

CONGRESO DE LA SEAP-AIP

ZARAGOZA 19-21 Mayo 2001

DETERMINACIÓN INMUNOHISTOQUÍMICA DE HER-2 EN
CÁNCER DE MAMA Y GÁSTRICO

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Angel Concha López

HUVN. Granada

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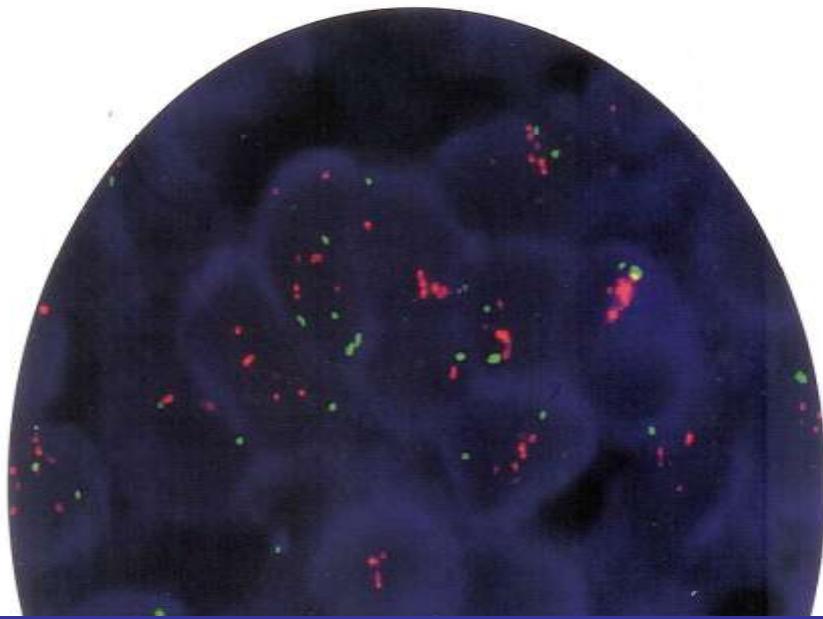
ASCO SPECIAL ARTICLE

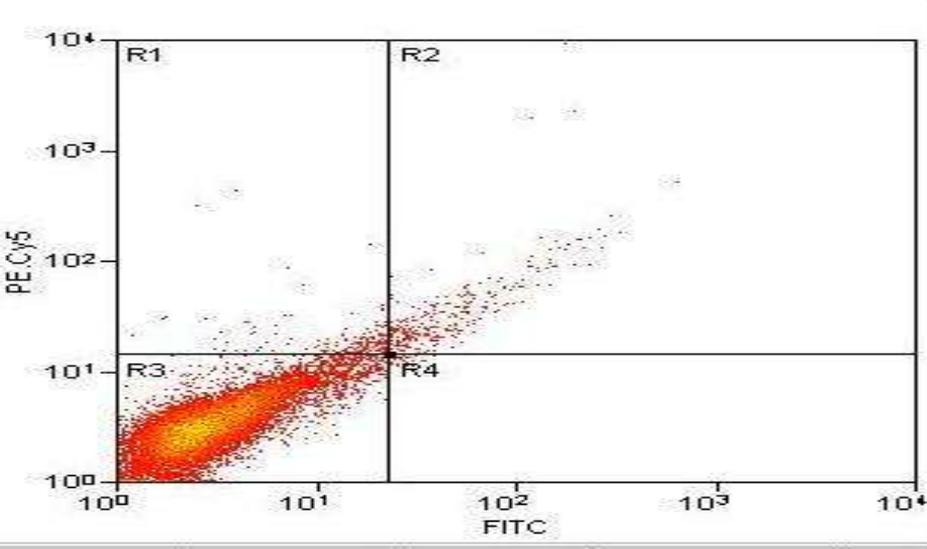
American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

Antonio C. Wolff, M. Elizabeth H. Hammond, Jared N. Schwartz, Karen L. Hagerty, D. Craig Allred, Richard J. Cote, Mitchell Dowsett, Patrick L. Fitzgibbons, Wedad M. Hanna, Amy Langer, Lisa M. McShane, Soonmyung Paik, Mark D. Pegram, Edith A. Perez, Michael F. Press, Anthony Rhodes, Catharine Sturgeon, Sheila E. Taube, Raymond Tubbs, Gail H. Vance, Marc van de Vijver, Thomas M. Wheeler, and Daniel F. Hayes

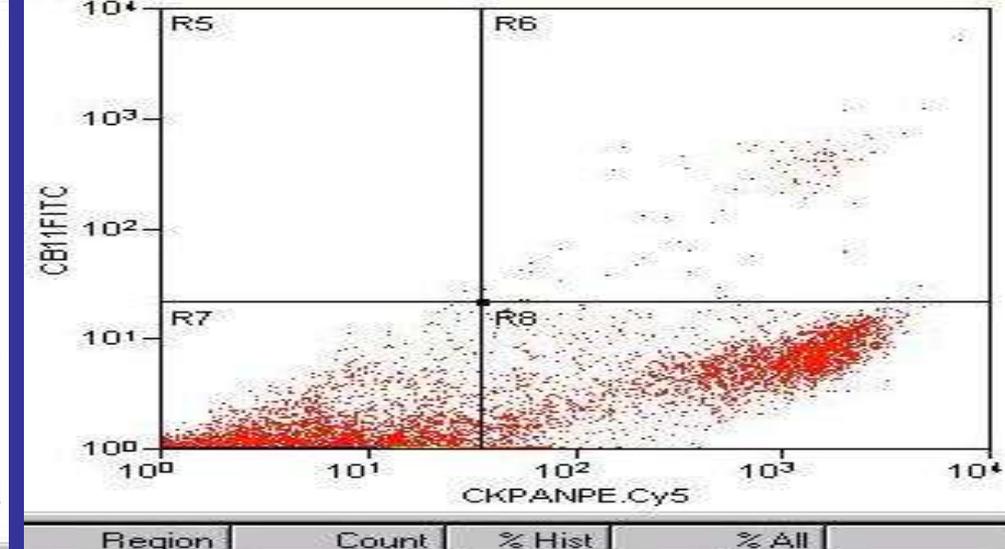
Recomendaciones para la determinación
de HER2 en cáncer de mama.

*Consenso nacional de la Sociedad
Española de Anatomía Patológica
(SEAP) y de la Sociedad Española
de Oncología Médica (SEOM)*

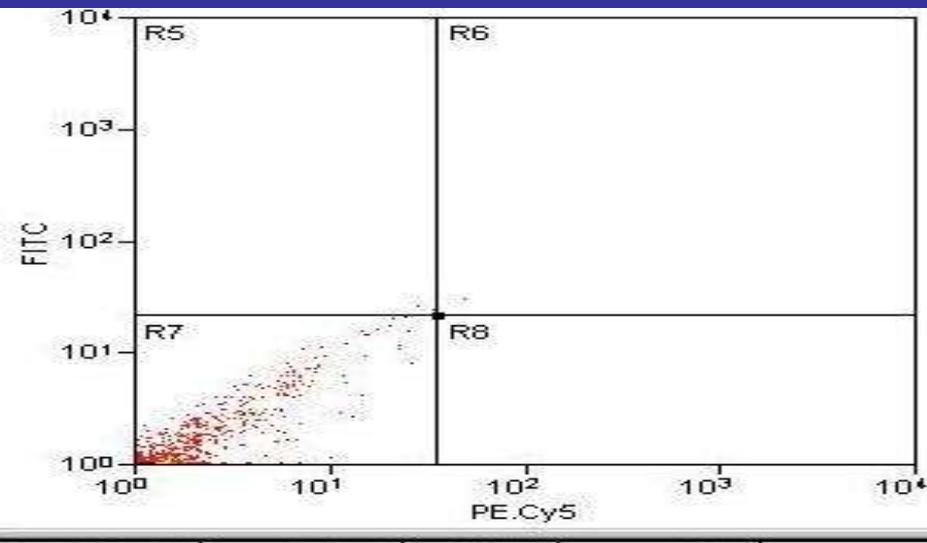




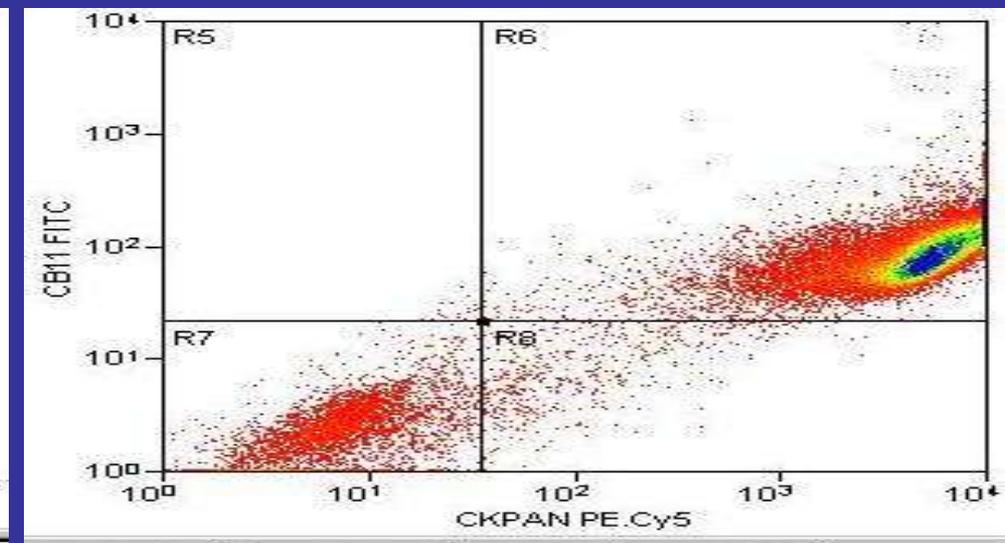
Region	Count	% All	Median
Total	13326	100.00	2.74, 3.05, 265.3
R1	170	1.28	16.55, 17.78, 39.13
R2	264	1.98	39.24, 27.38, 105.5
R3	12862	96.52	2.64, 3.05, 77.3
R4	30	0.23	27.38, 12.86, 13.0



Region	Count	% Hist	% All
Total	14685	100.00	100.00
R5	3	0.02	0.02
R6	103	0.70	0.70
R7	11849	80.69	80.69
R8	2730	18.59	18.59

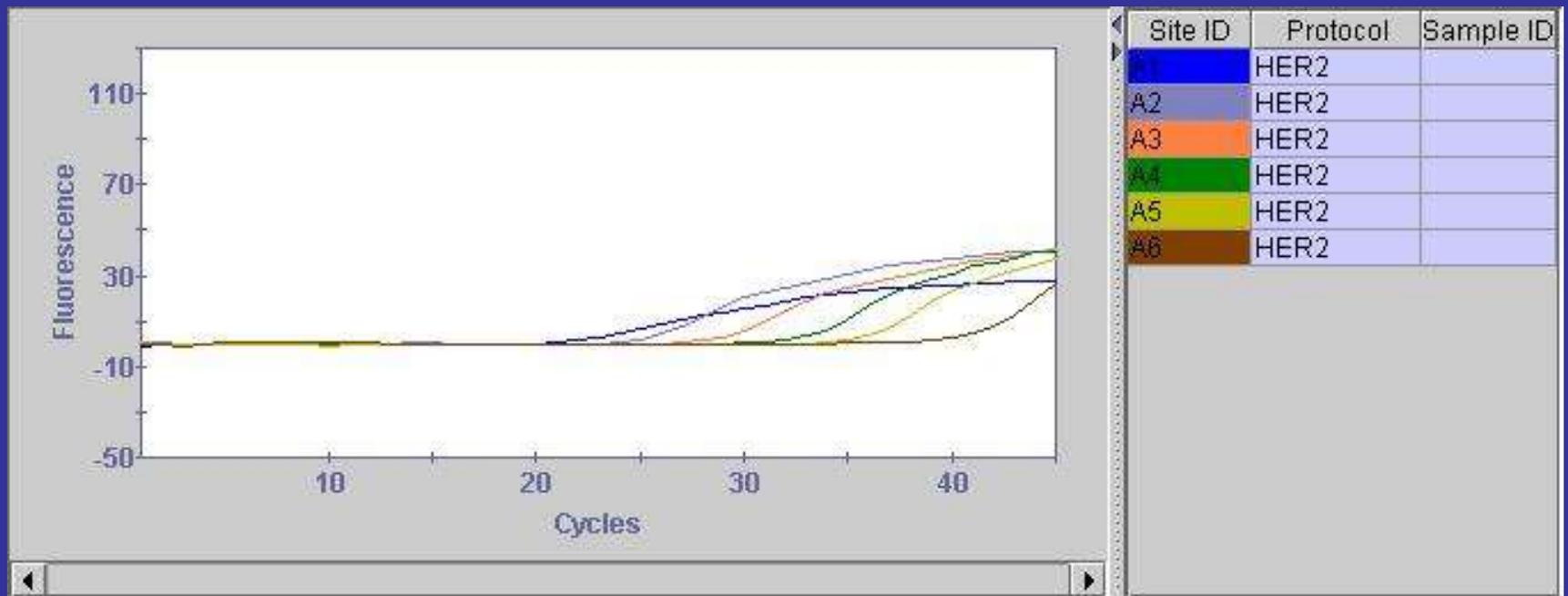


Region	Count	% Hist	% All
Total	2392	100.00	100.00
R5	3	0.13	0.13
R6	1	0.04	0.04
R7	2388	99.83	99.83
R8	0	0.00	0.00



Region	Count	% Hist	% All
Total	34116	100.00	100.00
R5	22	0.06	0.06
R6	29899	87.64	87.64
R7	3666	10.75	10.75
R8	529	1.55	1.55

ESTANDARES DE HER2 OBTENIDOS A PARTIR DE TEJIDO NORMAL DE MAMA



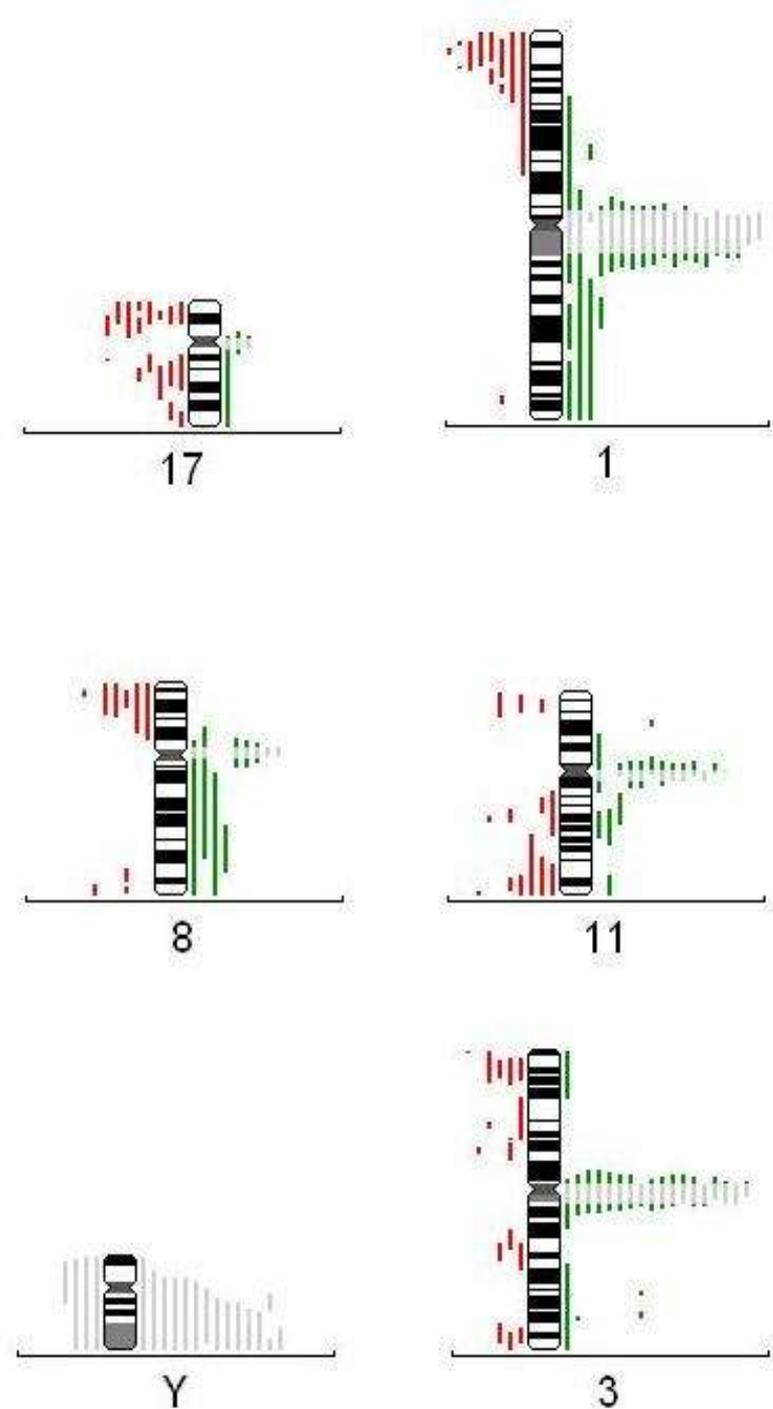
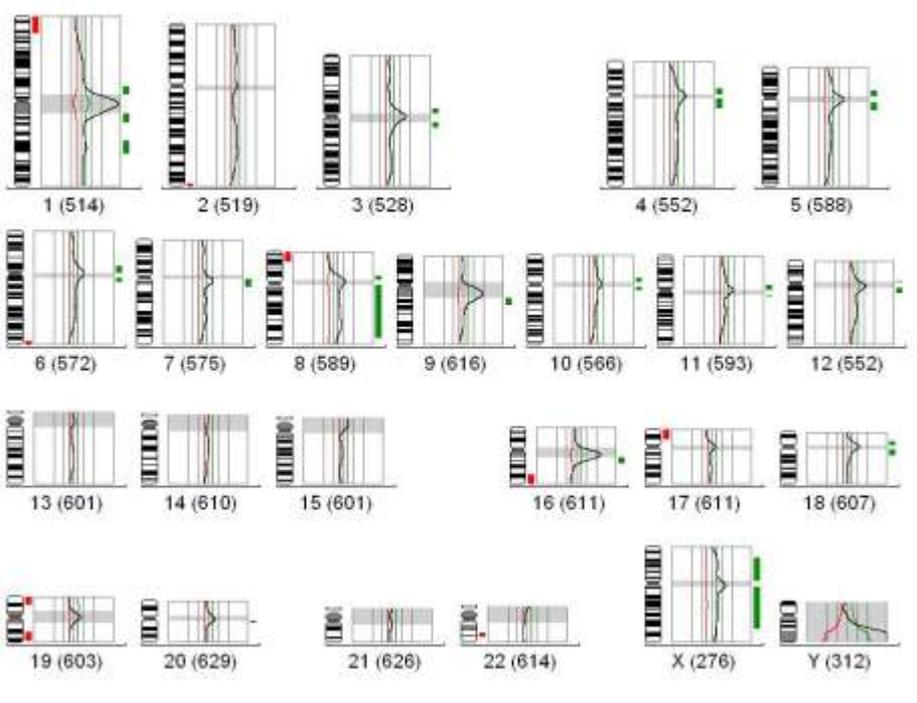
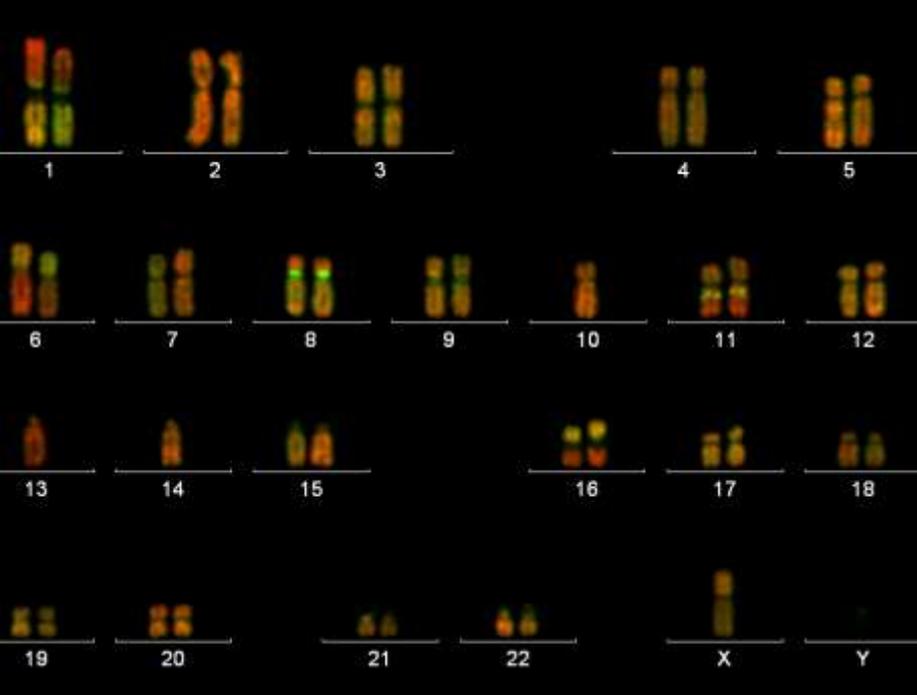
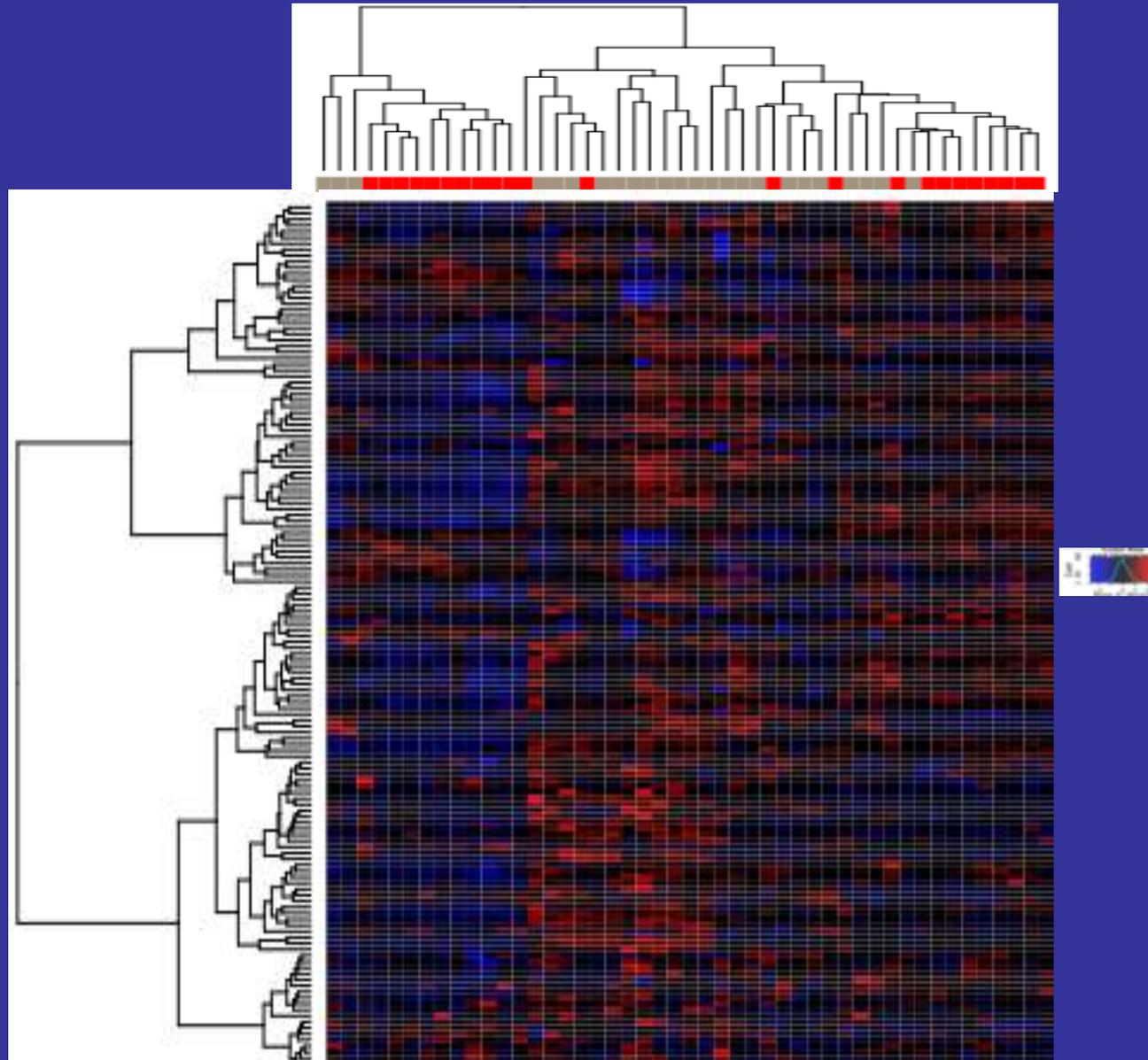


Figure 1. b) Clustering based on the expression of 143 genes with higher expression in HER2-positive tumors. Tumors with HER2 amplification appear in grey color while tumors without HER2 amplification are in red color. Blue color represents down-regulation in the gene expression and red means over-expression.

