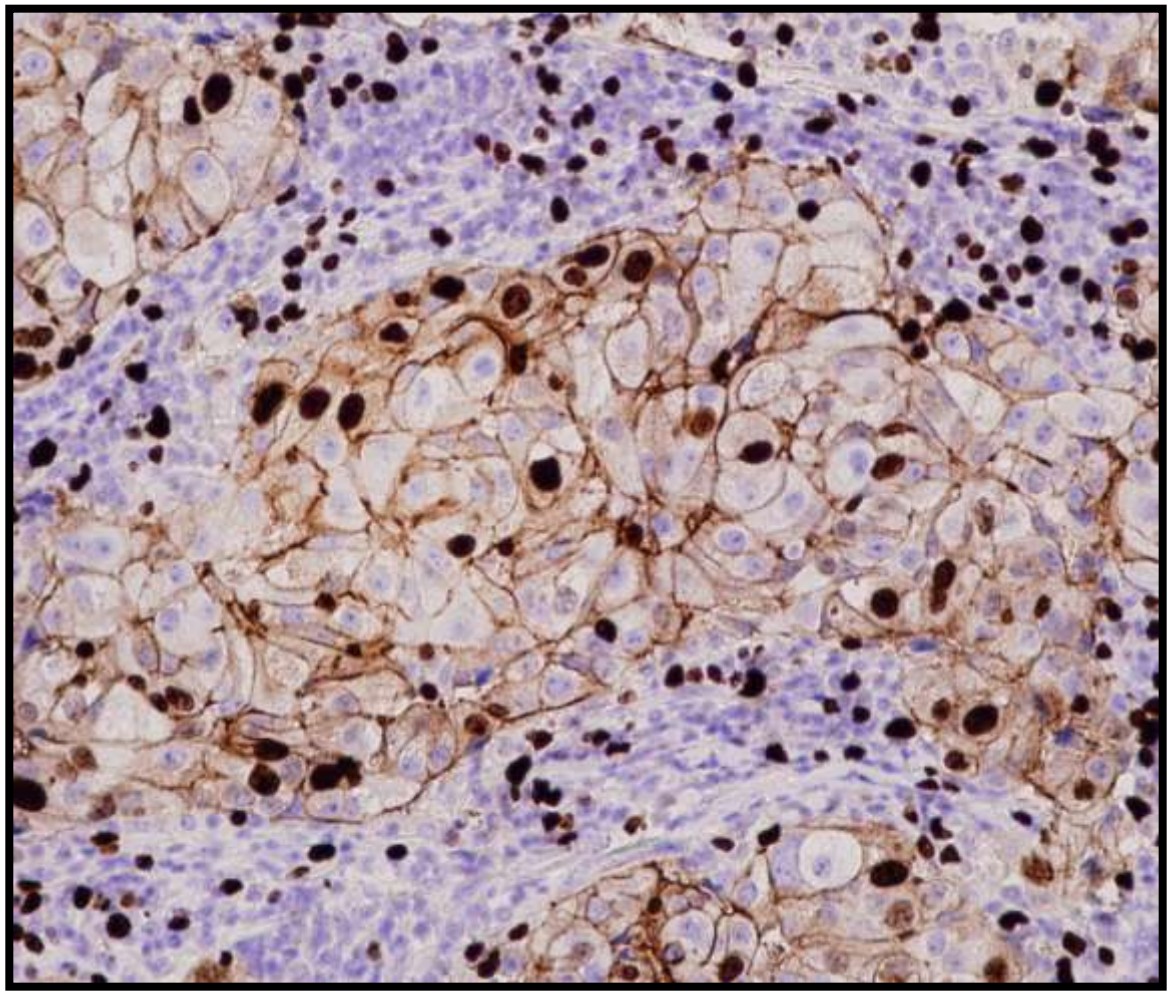


Ciclina D1



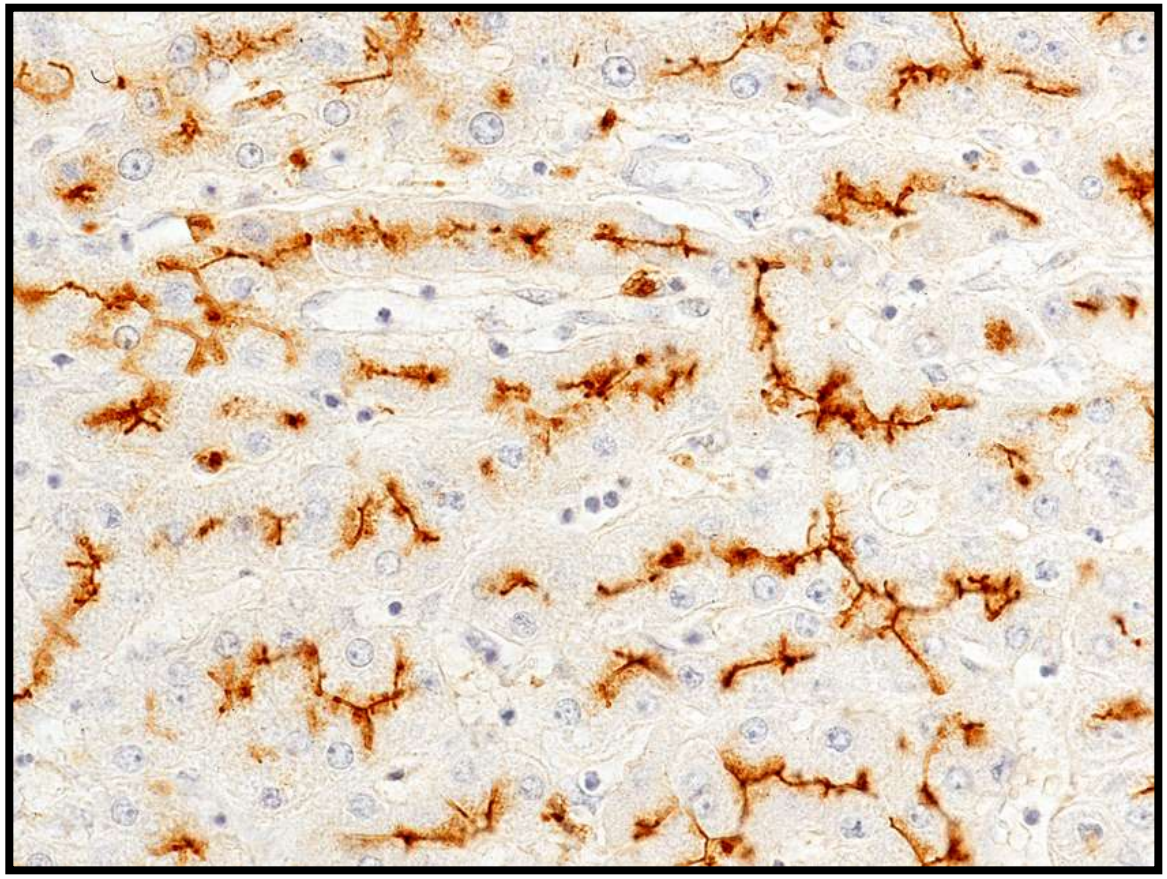
TTF1

Reactividad cruzada



MIB 1

Reactividad cruzada

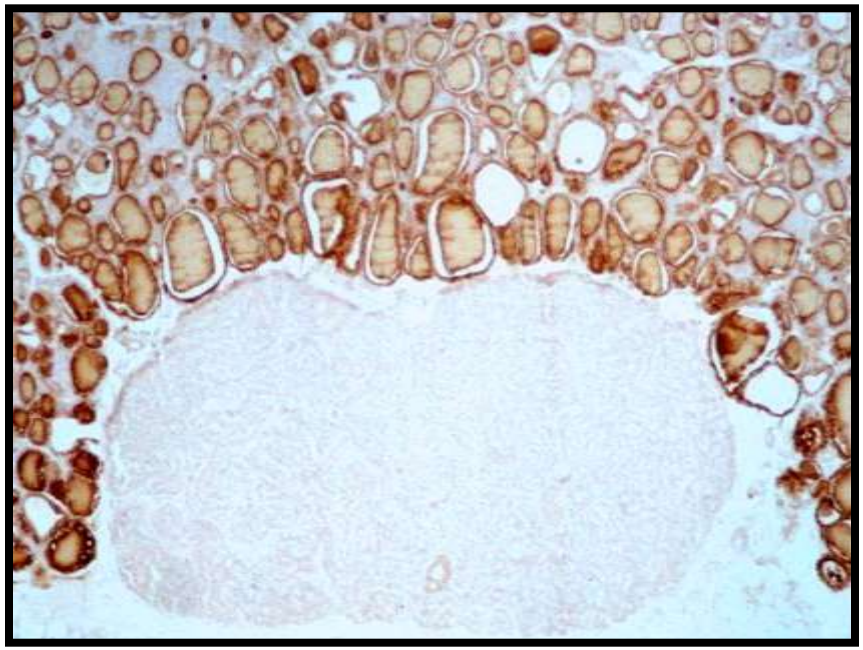


CEA

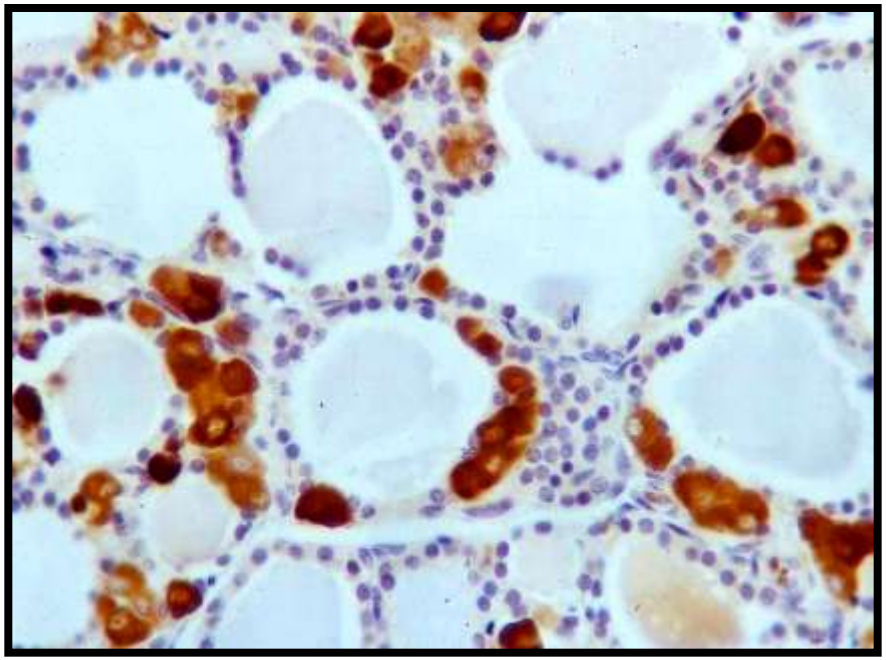
Glicoproteína biliar 1

7. Controles negativos

Interno

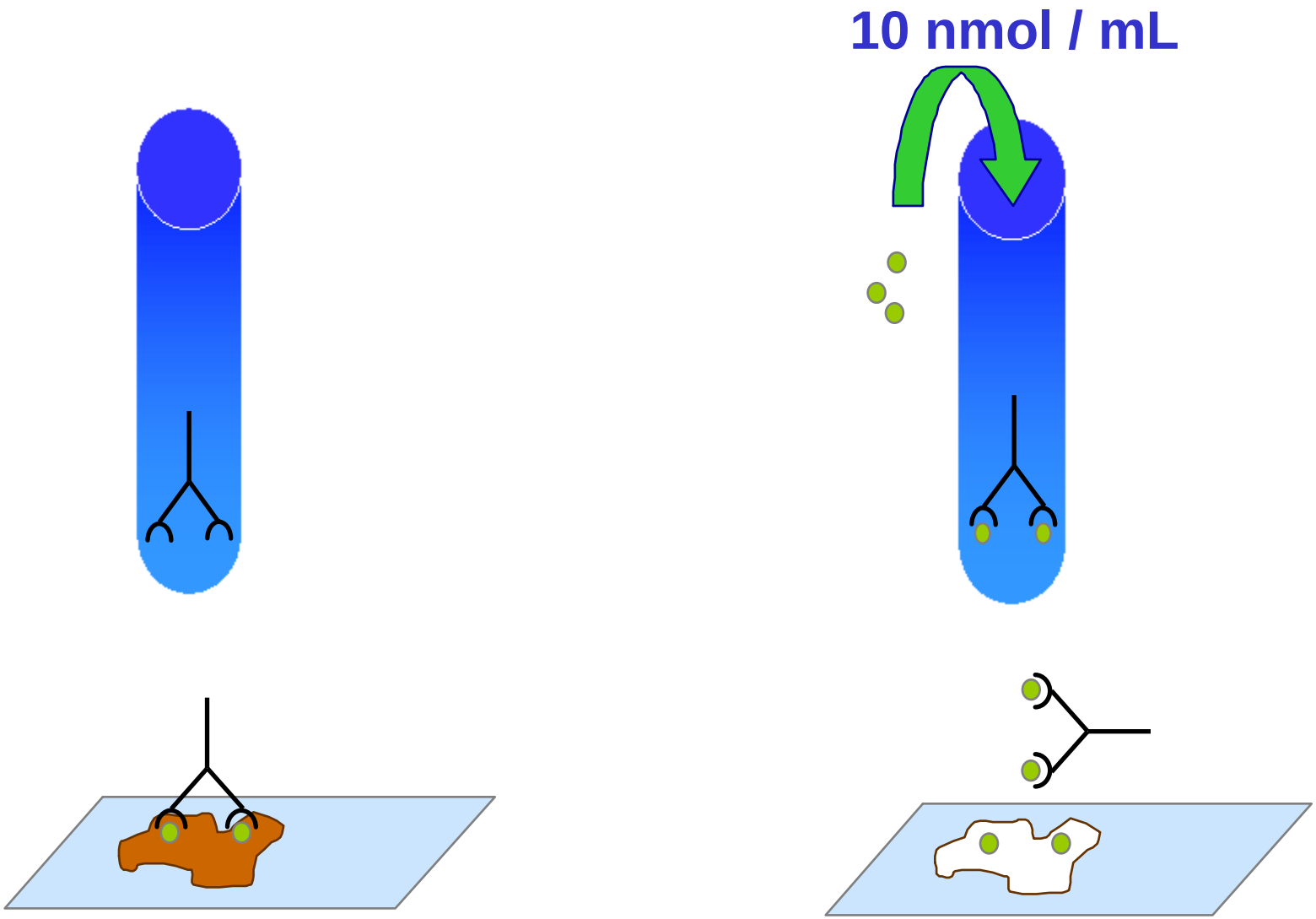


Tiroglobulina