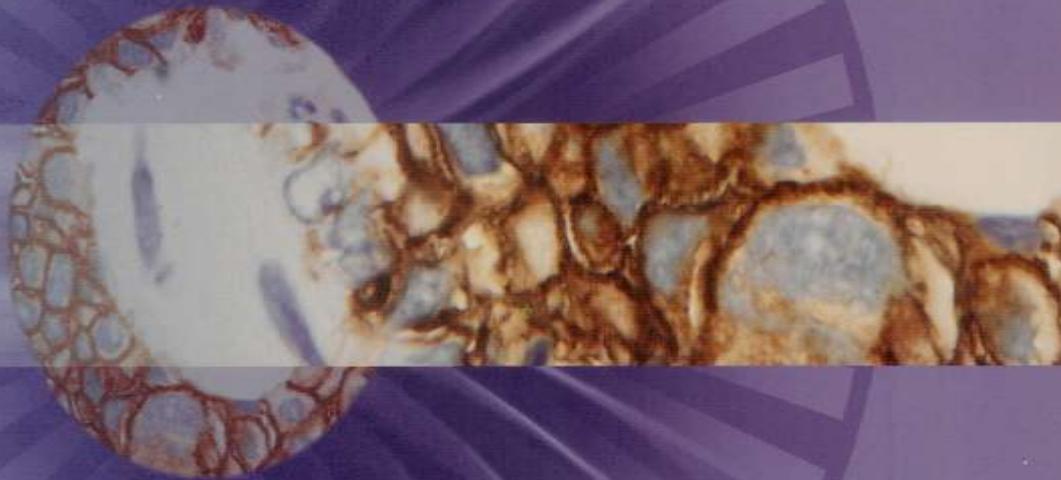


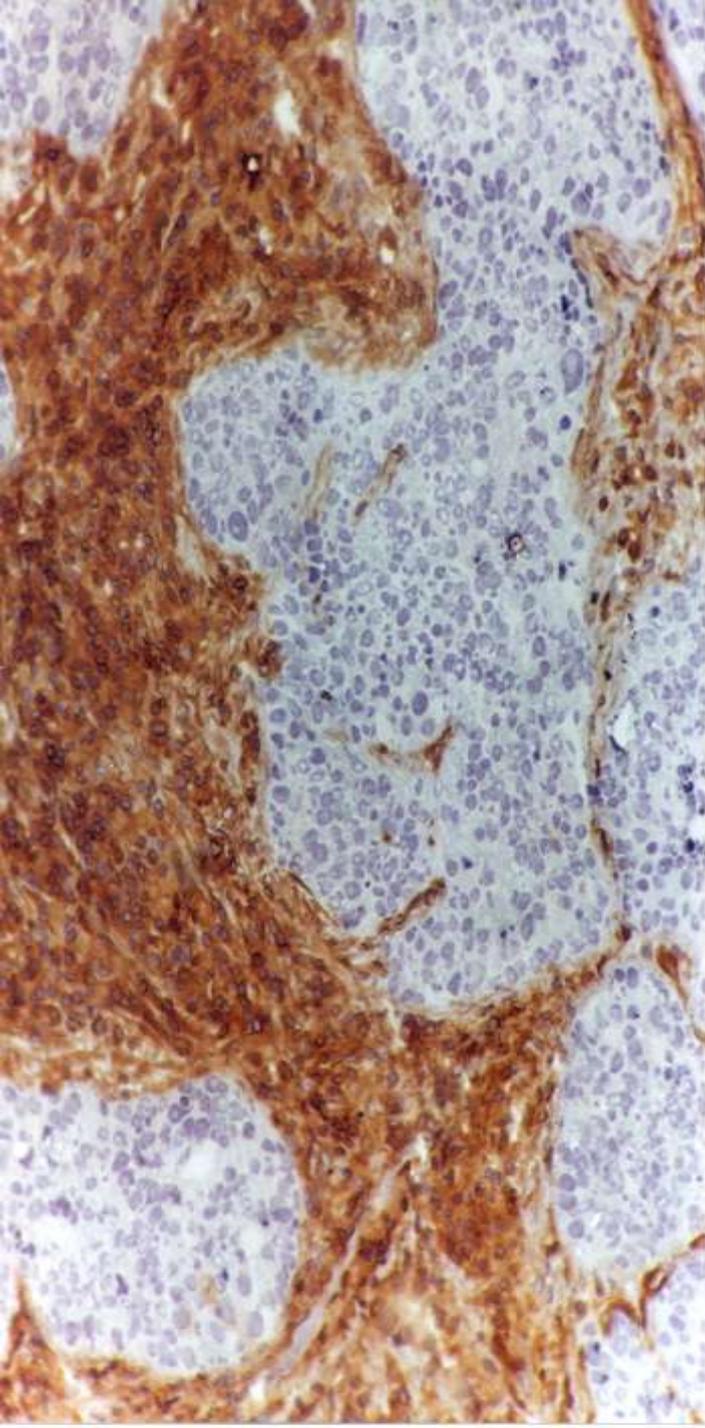
DIAGNÓSTICO POR INMUNOHISTOQUÍMICA DE LA EXPRESIÓN DE HER2 EN CÁNCER DE MAMA

AUTOR:

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Jefe de Servicio de Anatomía Patológica del Hospital Universitario Virgen de las Nieves





D3S1619

D2S123

6

1

2



L T

L T L T

D3S1619

D2S123

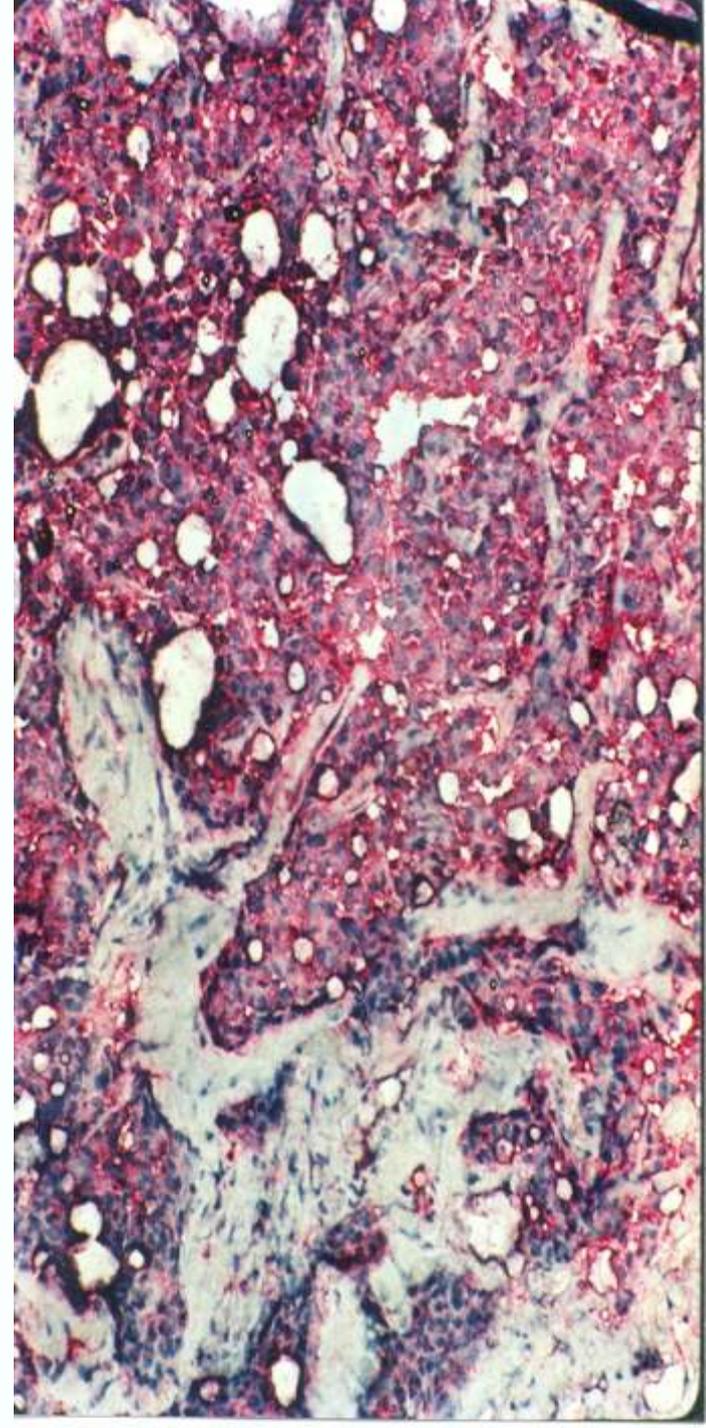
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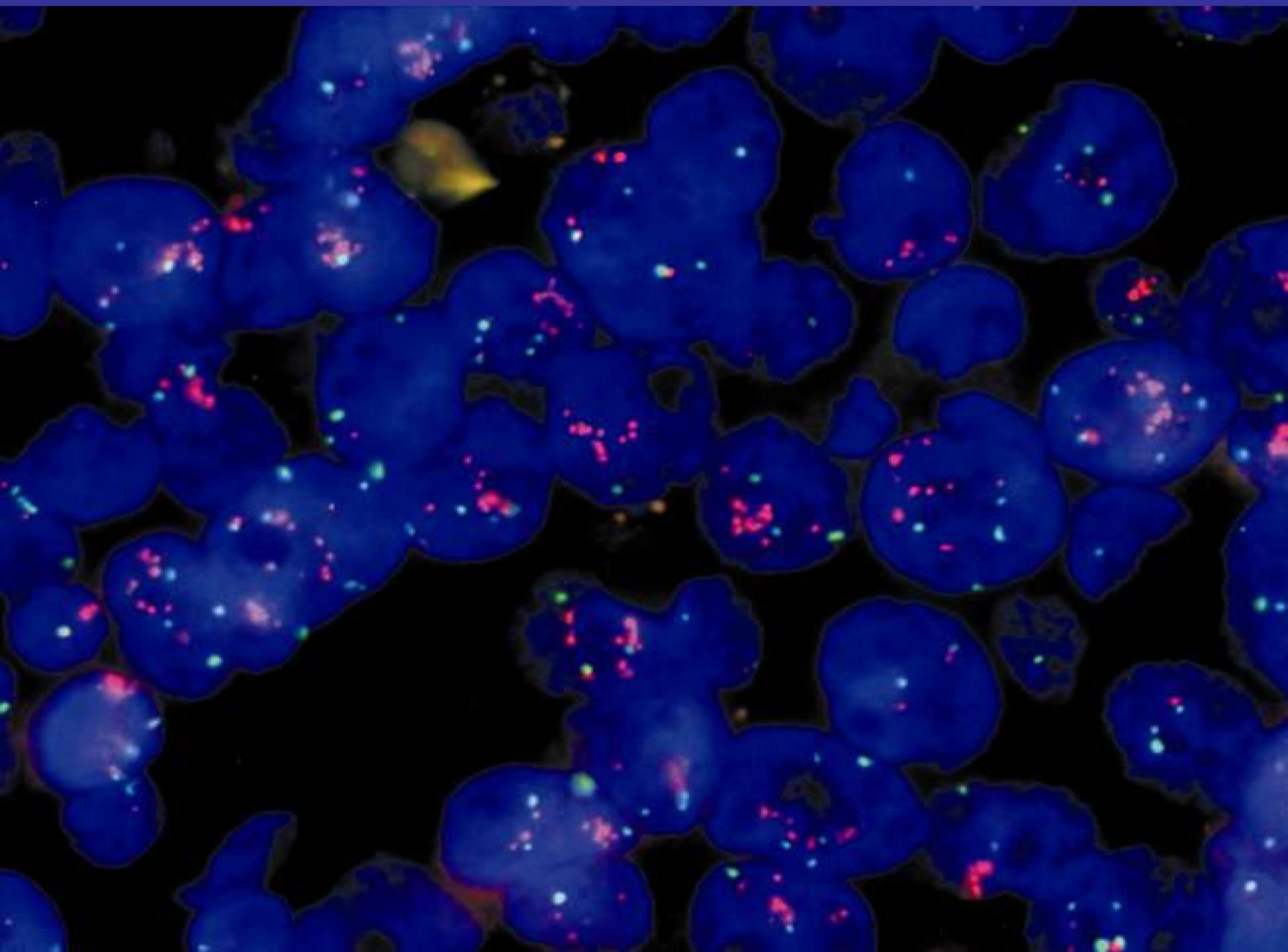
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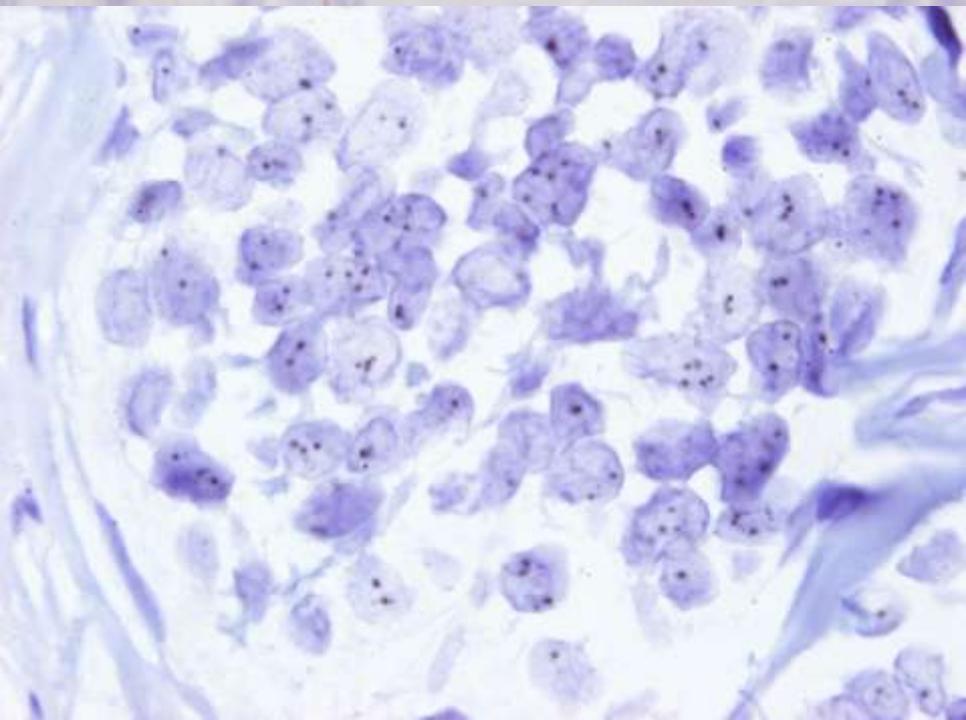
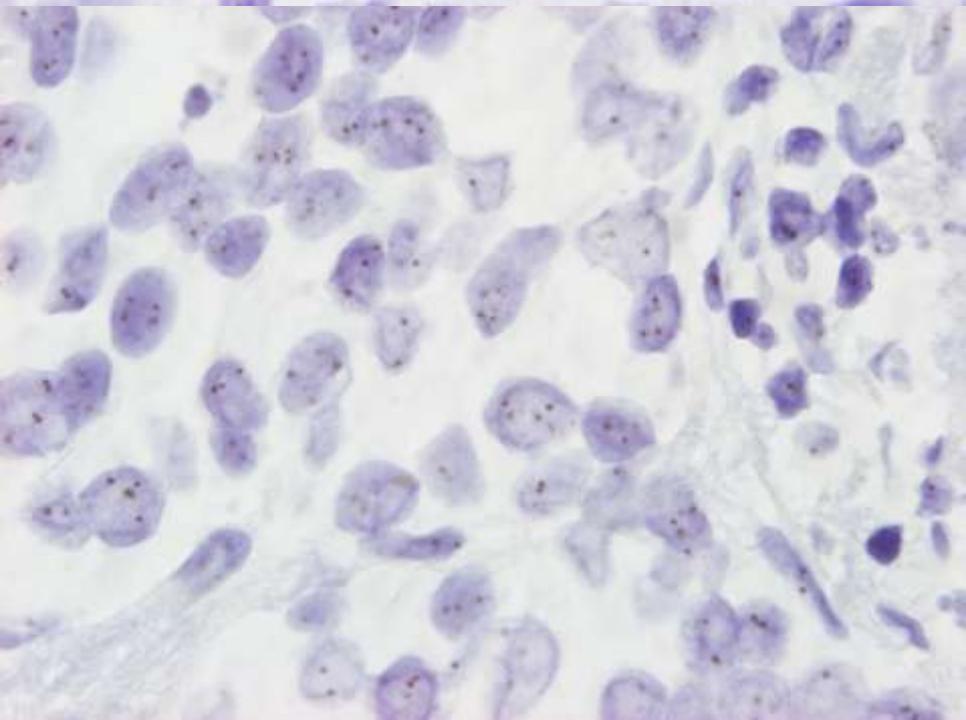
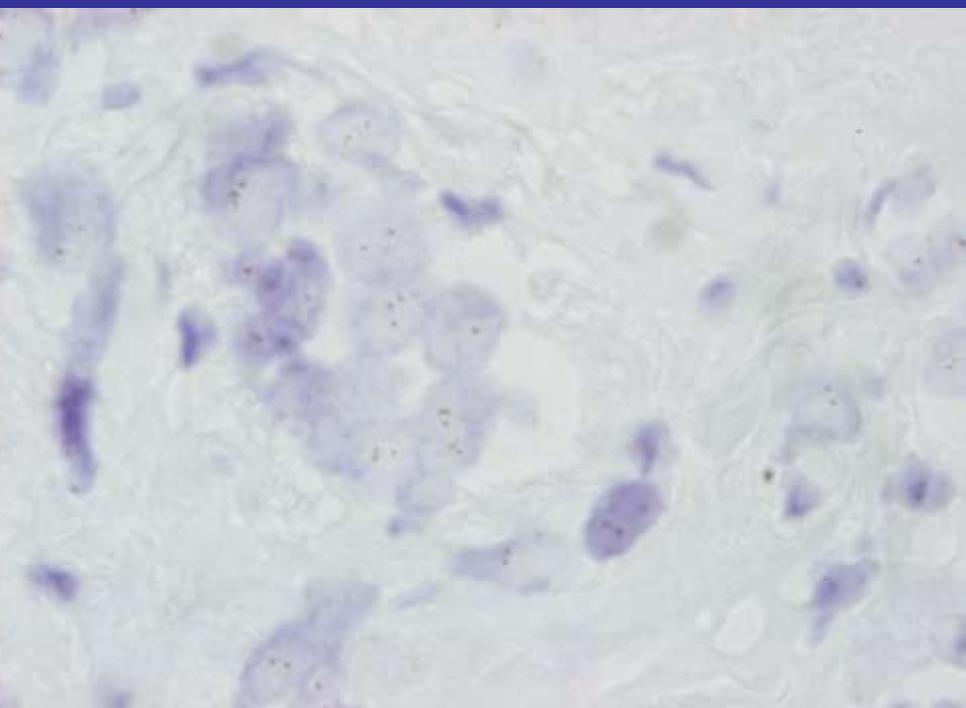
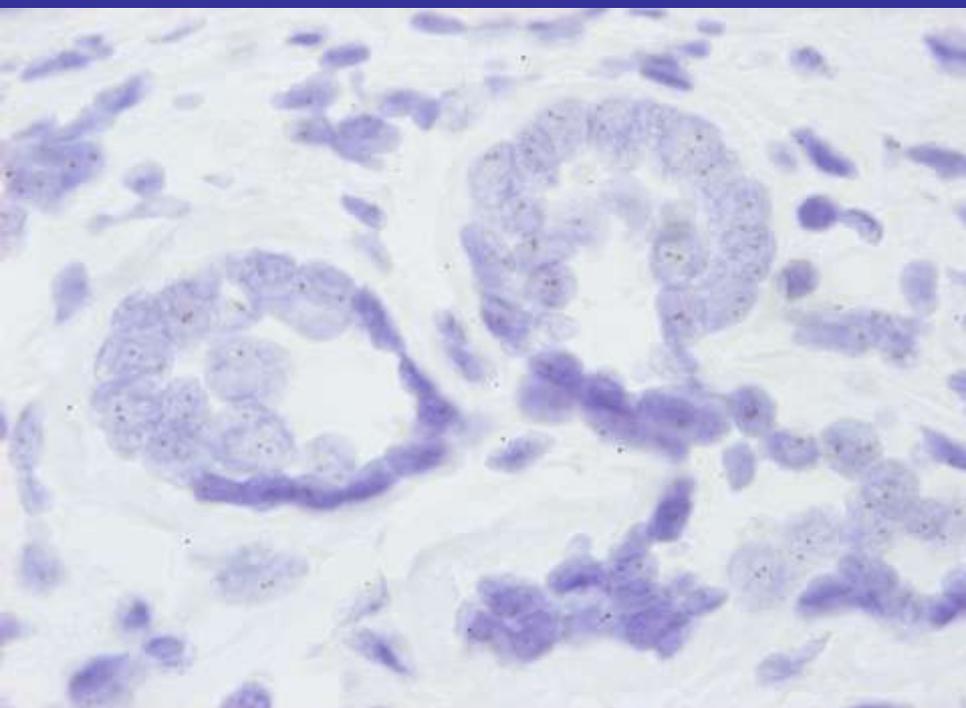


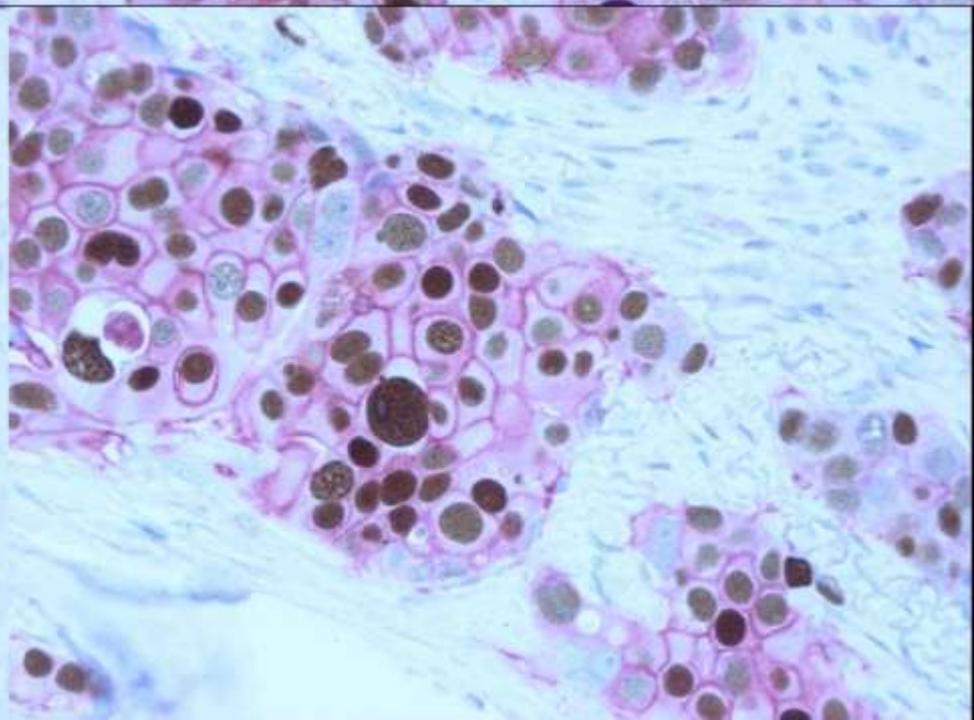
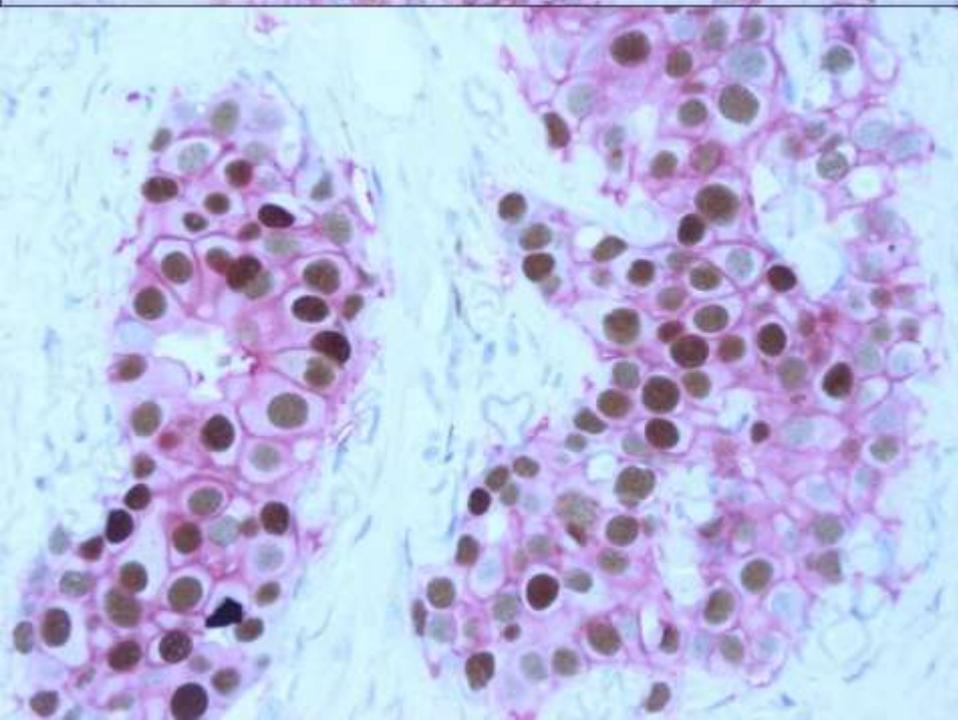
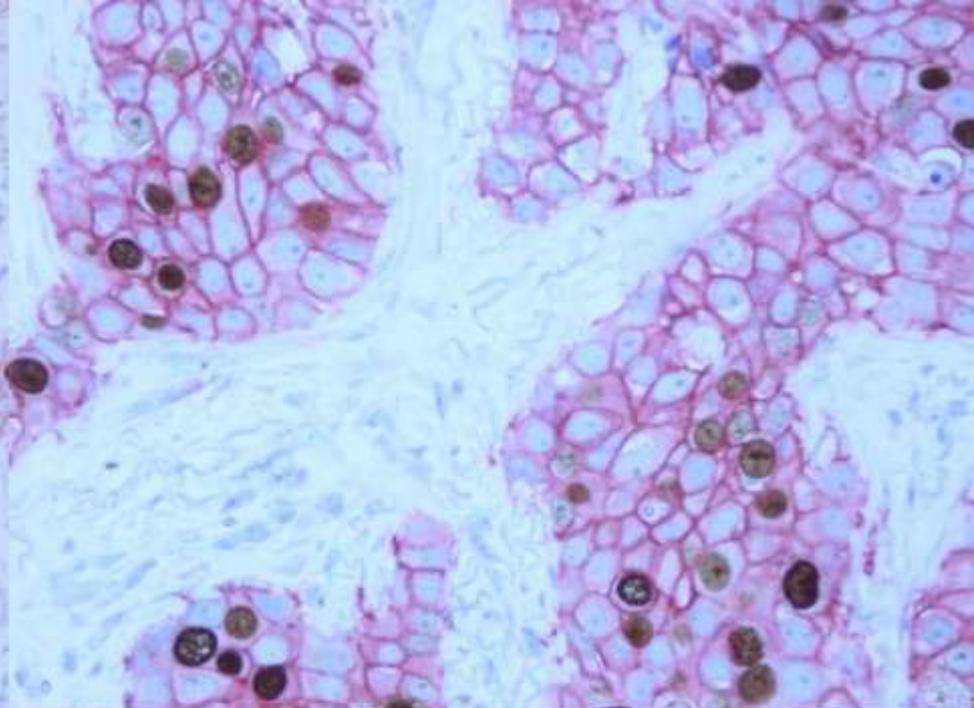
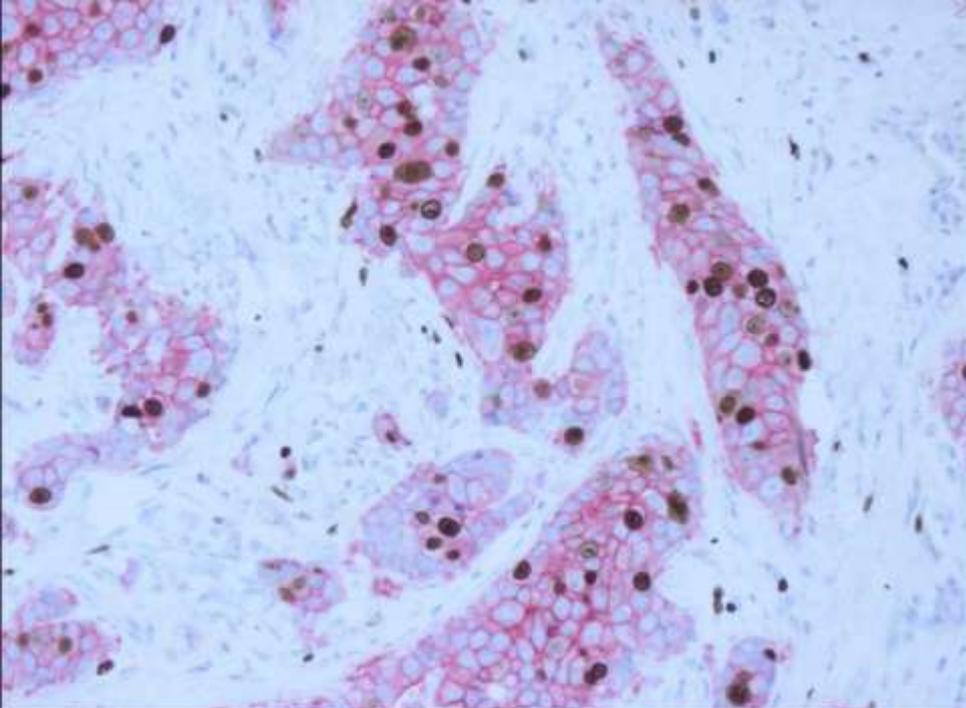
L T

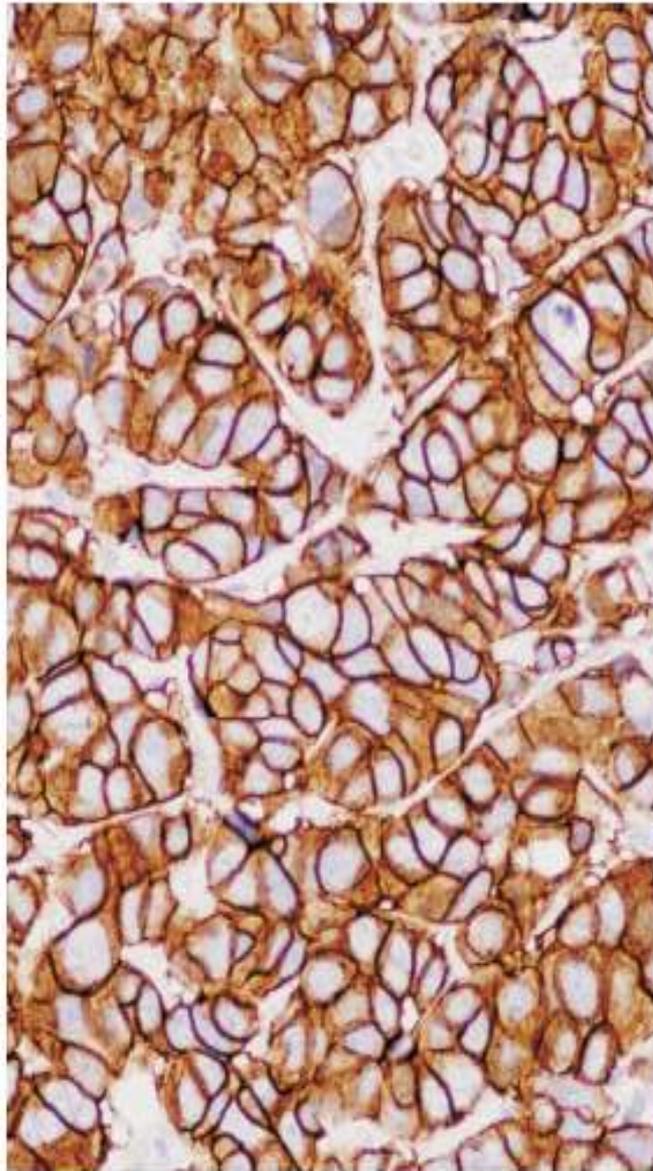
L T











ACTUALIZACION EN EL DIAGNOSTICO DE

HER-2 en Cancer de Mama Y CÁNCER DE ESTÓMAGO

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Adjunto Servicio de Anatomía Patológica,
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Jefe de Servicio de Anatomía Patológica
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12 de Marzo de 2010.

SALON CASTELAO DE LA FACULTAD DE MEDICINA
DE LA UNIVERSIDAD DE SANTIAGO DE COMPOSTELA

Status HER2/neu

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graph TD; A[Status HER2/neu] --> B[Pronóstico]; A --> C["Predictivo (Terapia)"]
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Pronóstico

**Predictivo
(Terapia)**

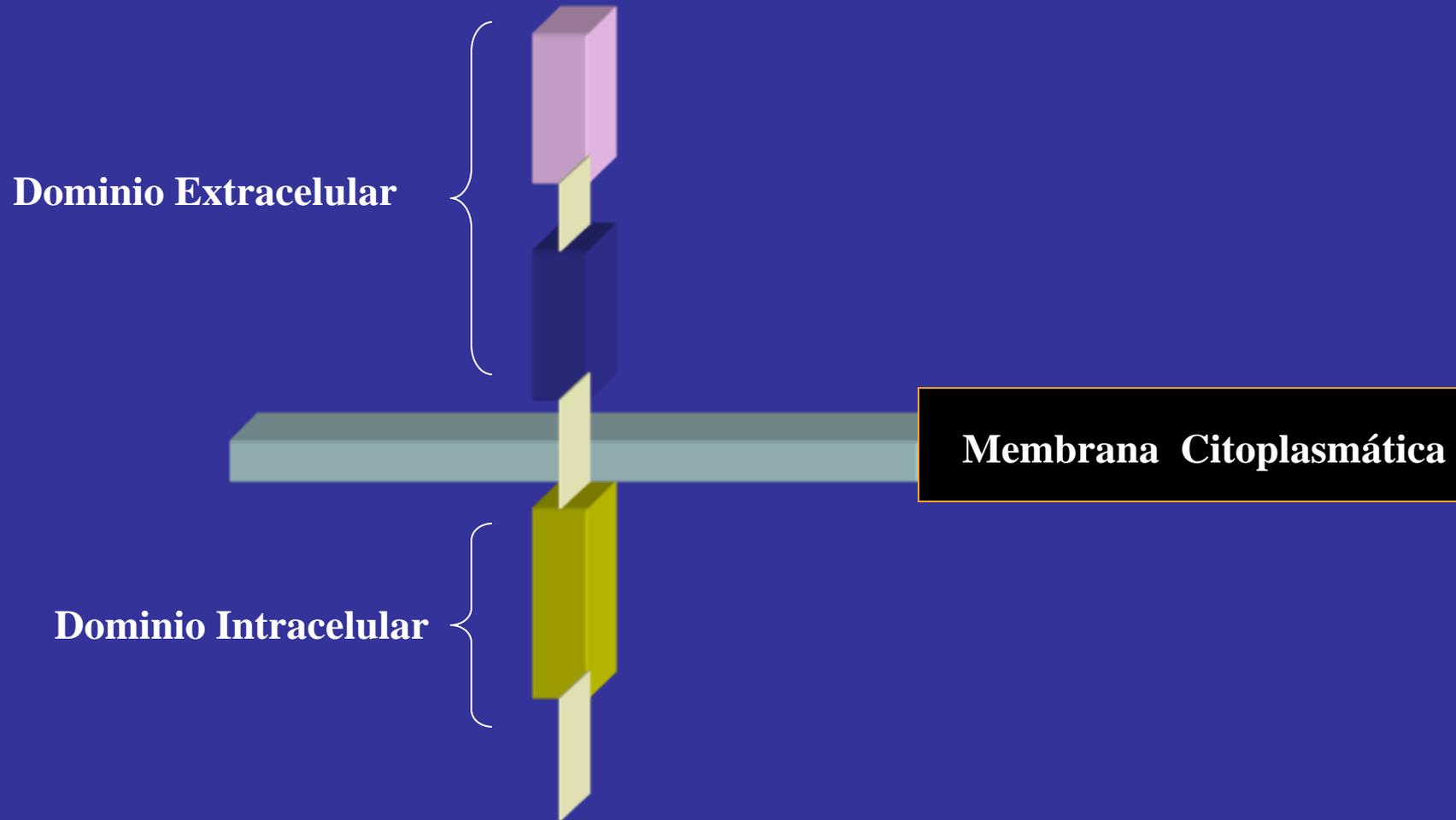
HER2, aspectos moleculares

- **Oncogen, localizado en el cromosoma 17q11.2-q12**
- **Codifica para una glicoproteína transmembrana de 185 kDa (p185^{HER2})**

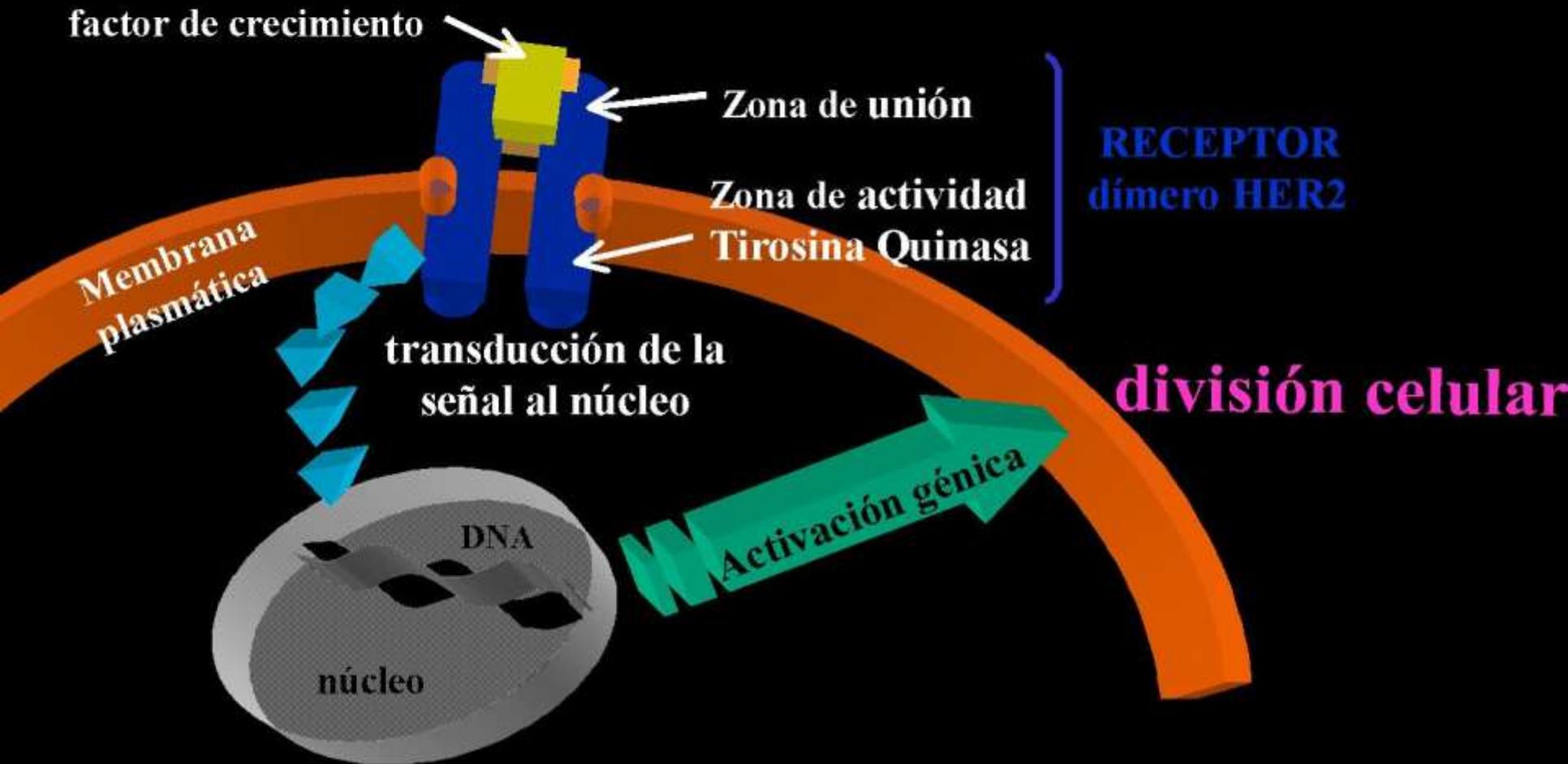
HER2, familia de receptores

- Proteína HER2 es un receptor de membrana perteneciente a la familia de los EGFR con actividad tirosín-quinasa.
 - EGFR (erbB-1, HER-1)
 - HER-2/neu (erbB-2)
 - HER-3 (erbB-3)
 - HER-4 (erbB-4)
- Formación de Heterodímeros
- Diferentes ligandos