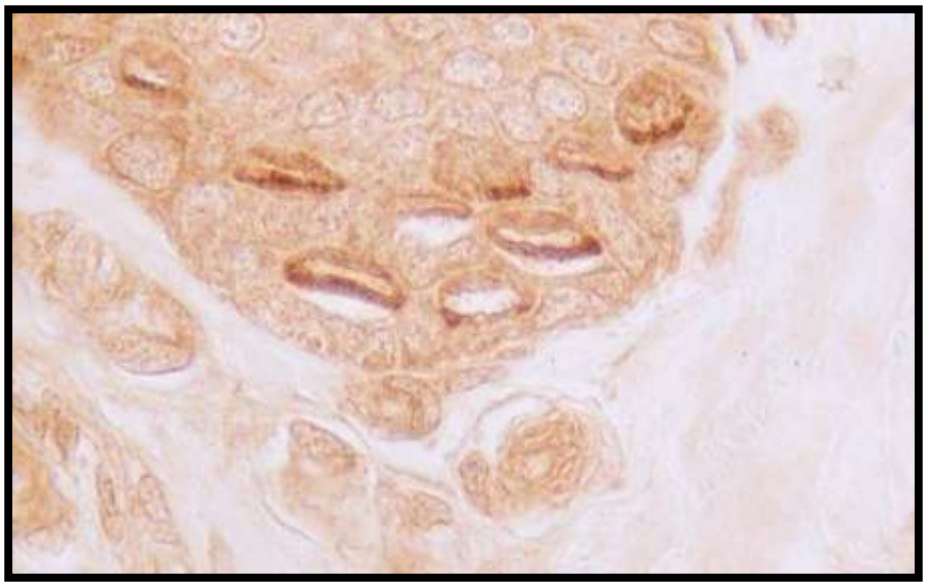


Calcitonina

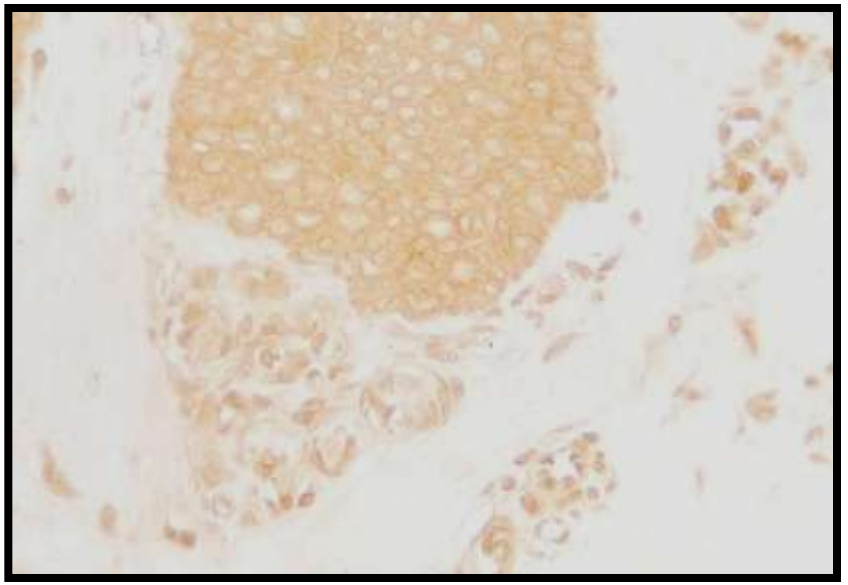
Control preadsorción



Control preadsorción



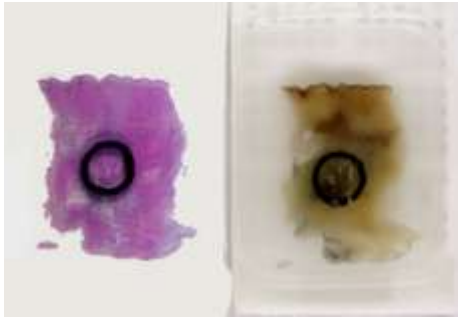
Anti-serotonina



Anti-serotonina + serotonina

Dos trucos...

Confección macromatrices



¡No queda material!

Immunohistochemistry on Cytologic Specimens and Previously Stained Slides (When No Paraffin Block Is Available)

Rodney T. Miller* and Patty Kubier
ProPath Laboratory, Inc., Dallas, Texas

Abstract

Most diagnostic immunohistochemistry is performed on histologic sections taken from paraffin blocks, but in certain cases only previously stained or unstained slides are available for study. This article discusses the approach to these specimens used in the authors' laboratory. Immunohistochemistry on cytologic smears and previously stained tissue sections is addressed, and the routine use of tissue-transfer techniques and tissue-protection immunohistochemistry as part of this approach is discussed and illustrated. This approach is effective and frequently obviates the need to rebiopsy the patient to obtain sufficient material for a definitive diagnosis. The techniques discussed are not technically demanding, and they can be performed in any histology or immunohistochemistry laboratory. Their widespread use has tremendous potential to lead to improved patient care. (*The J Histotechnol* 25:251, 2002)

Key words: immunohistochemistry, cytology, FNA, techniques, methods, cell transfer, tissue transfer, tissue protection