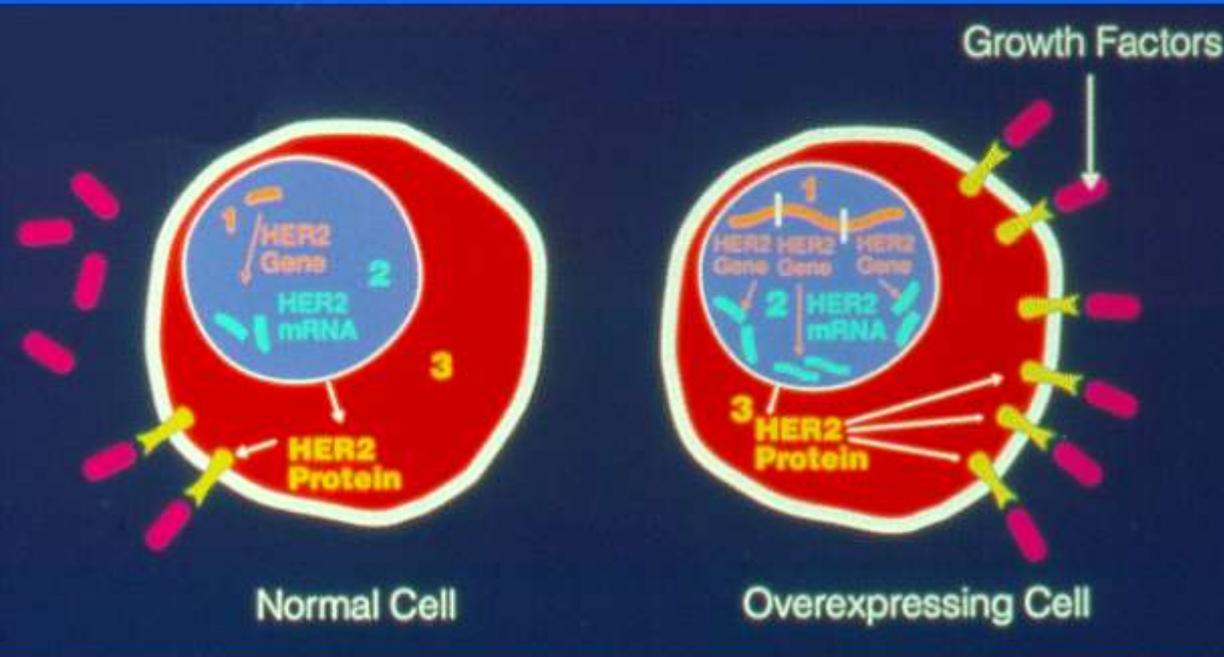
HER2, estructura de la proteína



HER2, actuación sobre el núcleo de la célula



HER2, amplificación y sobreexpresión



1.- Amplificación Génica

2.- Transcripción del mRNA

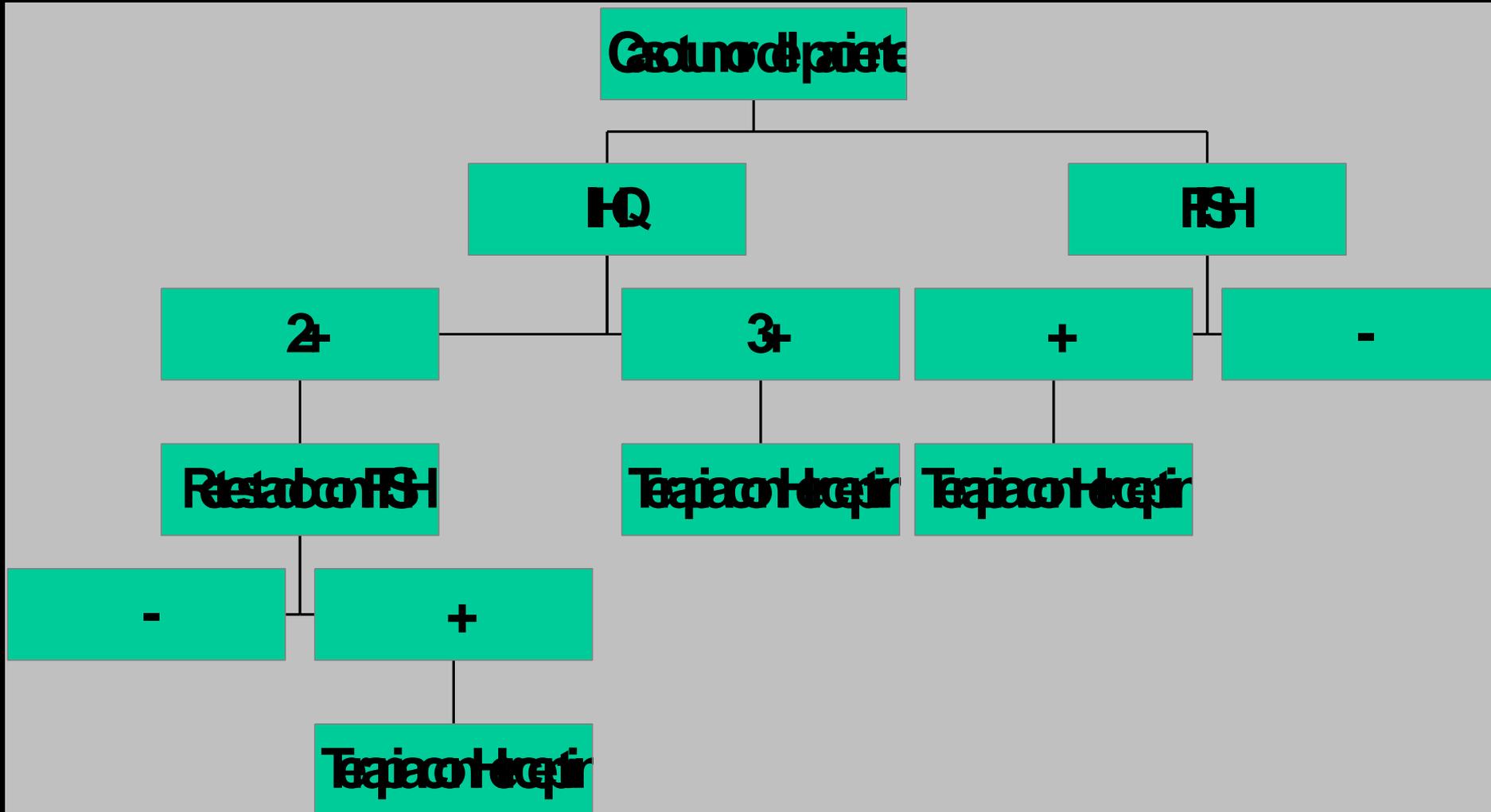
3.- Síntesis de Proteína

4.- Liberación del Dominio Extracelular

**SELECCIÓN DE PACIENTES
PARA HERCEPTIN :
¿IHQ, FISH O AMBOS?**

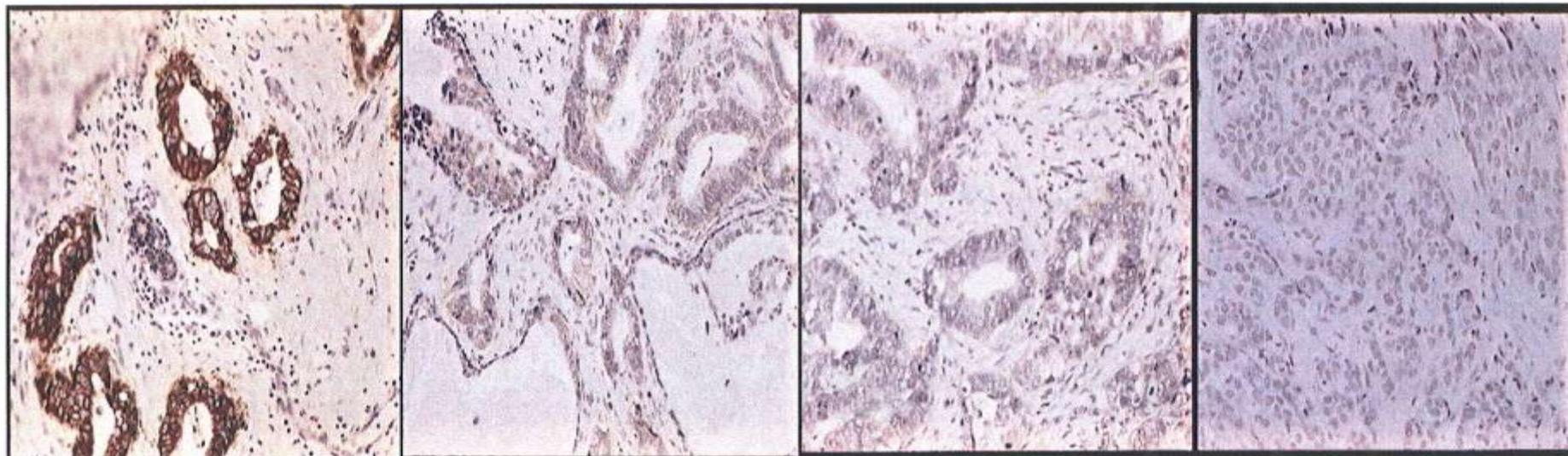


Algoritmo del estudio de HER2



Correlación entre el nº de receptores /copias del gen

<i>IHQ Score</i>	<i>Nº receptores/ célula</i>	<i>Nº copias del gen/ célula</i>
0	21,600 ± 6,700	2.4 ± 0.2
1	92,400 ± 12,000	3.0 ± 0.4
2	500,000 ± 130,000	7.8 ± 0.4
3	2,390,000 ± 1,000,000	15.3 ± 3.9

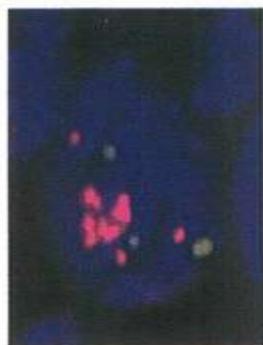


3+

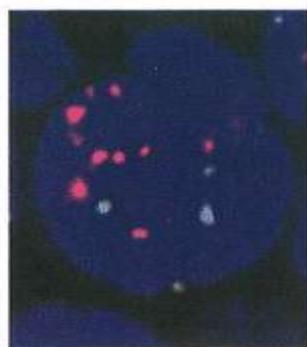
2+

1+

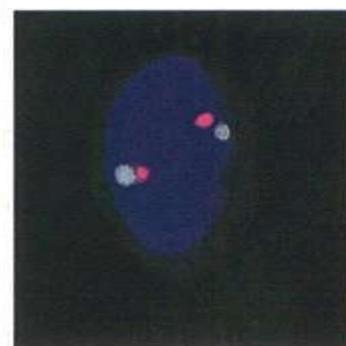
Negativo/0



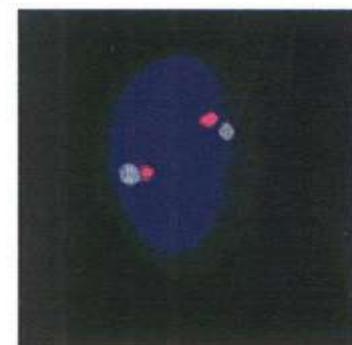
**Alta
amplificación**



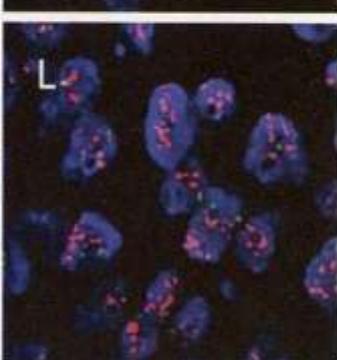
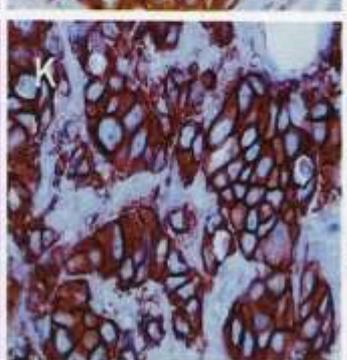
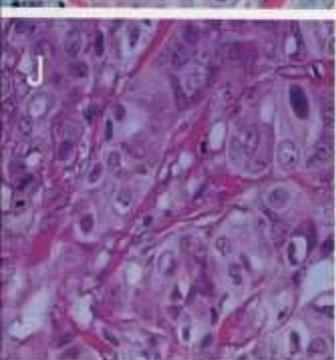
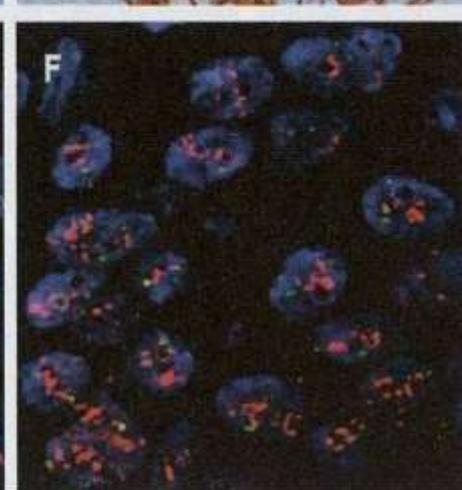
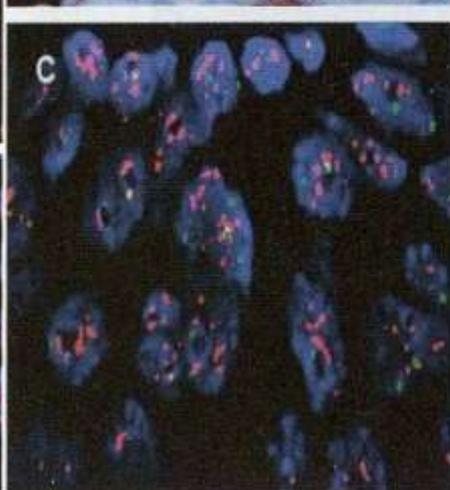
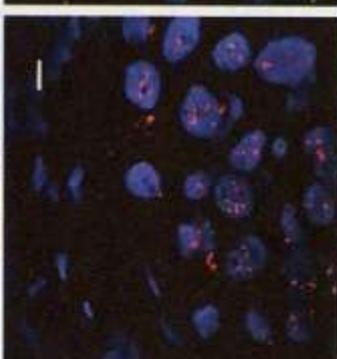
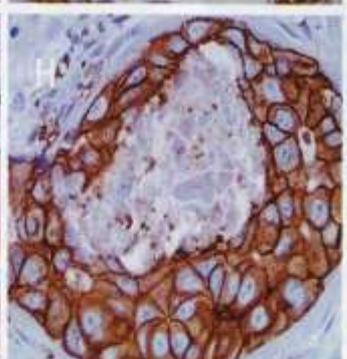
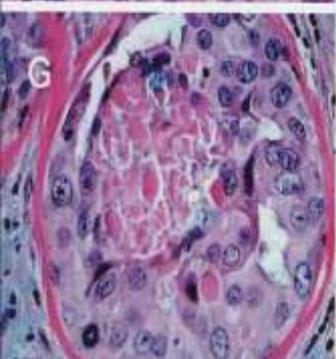
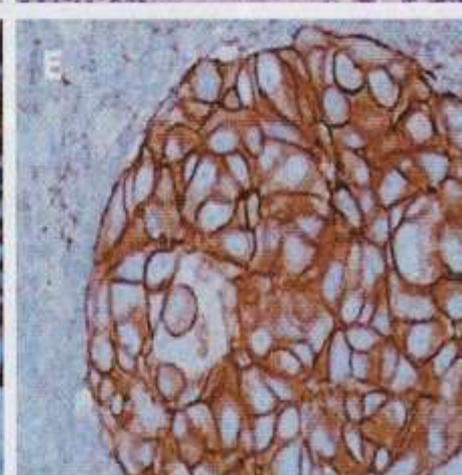
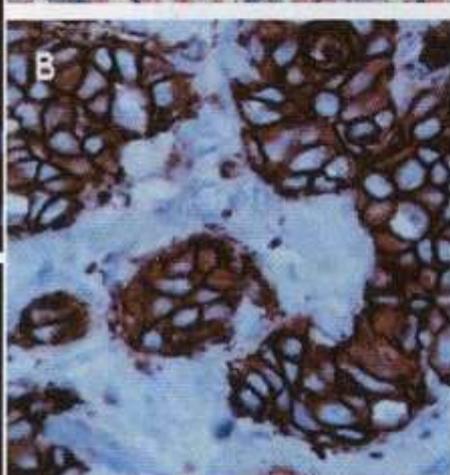
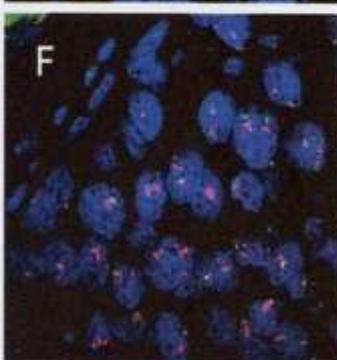
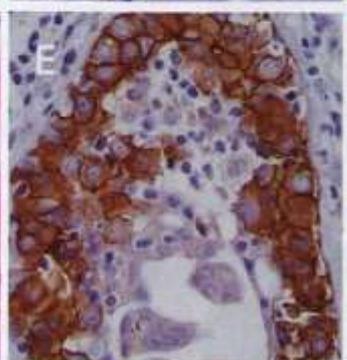
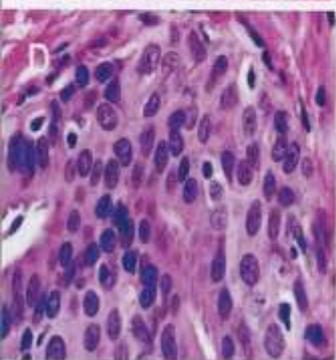
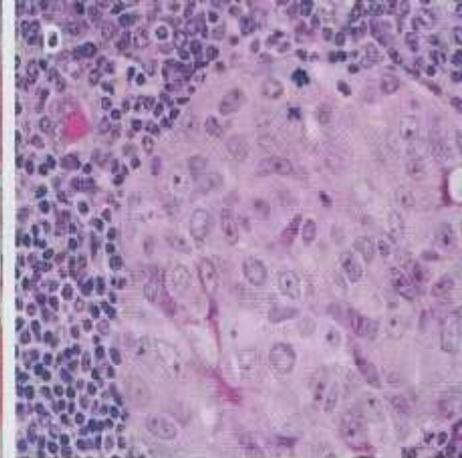
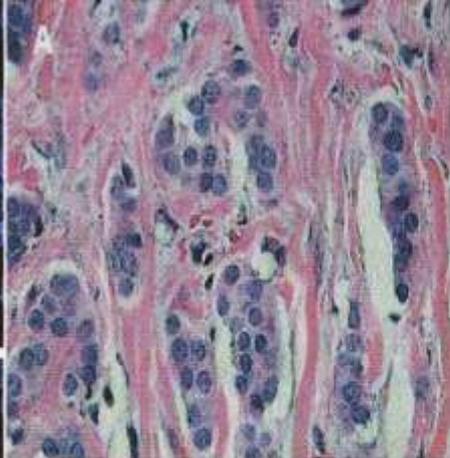
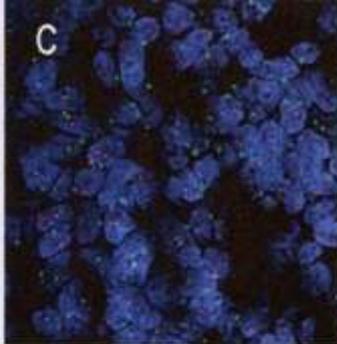
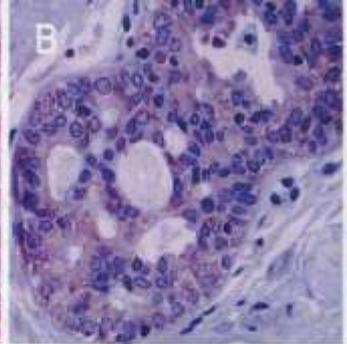
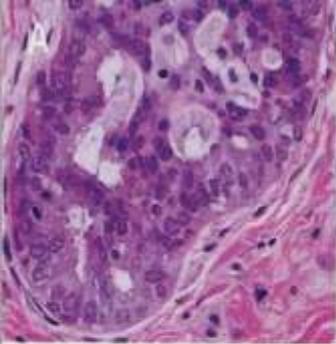
**Baja
amplificación**



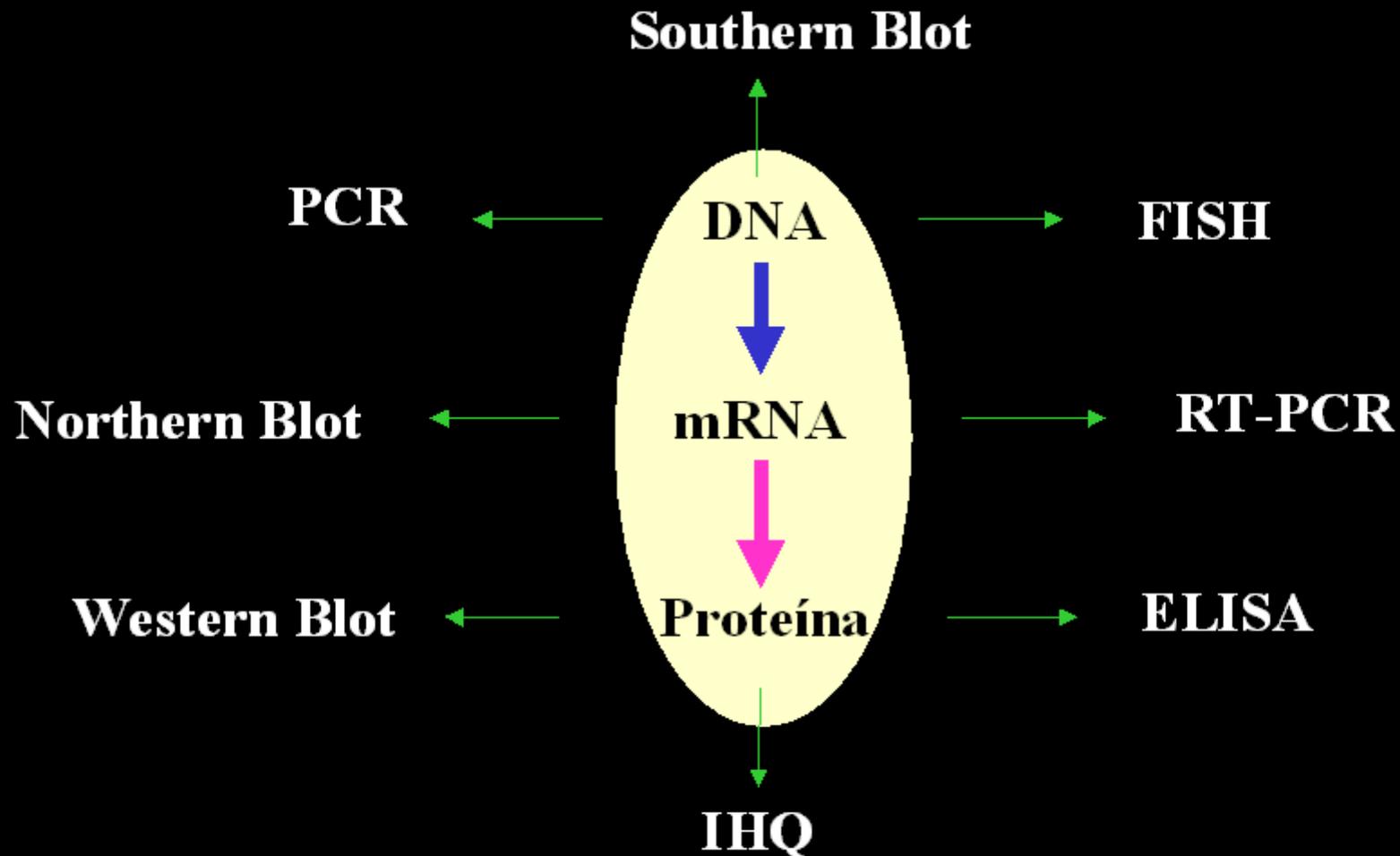
Normal



Normal



HER2, detección *in vitro*: distintas técnicas



HER2, detección *in vitro*: características

	Obtención muestra	Complejidad técnica	Complejidad Inter. Result.	Estandarización del método
Southern blot	++	+++	+++	+
PCR	++	+++	++	+
FISH	+	++	++	++
Northern blot	+++	+++	+++	+
RT-PCR	+++	+++	++	+
Western blot	+++	+++	++	+
IHQ	+	+	+	+++
ELISA	+	+	++	+

IHQ

- Método más común para el testado de HER2
- **-bien establecido, relativamente simple de usar**
- **-ampliamente disponible**
- Metodología y puntuación/interpretación
- **-diferentes anticuerpos (TAB250, CB11, A0485, 4D5), sistemas de puntuación (% positividad, intensidad de tinción, puntuación) y fijadores**
- **-necesidad para optimizar los reactivos y controles apropiados; interpretación subjetiva; incertidumbre sobre la puntuación 2+**

Hercep Test-pros y contras

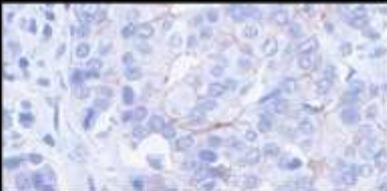
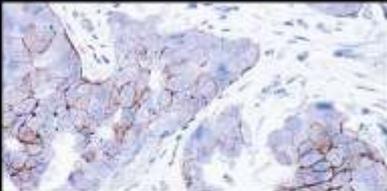
- PROS

- Estandarizado
- Controles positivos
- Sistema de puntuación
- Test sensible

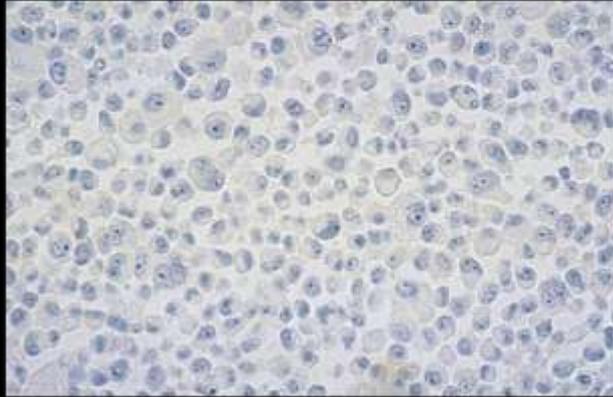
- CONTRAS

- Costoso
- Problemas con los fijadores alcohólicos
- Número limitado de controles
- Falsos positivos

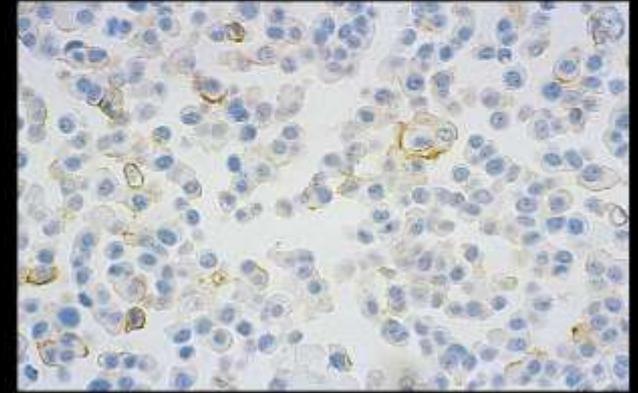
HerceptestTM: guía para la interpretación

<u>Patrón de tinción</u>	<u>Score</u>	<u>Valoración</u>	<u>Ejemplo</u>
No se observa tinción, o sólo se observa tinción de membrana en menos del 10% de células tumorales	0	Negativo	
Se observa tinción de membrana incompleta en más del 10% de células tumorales	1+	Negativo	
Se observa tinción completa de membrana y de intensidad débil o moderada, en más del 10% de células tumorales	2+	Positivo débil	
Se observa tinción completa de membrana y de intensidad fuerte, en más del 10% de células tumorales	3+	Positivo fuerte	

Herceptest™: guía para la interpretación



MDA-231
21.600 Rec./cél.
Score 0

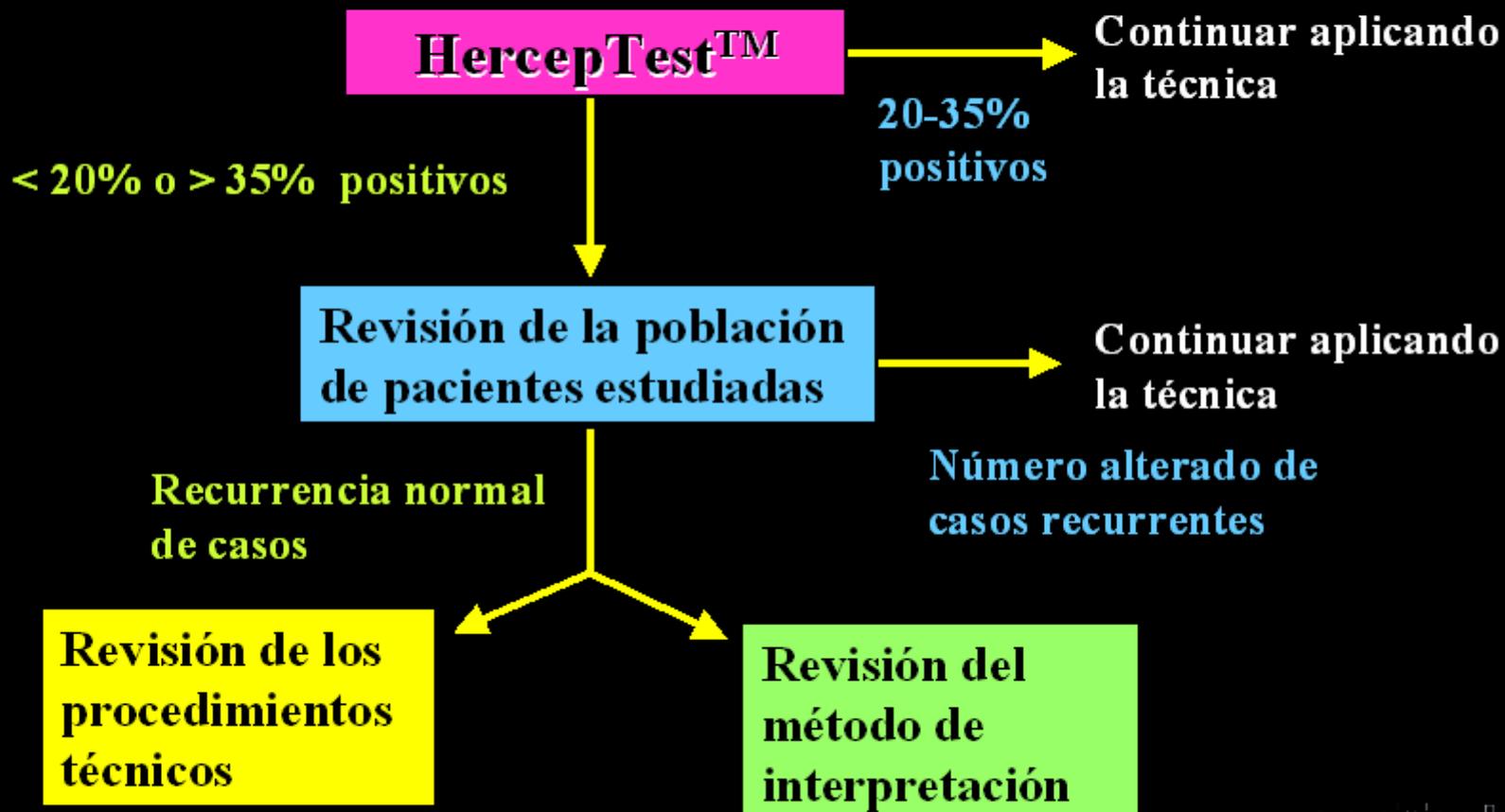


MDA-175
92.400 Rec./cél.
Score 1+



SK-BR-3
>2.000.000 Rec/cél.
Score 3+

Herceptest™: procedimiento de actuación recomendado



INMUNORREACTIVIDAD DE HER2/neu EN 142 CARCINOMAS DE MAMA INVASIVOS: ESTUDIO COMPARATIVO USANDO CUATRO ANTICUERPOS

Intensidad de tinción	DAKO policlonal		PAB-1 policlonal		TAB250 monoclonal		CB11 monoclonal	
	#	%	#	%	#	%	#	%
0/1+	76	54	60	42	110	77	99	70
2+	25	18	33	23	10	7	12	8
3+	41	29	49	35	22	15	31	22
2/3+	66	46	82	58	32	23	43	30
TOTAL	142		142		142		142	

FISH

- **100% específico**
- **96.5% sensible**
- **Baja variación interlaboratorio**
- **Equipamiento costoso y especializado**
- **Disponibilidad limitada**
- **Algunos problemas con fijadores**

Estudio de la concordancia FISH/IHQ de cortes de tejido

		IHQ			
FISH		207	28	67	21
		7	2	21	176
		3%	7%	24%	9%

COMPARACIÓN DE DIFERENTES ANTICUERPOS EMPLEADOS EN IHQ PARA ESTUDIO DE HER2/neu EN CÁNCER METASTÁTICO DE MAMA.

N=42	DAKO		PAB1		TAB250		CB11	
FISH	-	+	-	+	-	+	-	+
<2.0	15	4	13	6	19	0	19	0
>/=2.0	2	21	1	22	5	18	2	21
Concordancia	36/42 (86%)		35/42(83%)		37/42(88%)		40/42(95%)	

Comparación entre CISH y FISH en la detección de la amplificación de HER2/neu en 157 cánceres de mama

	CISH	
FISH	No amplificación (%)	Amplificación (%)
No amplificación	120 (76.4)	0 (0)
Amplificación	10 (6.4)	27 (17.2)

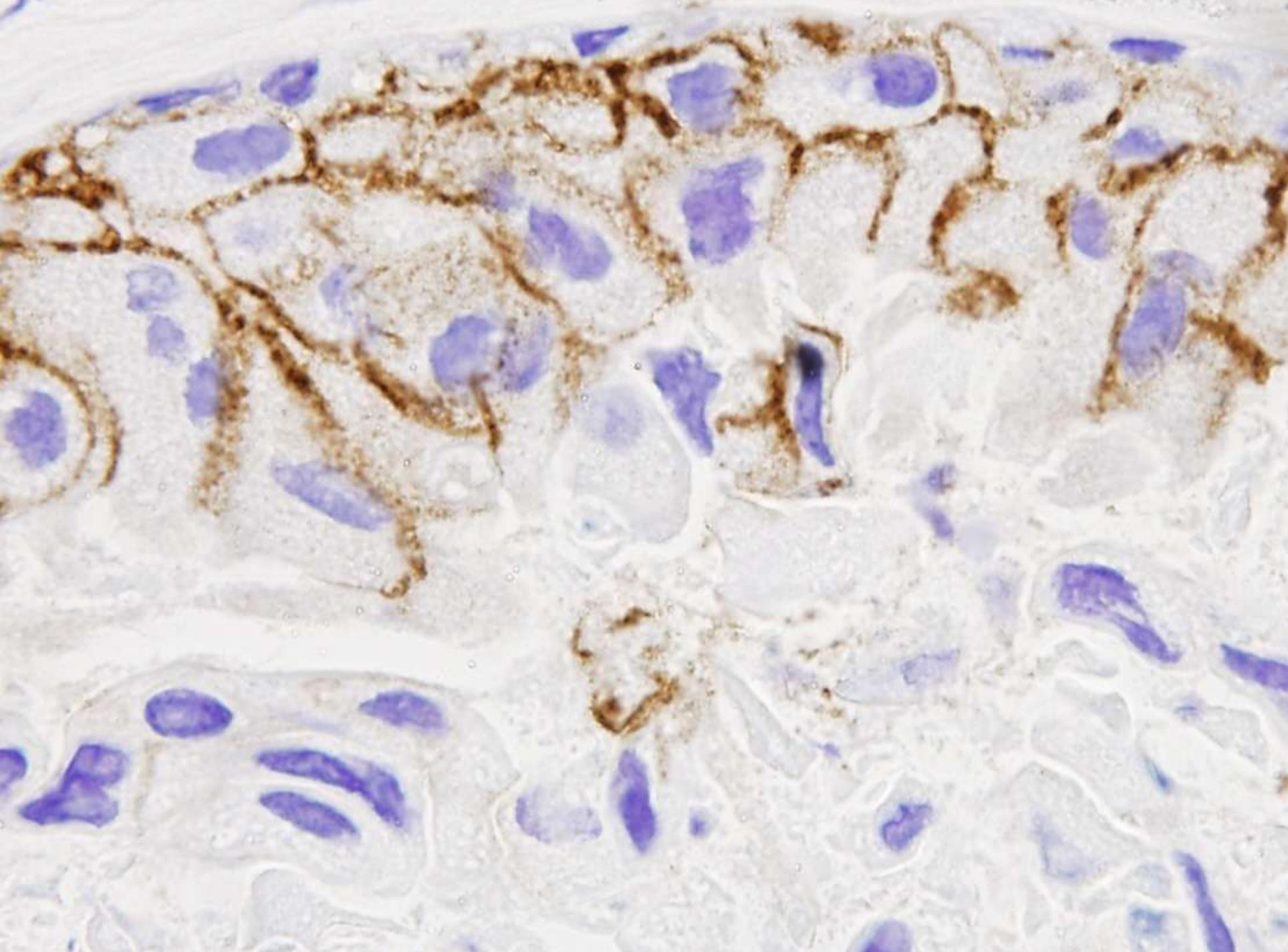
DISCORDANCIA ENTRE DIFERENTES TÉCNICAS PARA DETERMINAR EL STATUS DE HER2/neu EN CARCINOMA DE MAMA

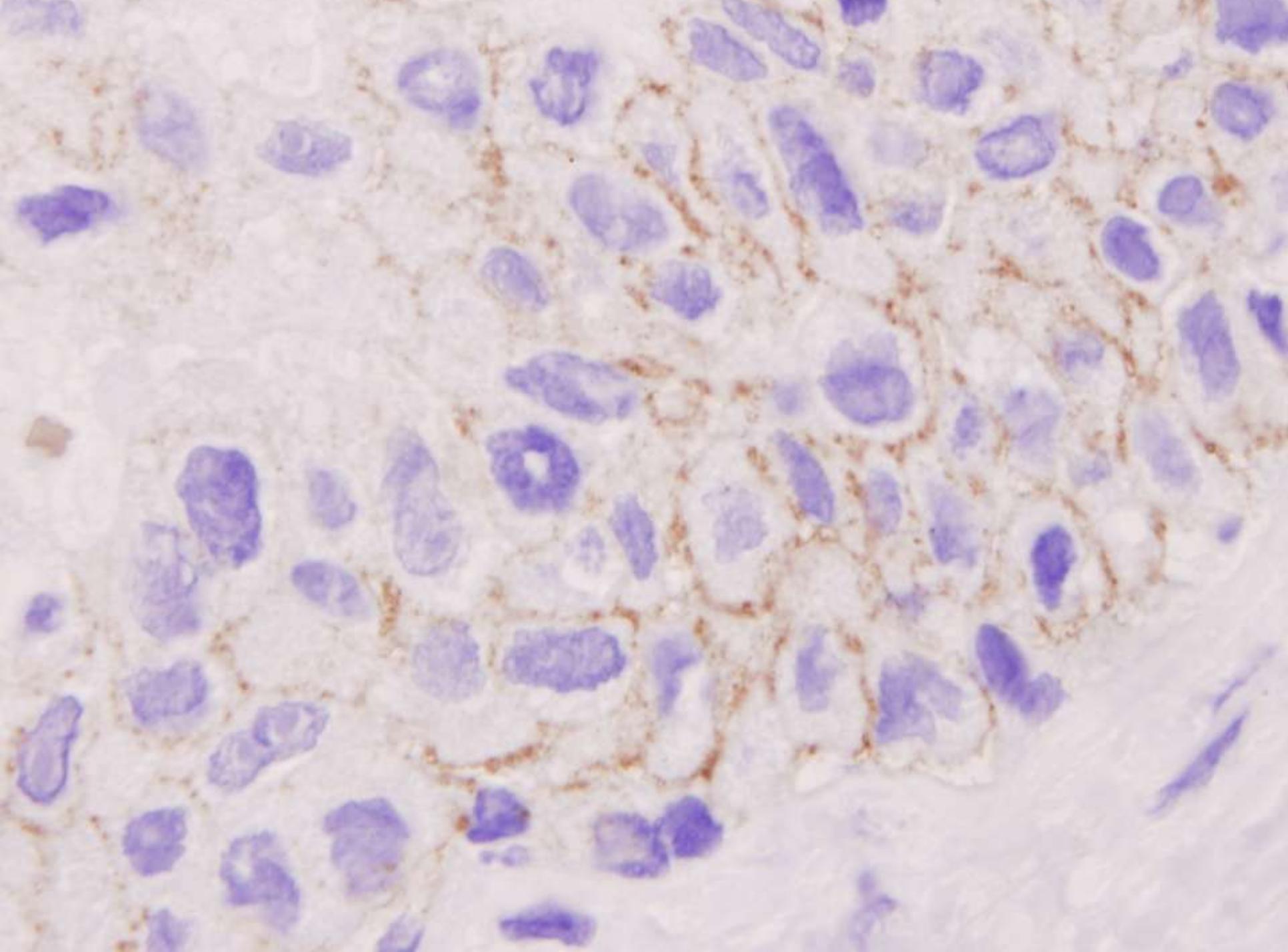
CONCORDANCIA		DISCORDANCIA	
95%		5%	
14%	81%	3%	2%
IHQ+/PCR+	IHQ-/PCR-	PCR-/FISH+	IHQ-/FISH+

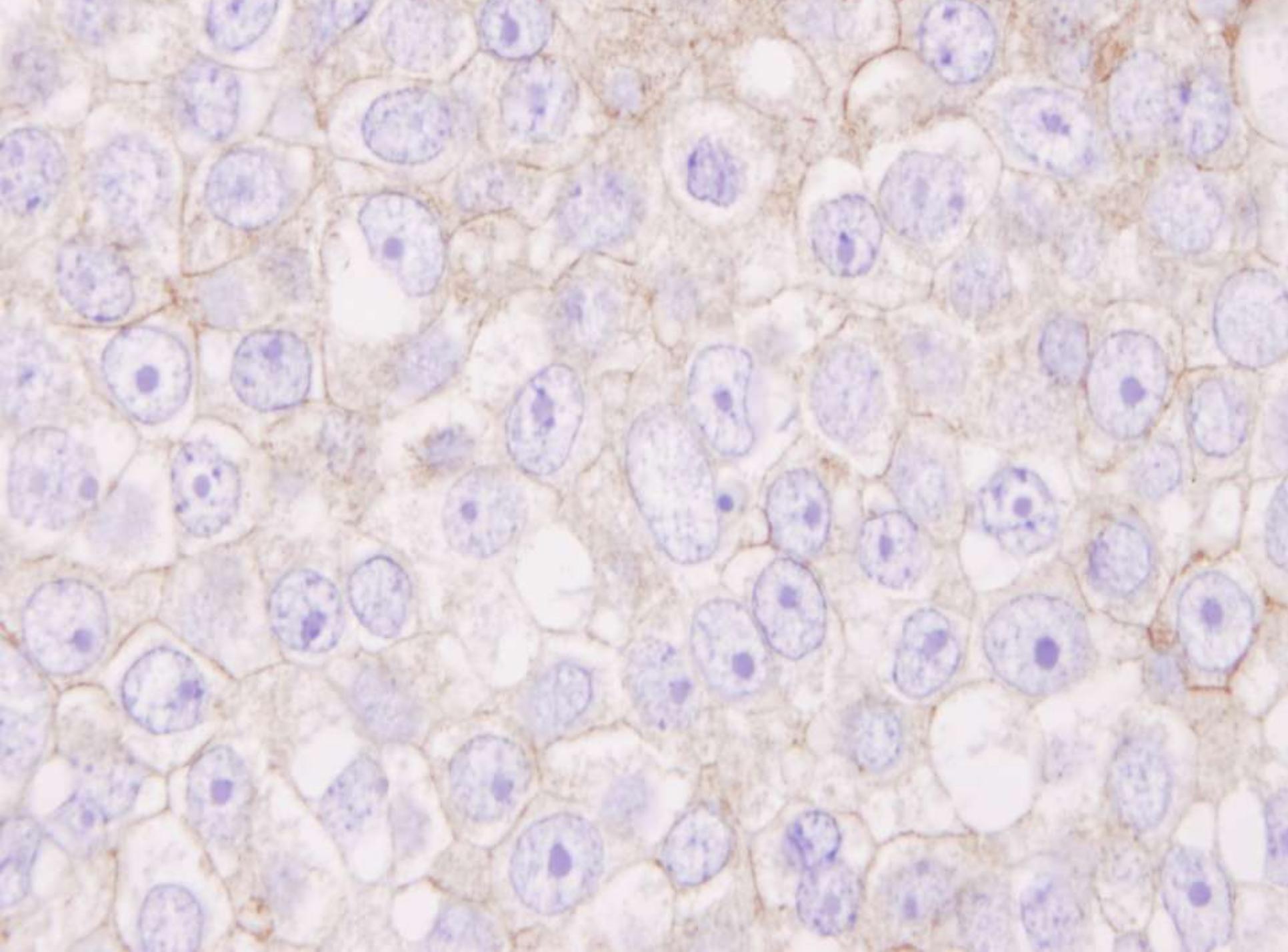
DISCORDANCIA EN LA IHQ DE HER2/neu EN CARCINOMA DE MAMA PRIMARIO (CP) Y METASTÁTICO (CM)

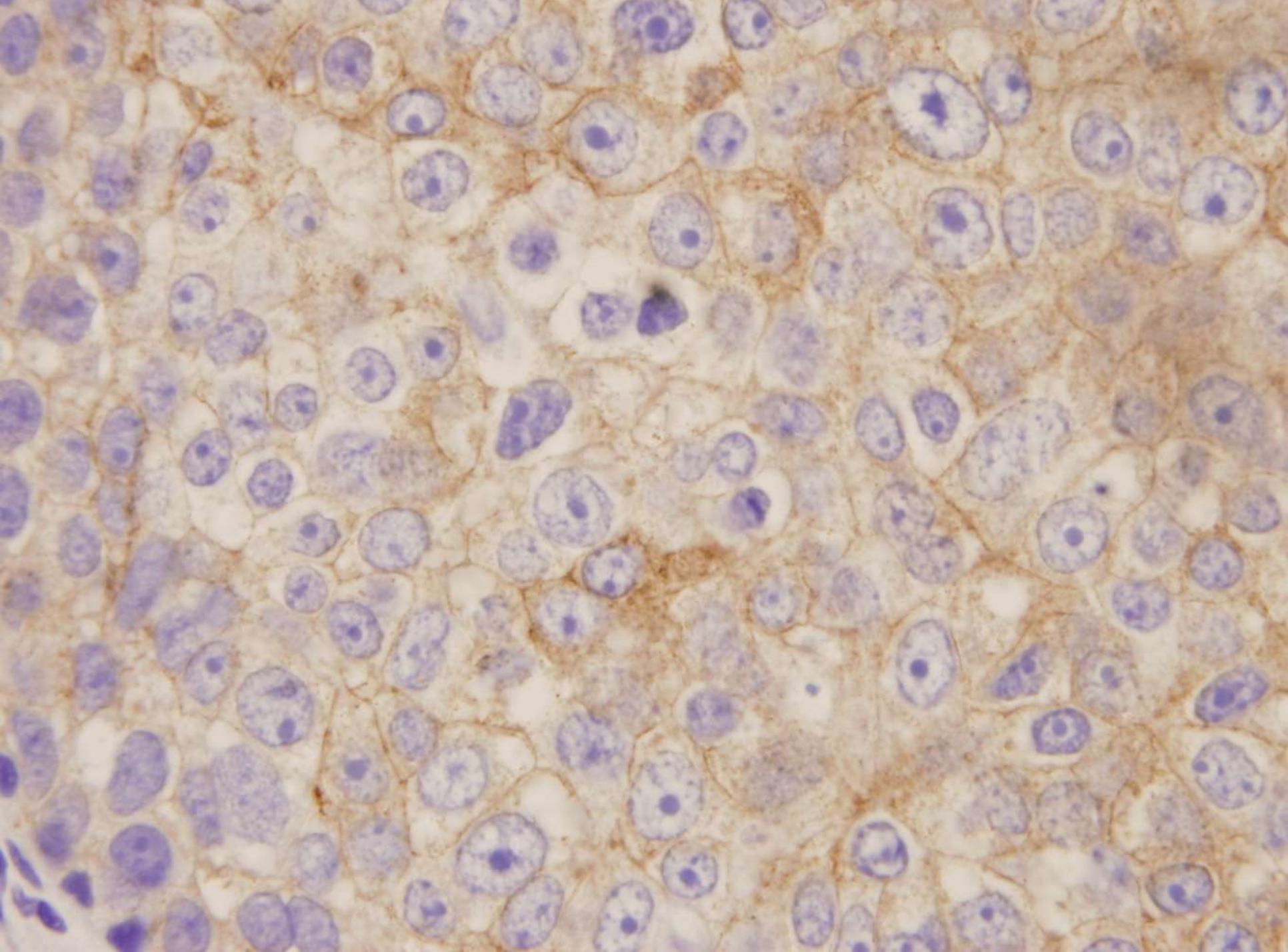
CONCORDANTES	23/36	9/23	CP-/CM-
		14/23	CP+/CM+
DISCORDANTES	13/36	9/13	CP-/CM+
		4/13	CP+/CM-