Introduction

Immunohistochemistry (IHC) on sections taken from paraffin-embedded material is well established as a routine method to assist in the diagnosis of cases that cannot be confidently interpreted on standard H&E morphologic examination. However, in situations when a paraffin block is not available for analysis, attempting IHC can be problematic for a variety of reasons. If the slide material has been previously placed on nonadhesive slides, attempts to perform IHC frequently lead to detachment of the diagnostic material from the slide. Additionally, only one or two slides containing diagnostic material may be available for study, and in most situations more than one or two immunostains are needed to properly evaluate a diagnostic problem. However, by routinely using tissue-transfer techniques (1,2) or tissue-protection techniques (3) in the approach to these types of specimens, a definitive diagnosis can be rendered in a high percentage of these cases. In this review, an approach to IHC on cytologic smears will be discussed, followed by an approach to immunostaining of previously stained H&E slides of paraffin section material (where diagnostic material is no longer present in the corresponding paraffin block).

IHC Cytologic Specimens

Common Problems With Cytologic IHC

The most frequent problem that is experienced when dealing with cytologic IHC is the lack of satisfactory or sufficient material. Only a small number of slides may be available for immunostaining, and it frequently seems that the number of slides available varies inversely with the diagnostic difficulty of the case. The quality of the smears is also highly variable, and may contain inappropriately thick areas with poor cell distribution on the slide. Variable fixation and drying is also a common problem. Finally, nonadhesive slides are often used for cytologic IHC, compromising their utility for standard immunostaining techniques because diagnostic material frequently detaches from nonadhesive slides during the immunostaining process.

Importance of Attempts to Obtain Cell Blocks

Before discussing the approach to IHC on cytologic specimens, the importance of attempting to obtain cell blocks on all cytologic specimens needs to be stressed. There is no question in our minds that the best advice that can be given to those involved in obtaining and preparing cytologic specimens is the following: GET A CELL BLOCK! If one has a satisfactory cell block, it is frequently possible to perform a large battery of immunostains if needed, using routine well-established techniques that are

* Address correspondence to Rodney T. Miller, ProPath Laboratory, Inc., 8267 Elmbrook Drive, Suite 100, Dallas, TX 75247. E-mail: rmiller@propathlab.com

Tissue Protection Immunohistochemistry

A Useful Adjunct in the Interpretation of Prostate Biopsy Specimens and Other Selected Cases in Which Immunostains Are Needed on Minute Lesions

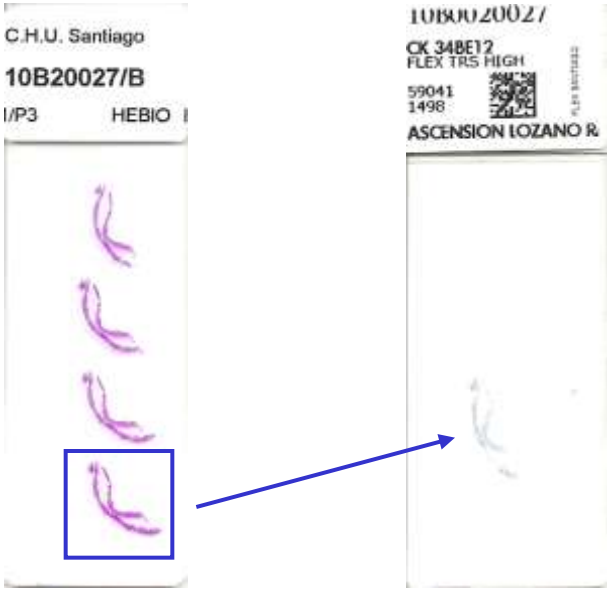
Patty Kubier, HT(ASCP), AAS, QIHC, and Rodney T. Miller, MD

Key Words: Immunohistochemistry; Prostate biopsy specimens; Immunoperoxidase; Techniques; Methods

Am J Clin Pathol 117:194-8, 2002

Mount Quick

(Electron Microscopy Sciences, Hatfield, PA, USA)



- Necesidad de especialización - Técnicos fijos de IHQ
- Valoración microscópica de los resultados
- Listado de controles positivos
  - Problemas
  - Controles de calidad
- Formación continuada


¡Muchas gracias!