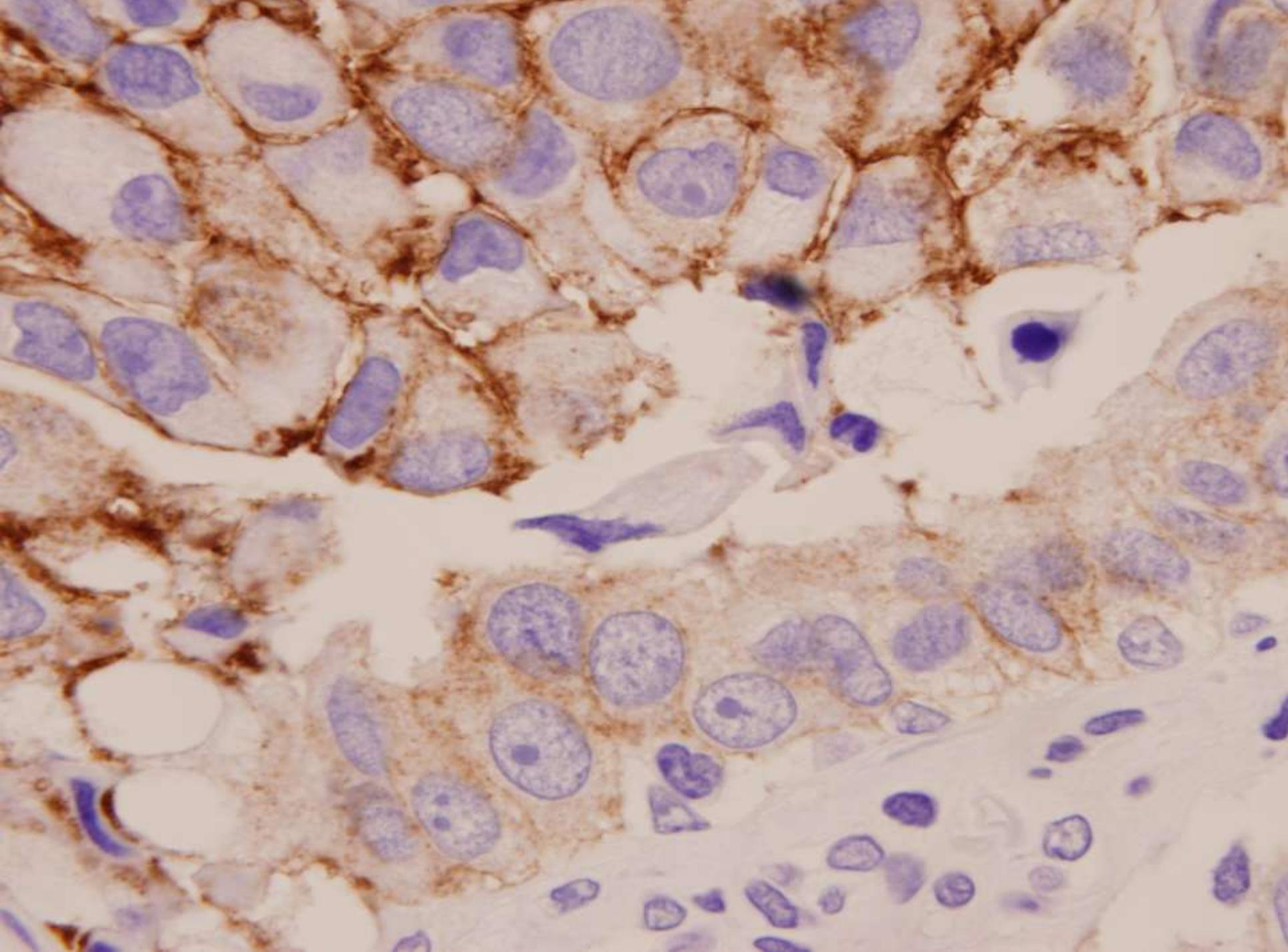


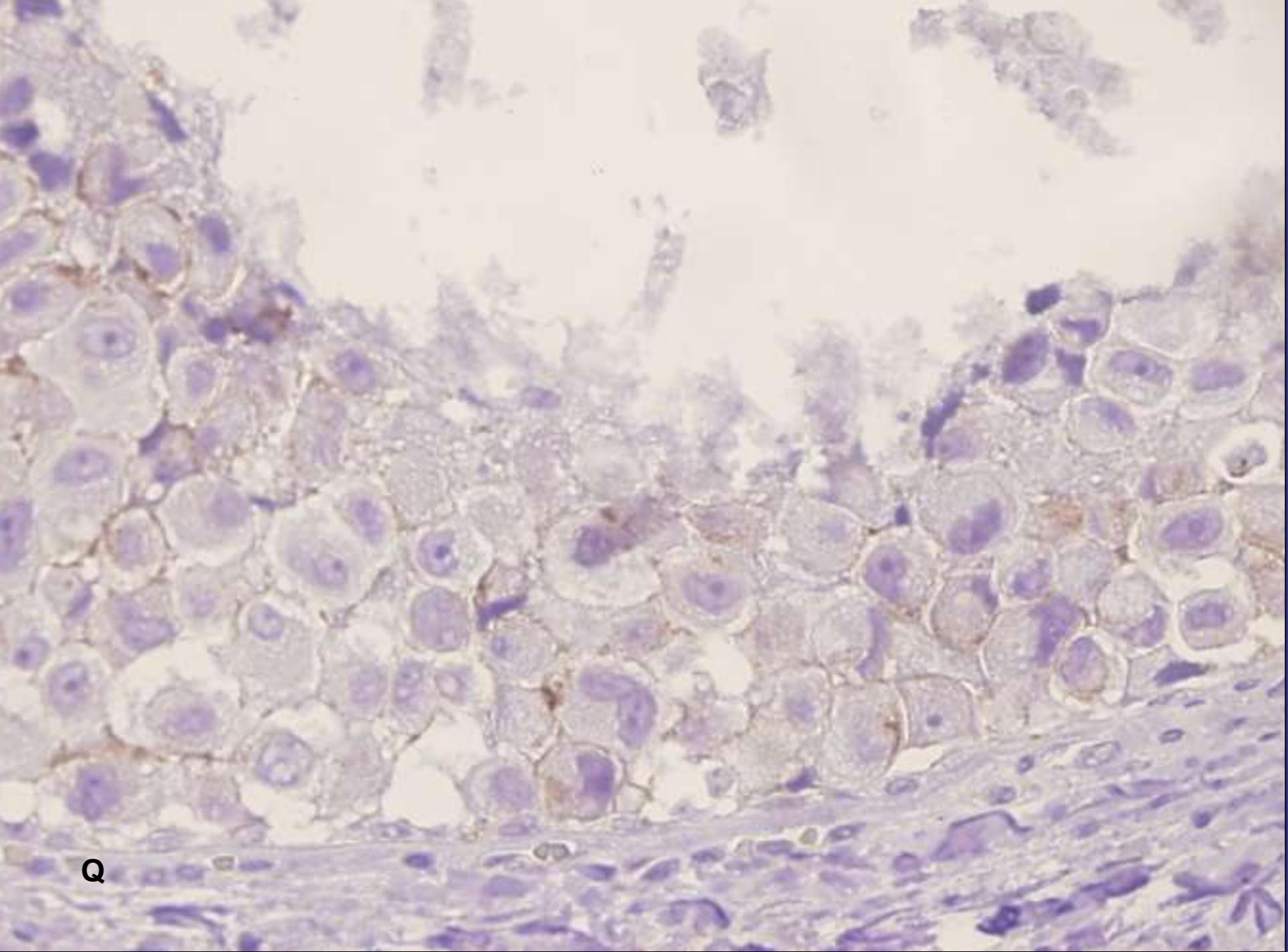




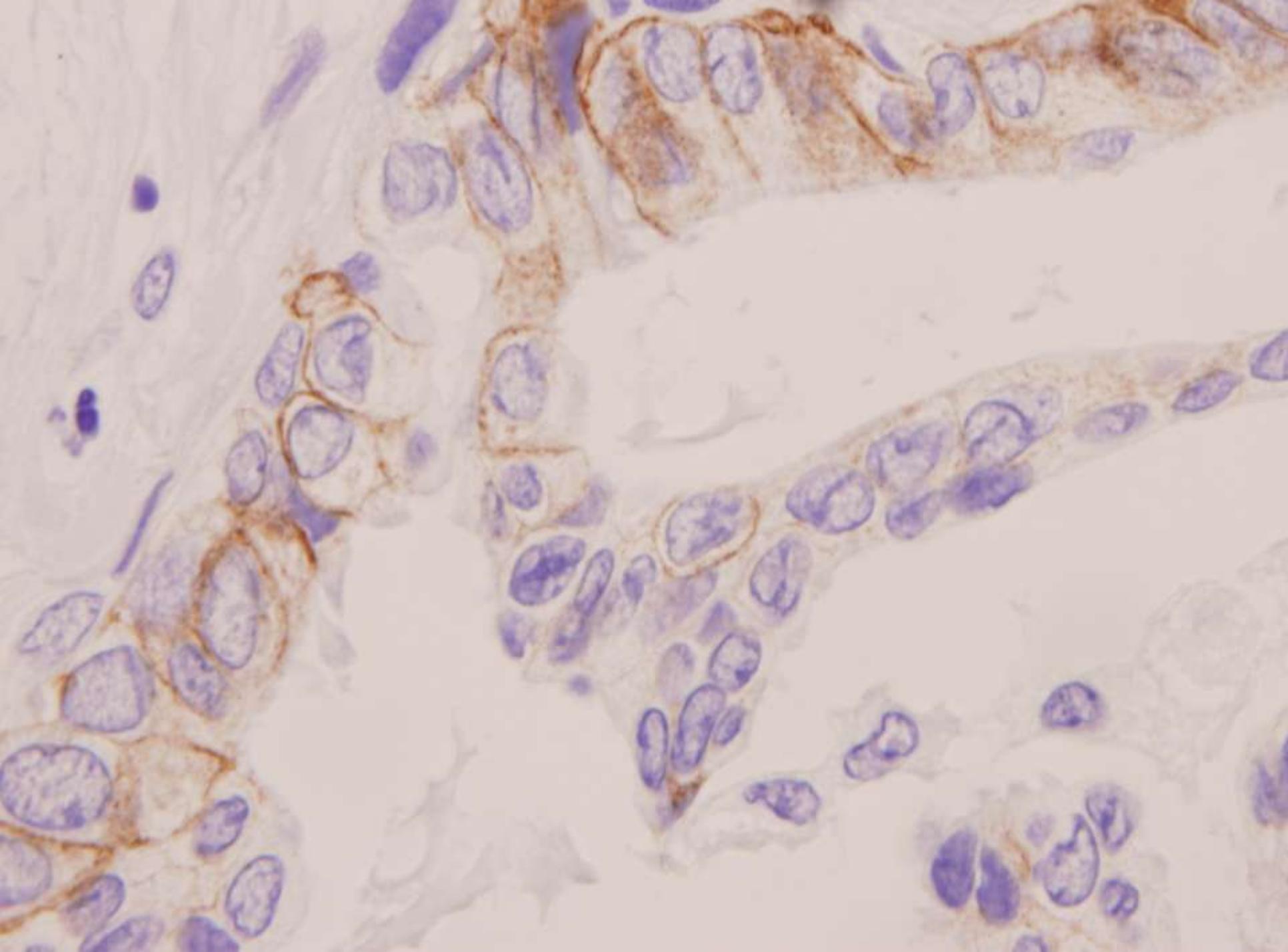


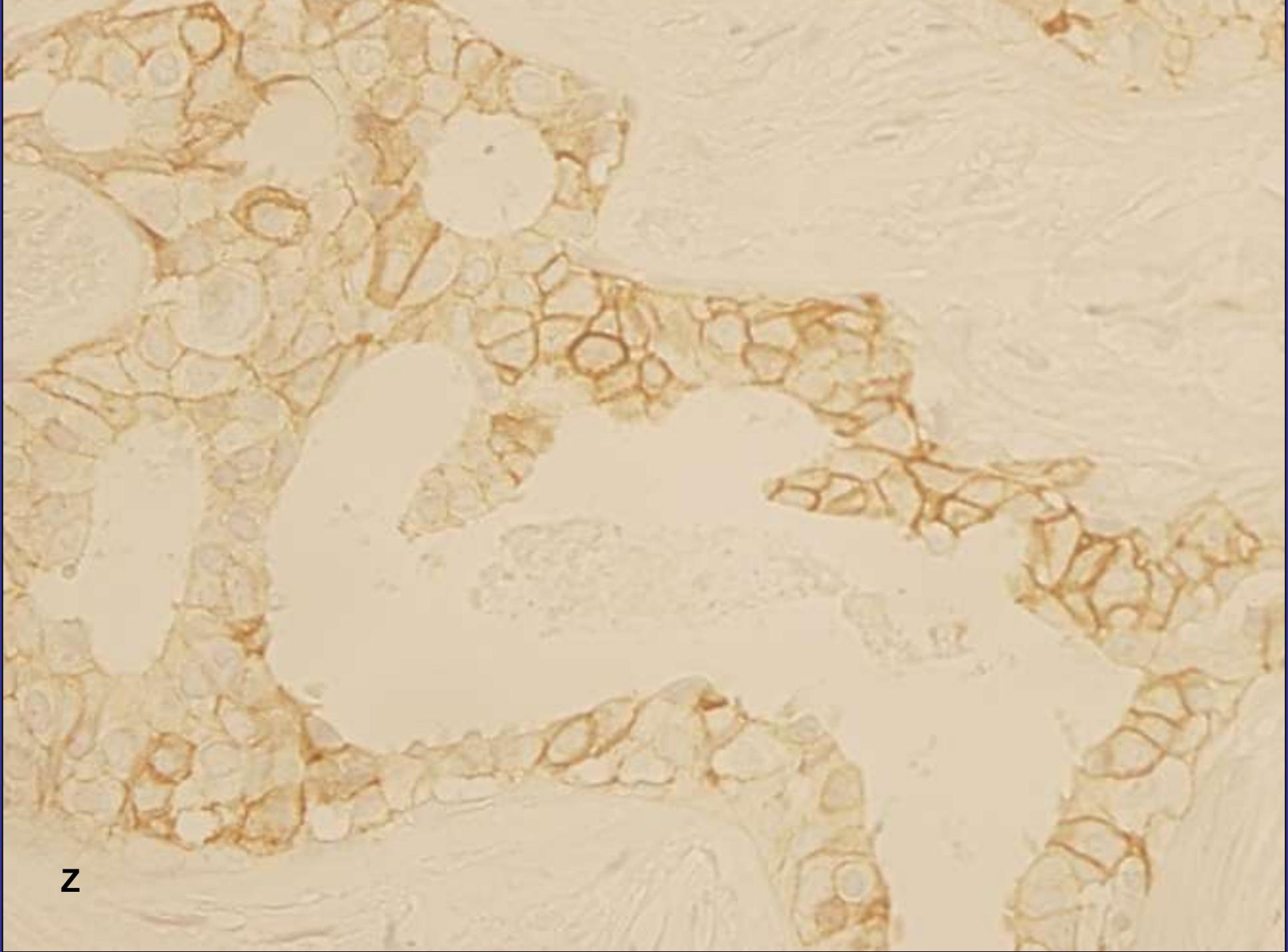




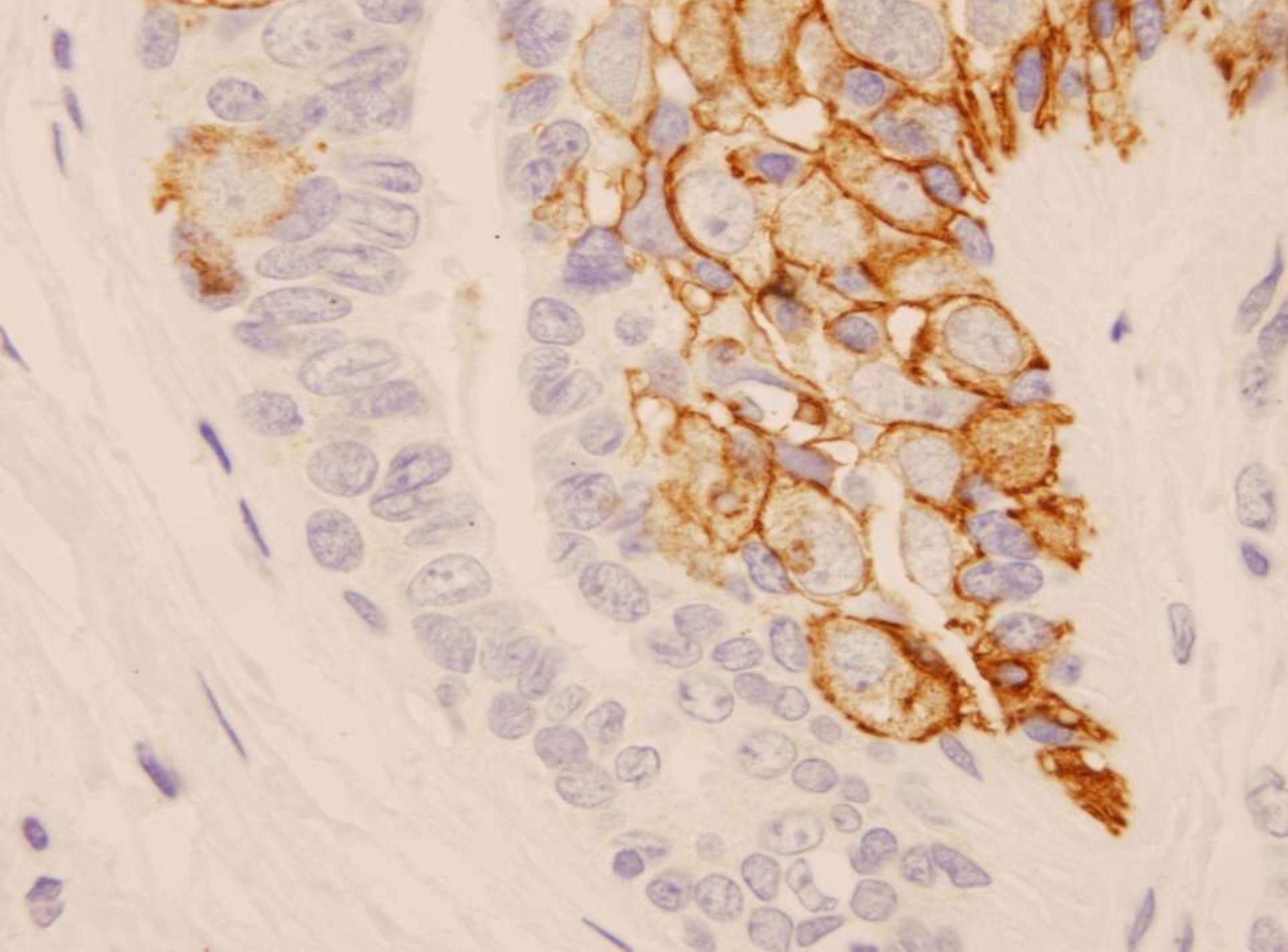


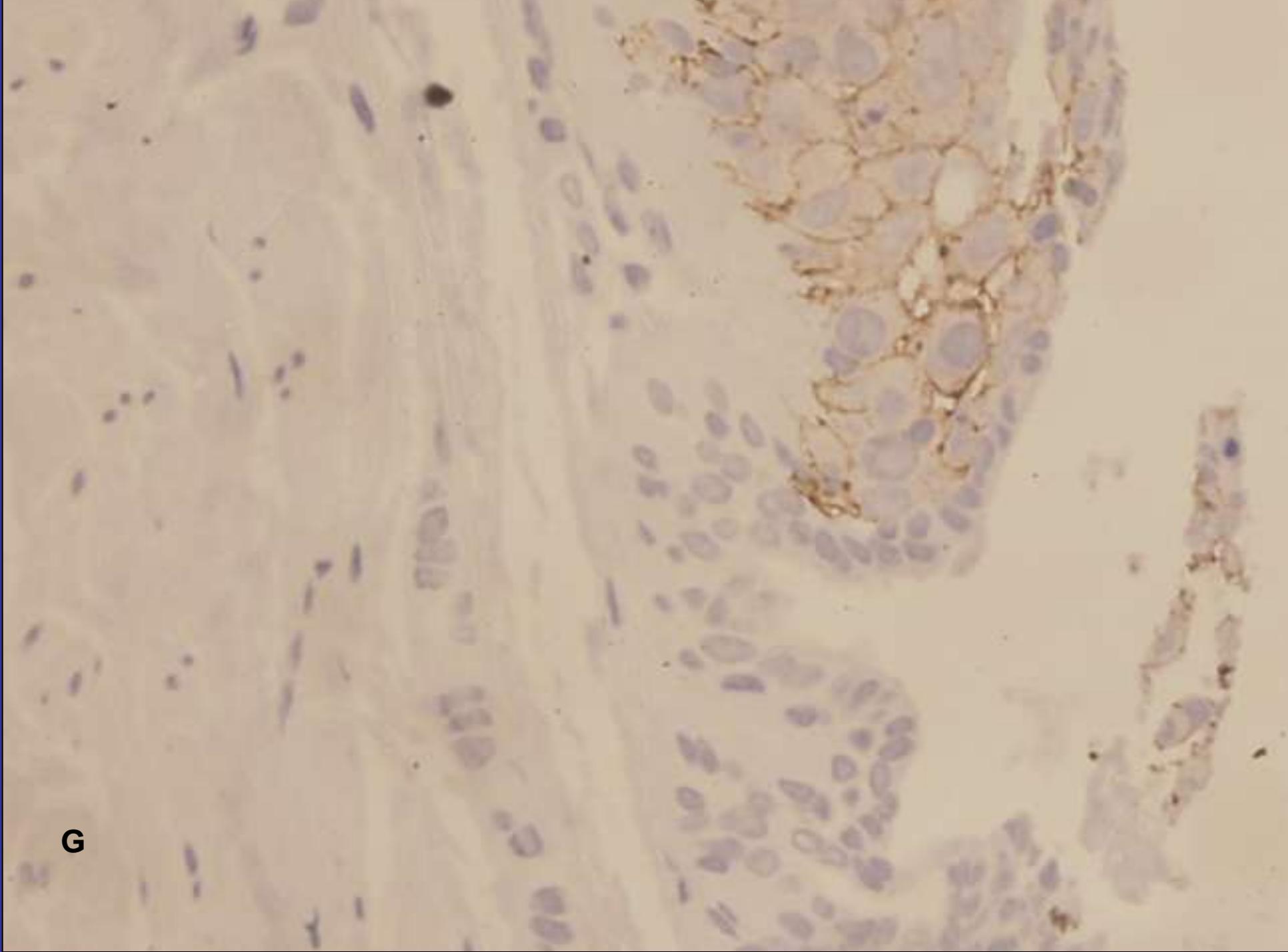
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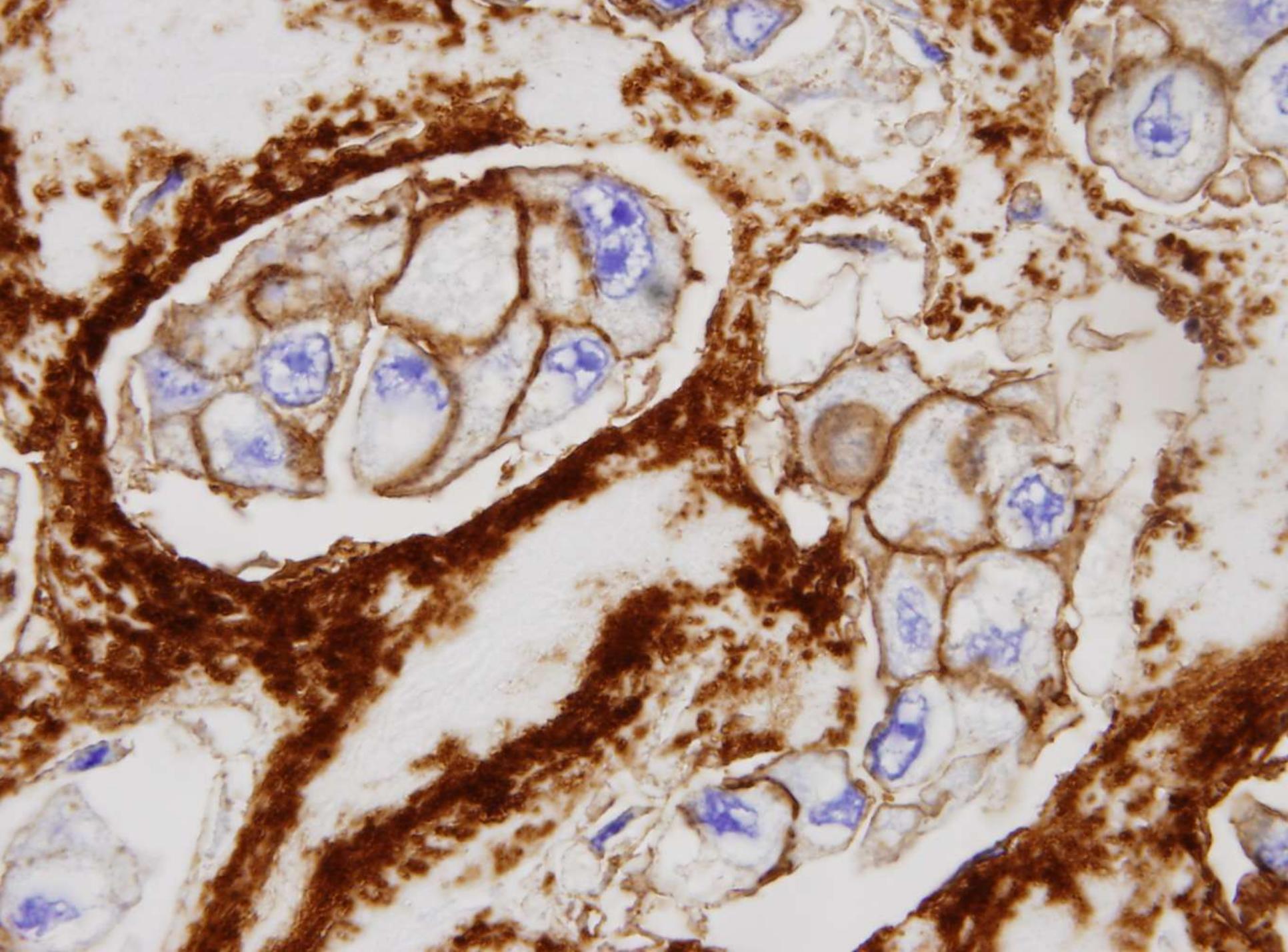


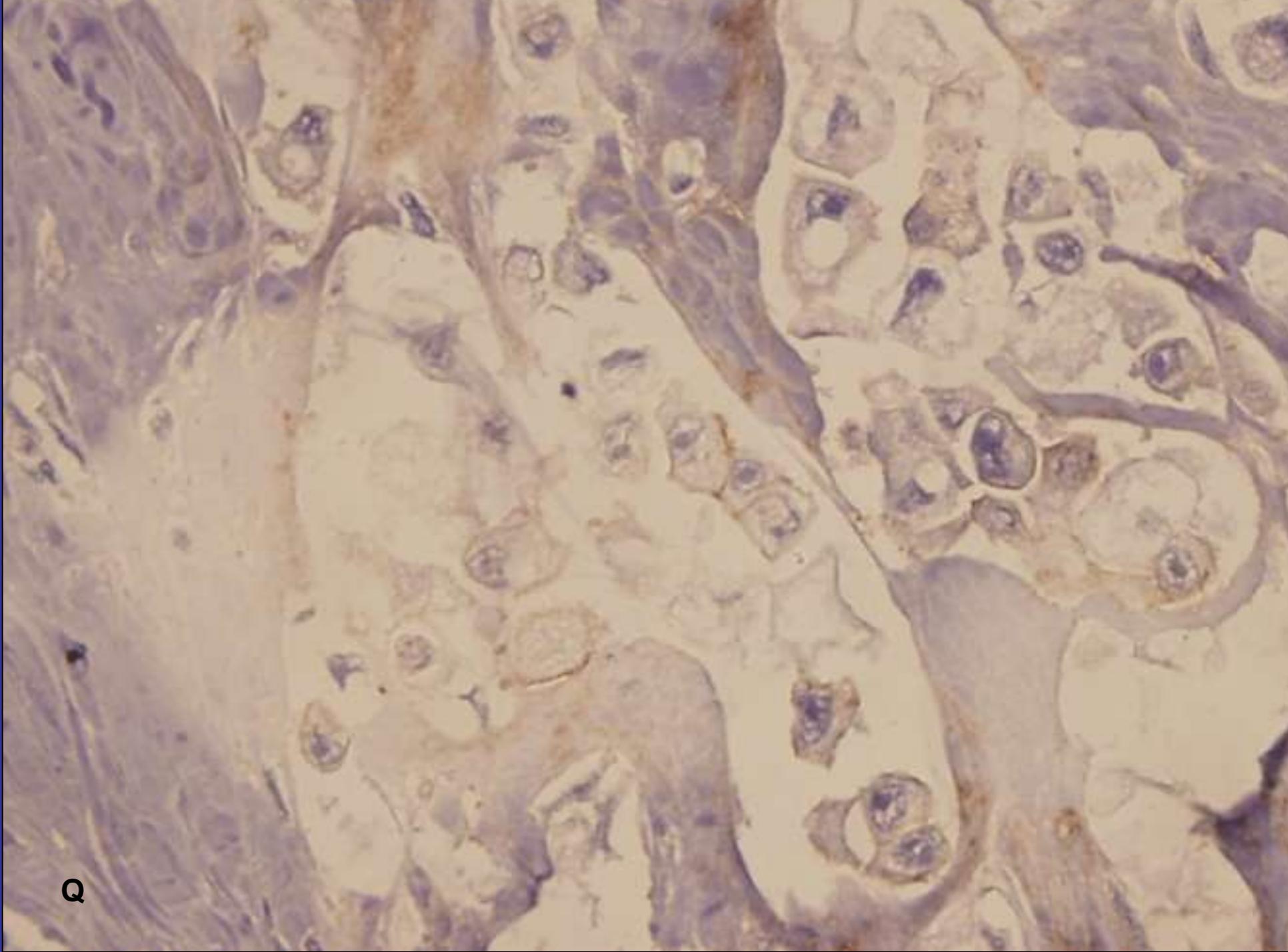
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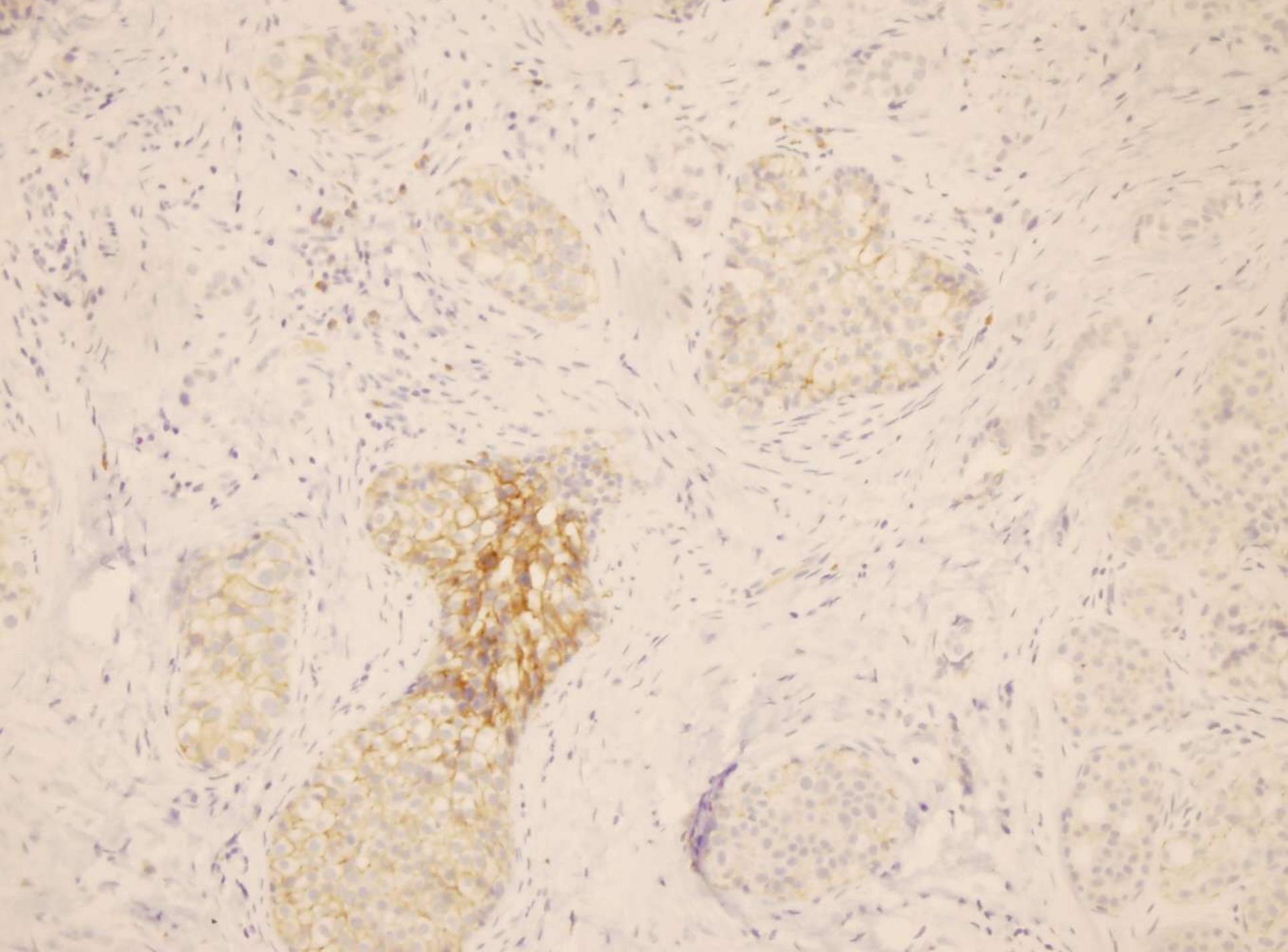


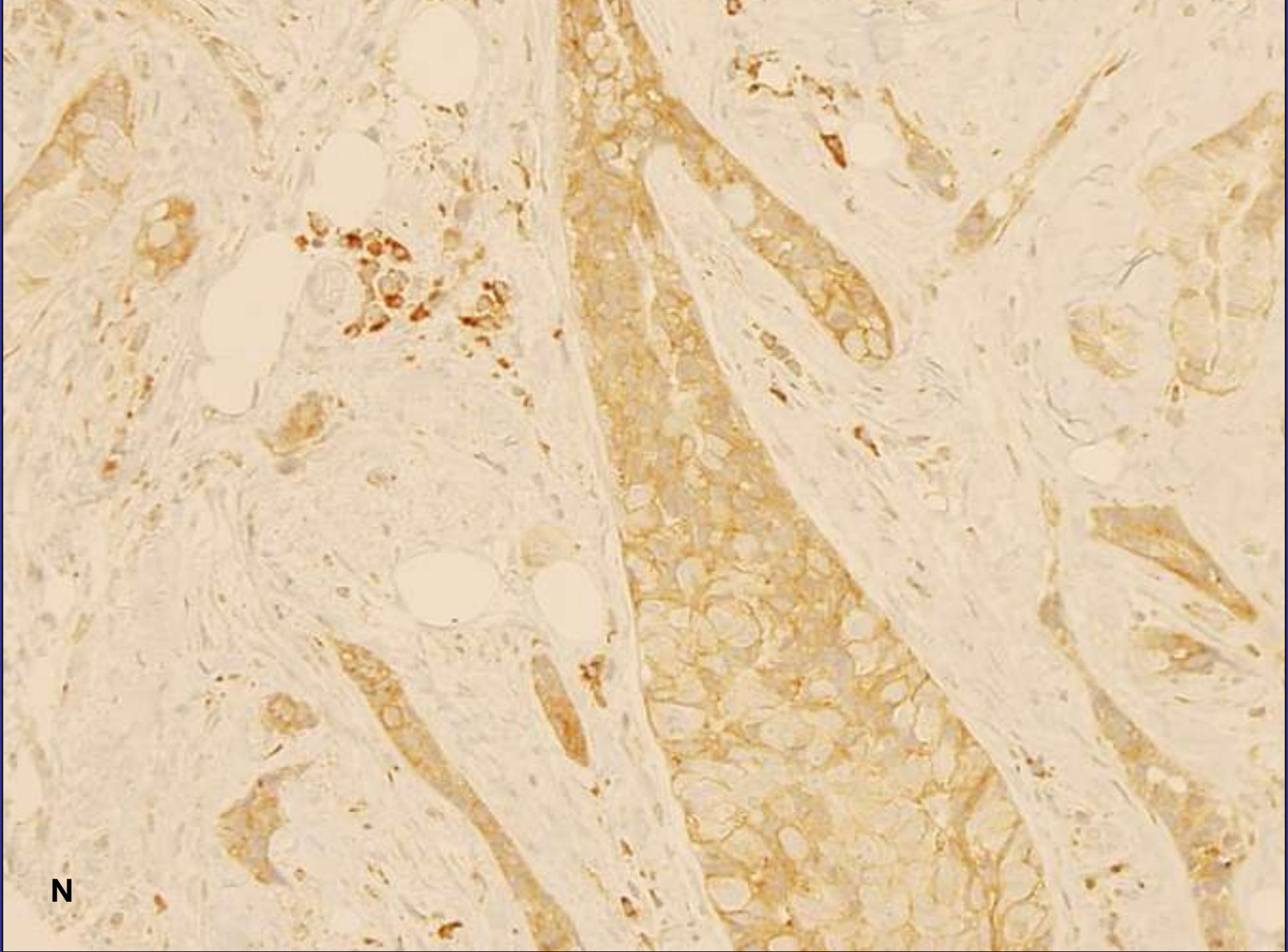


G

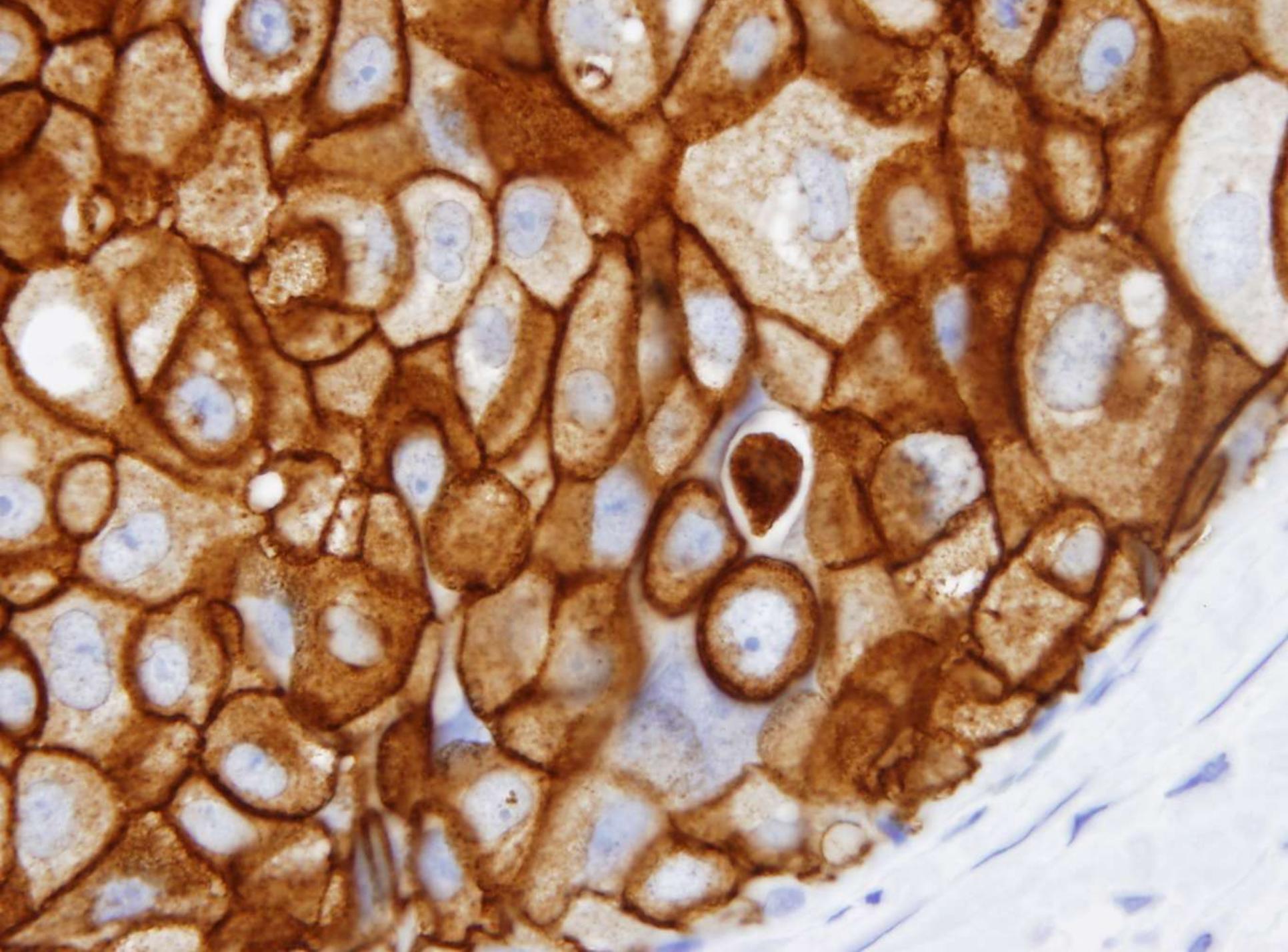


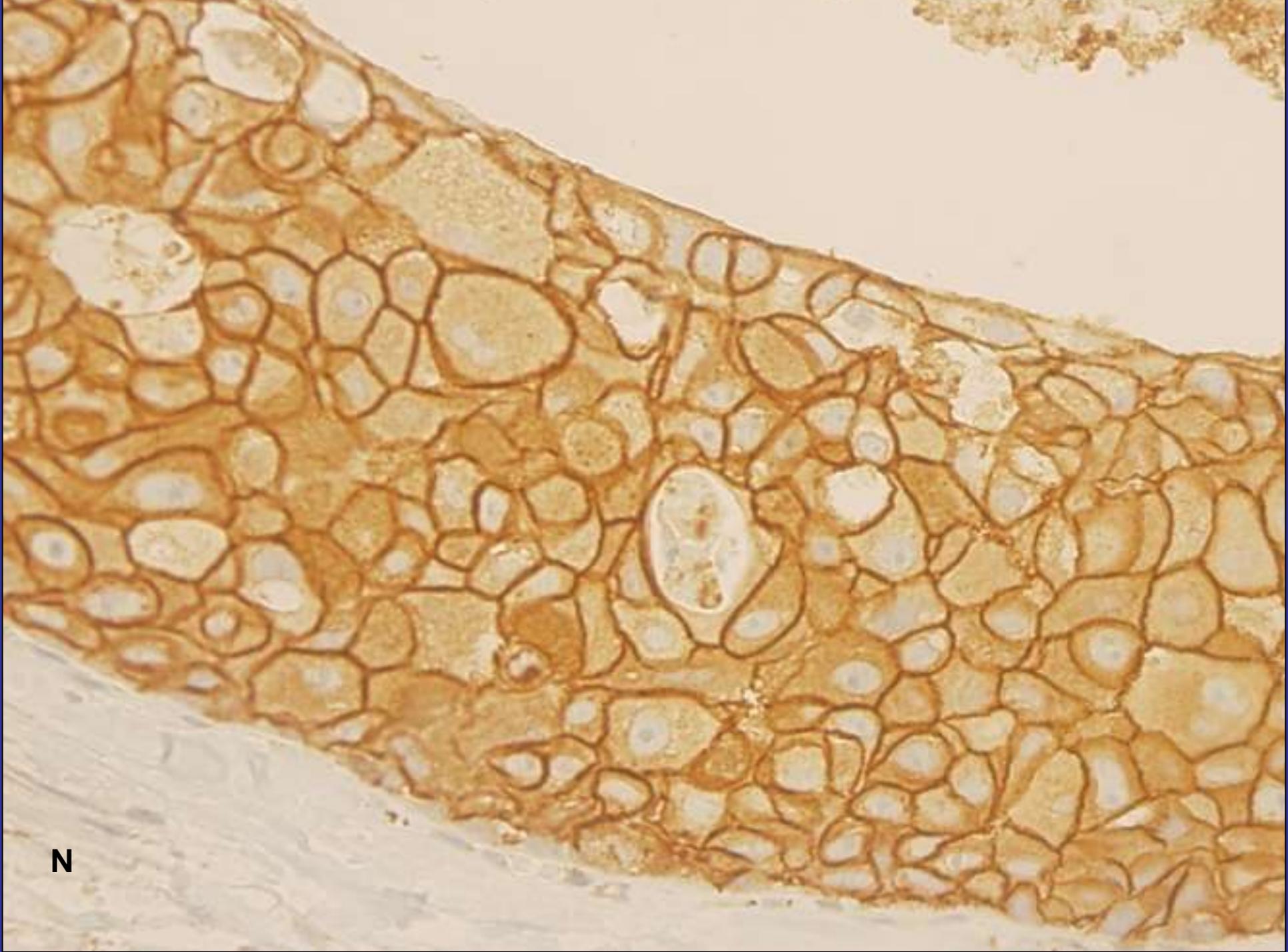




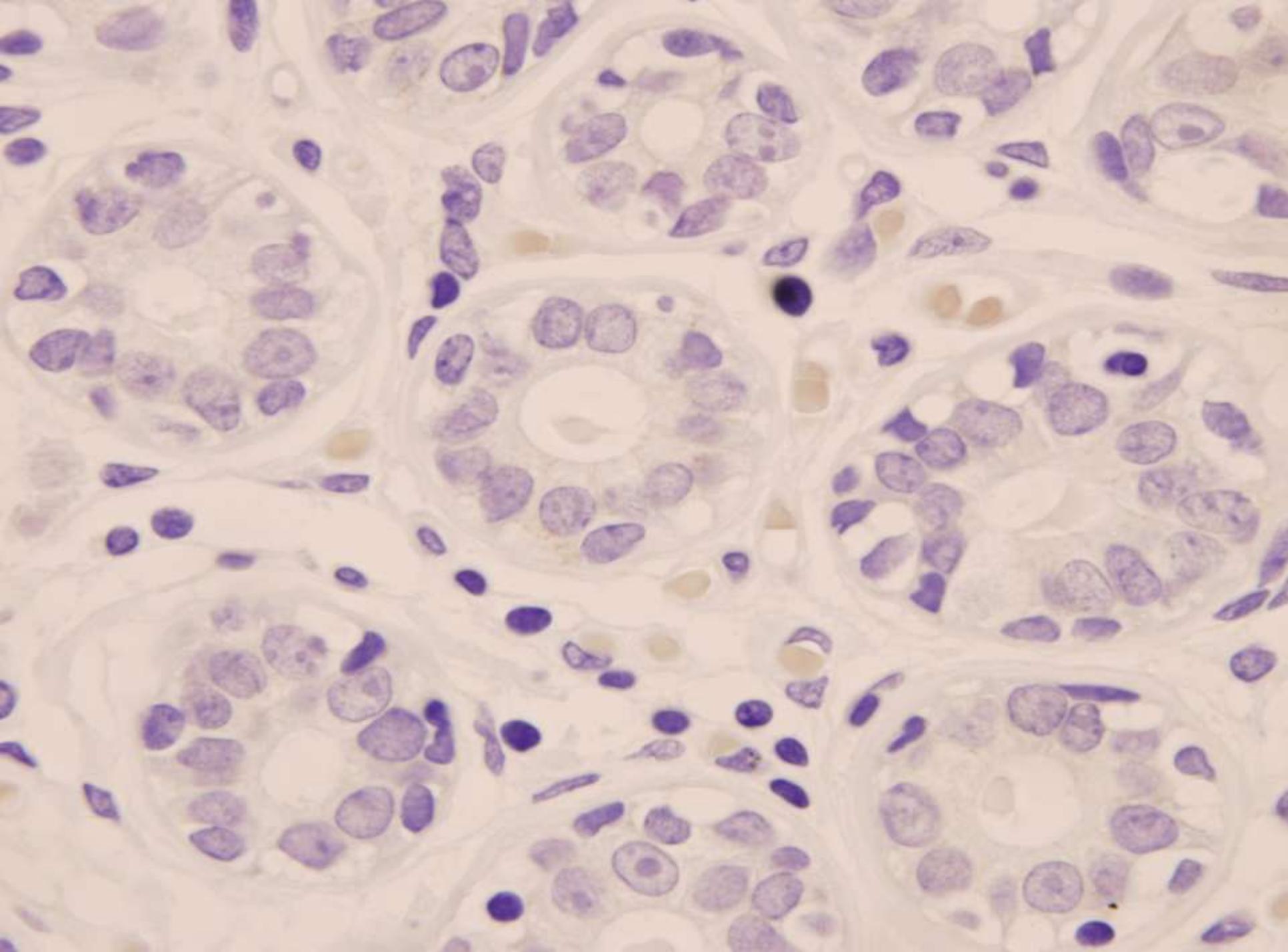


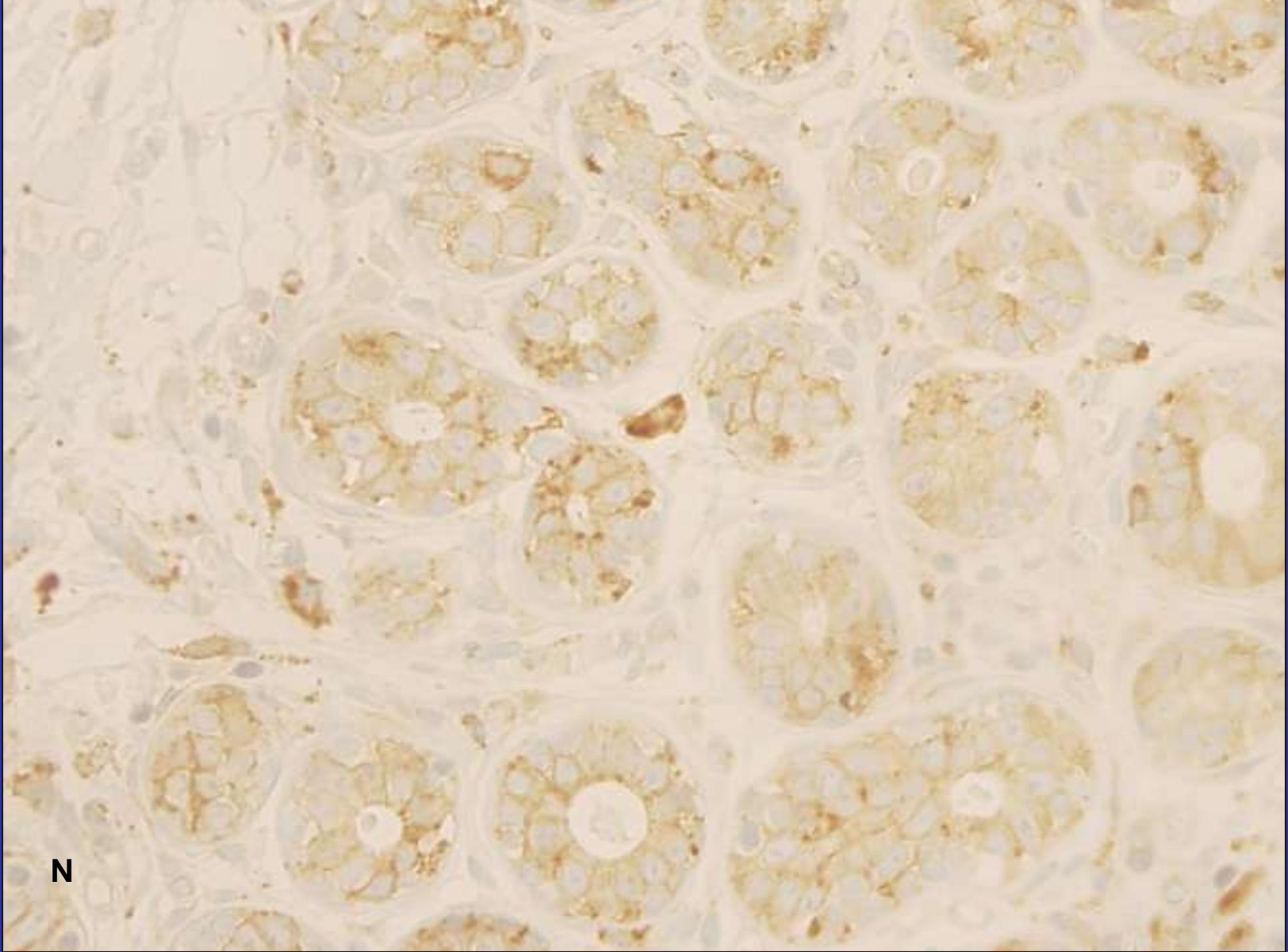
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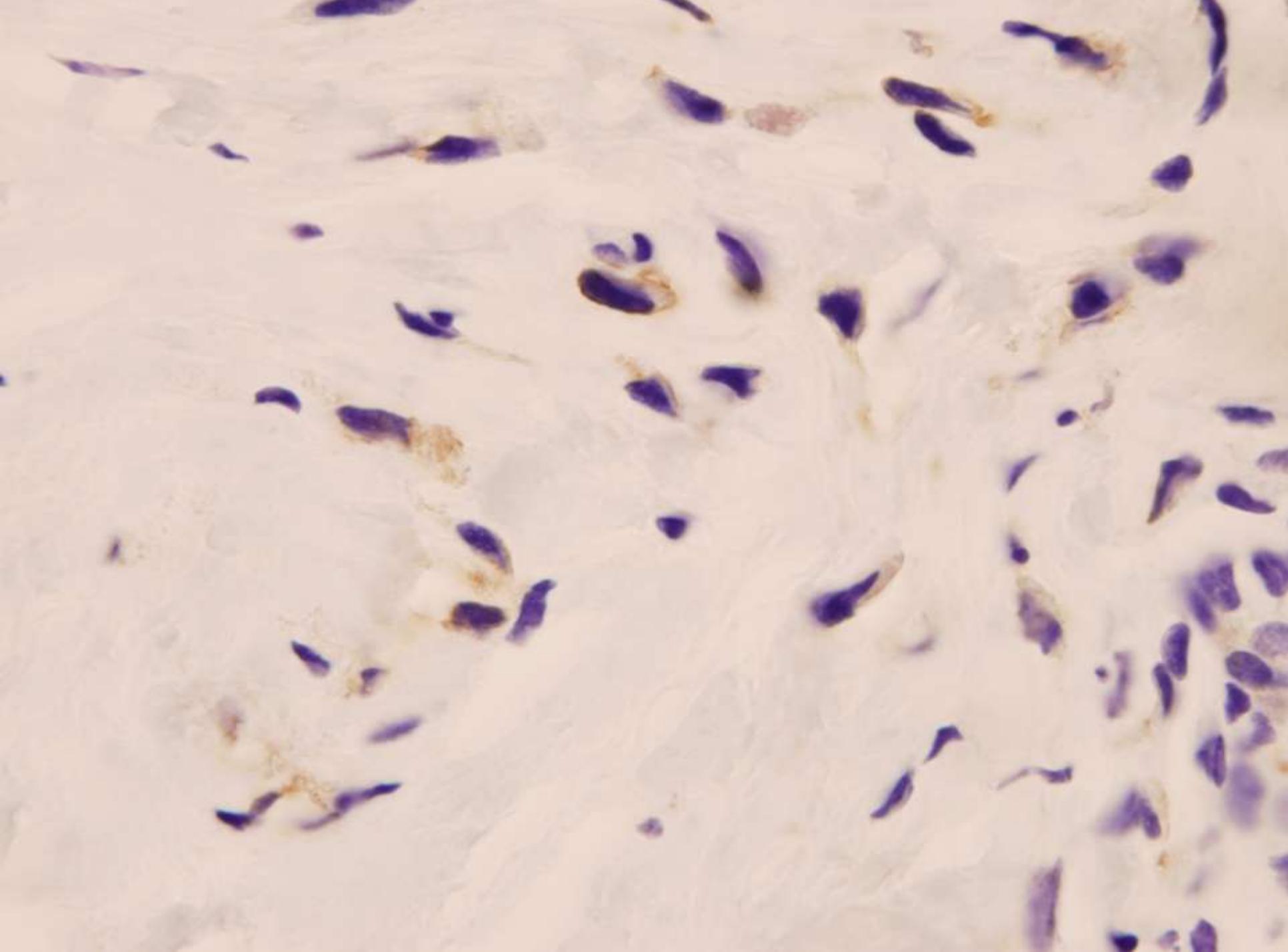


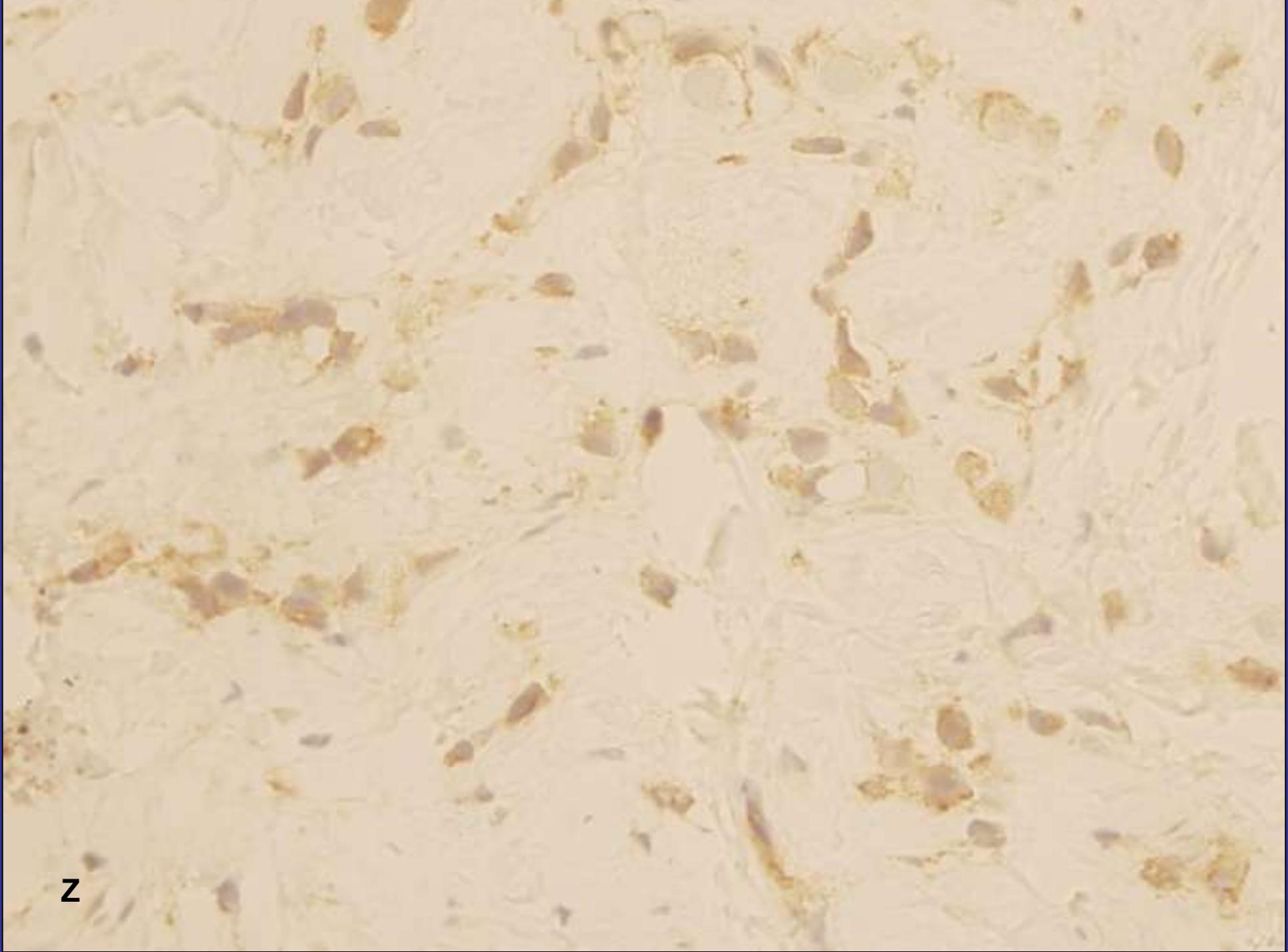
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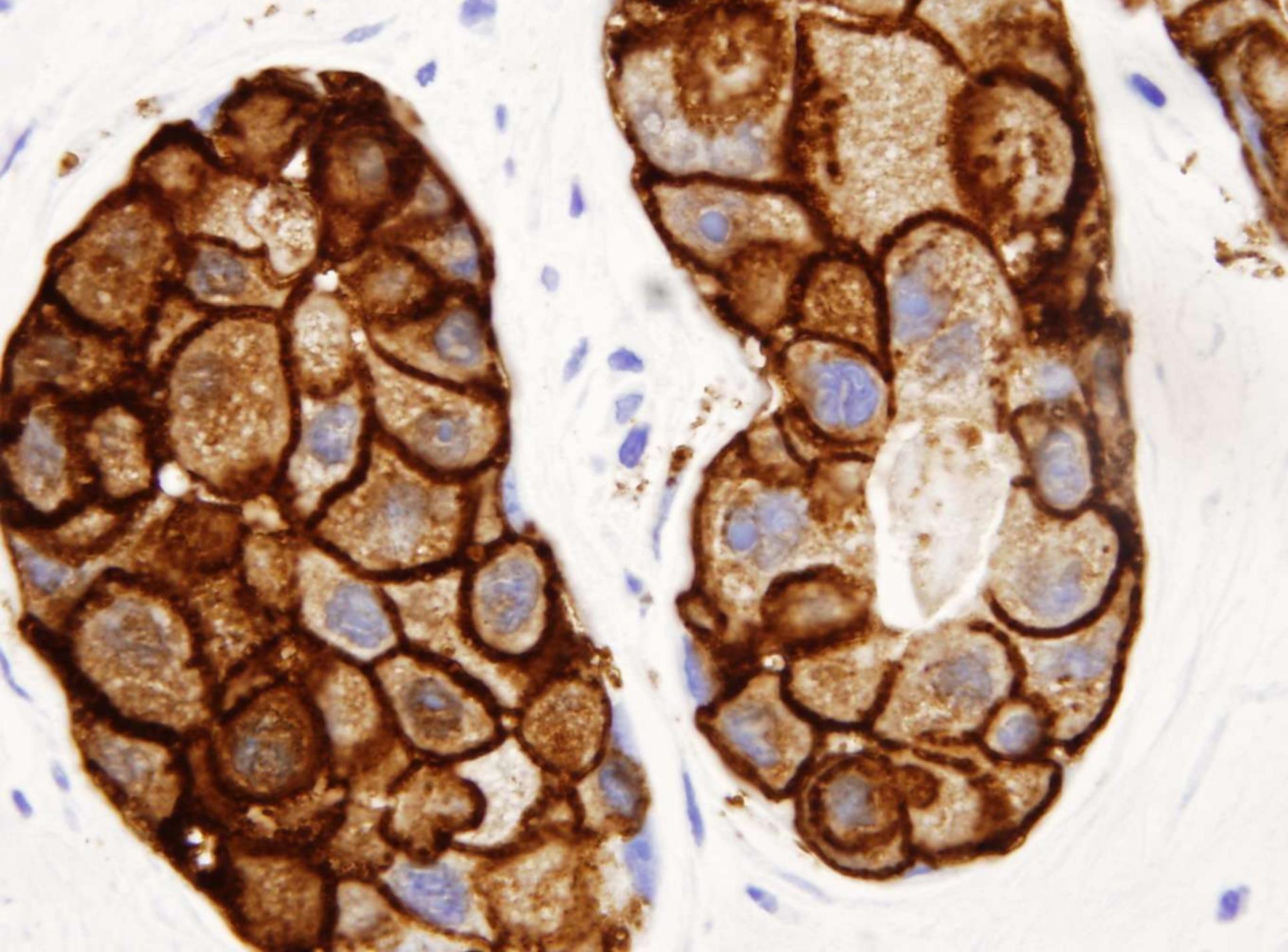


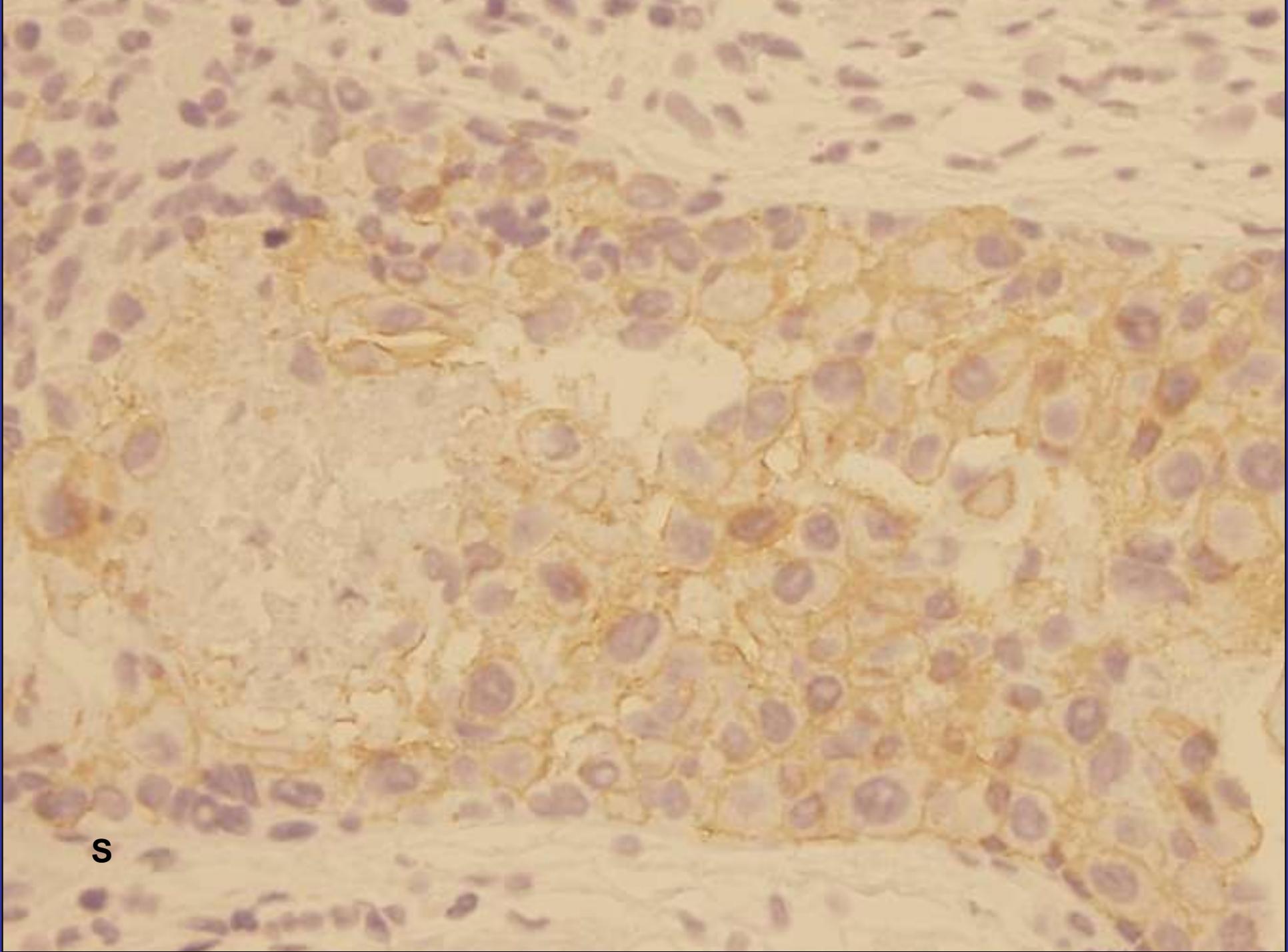
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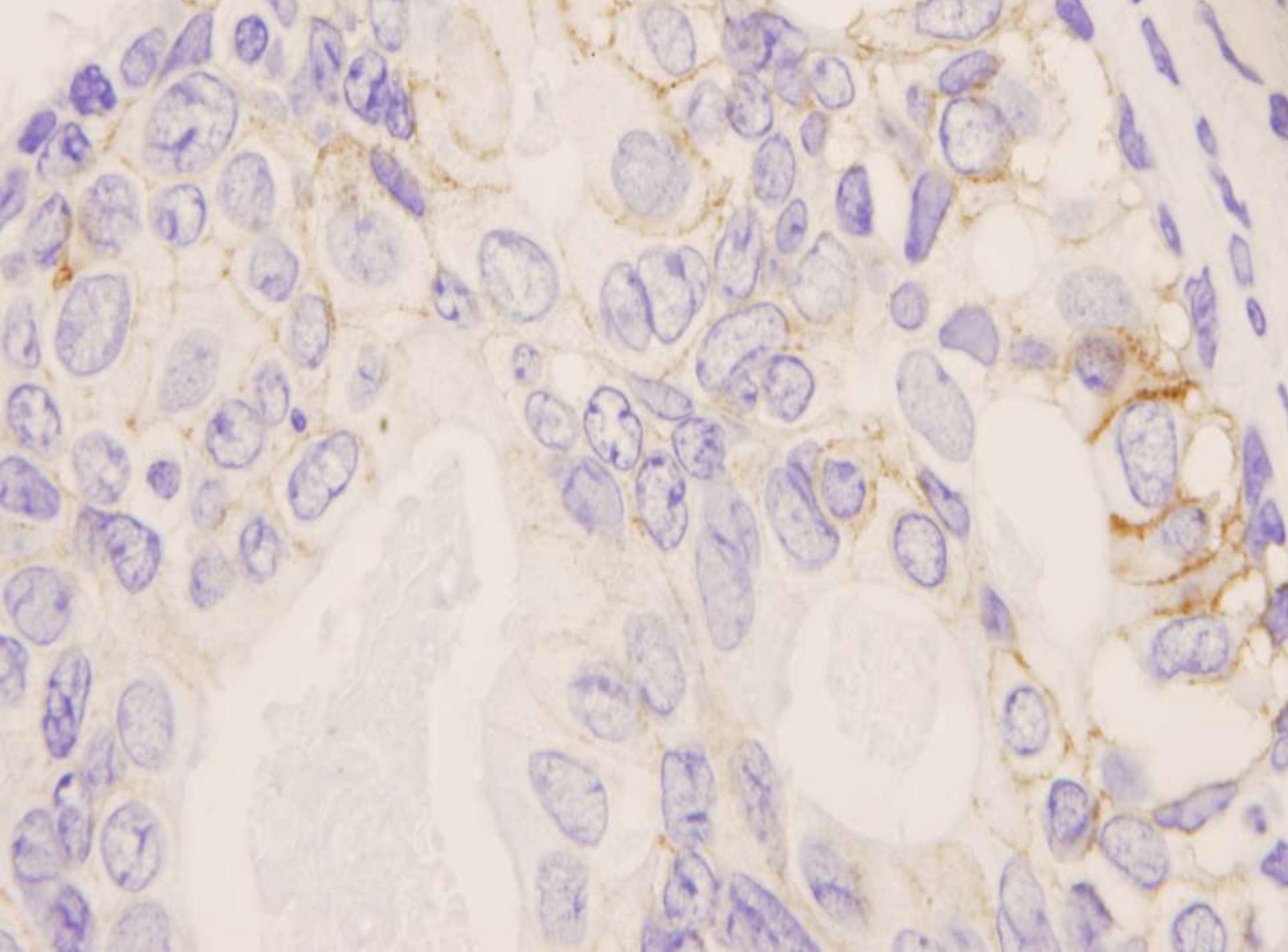


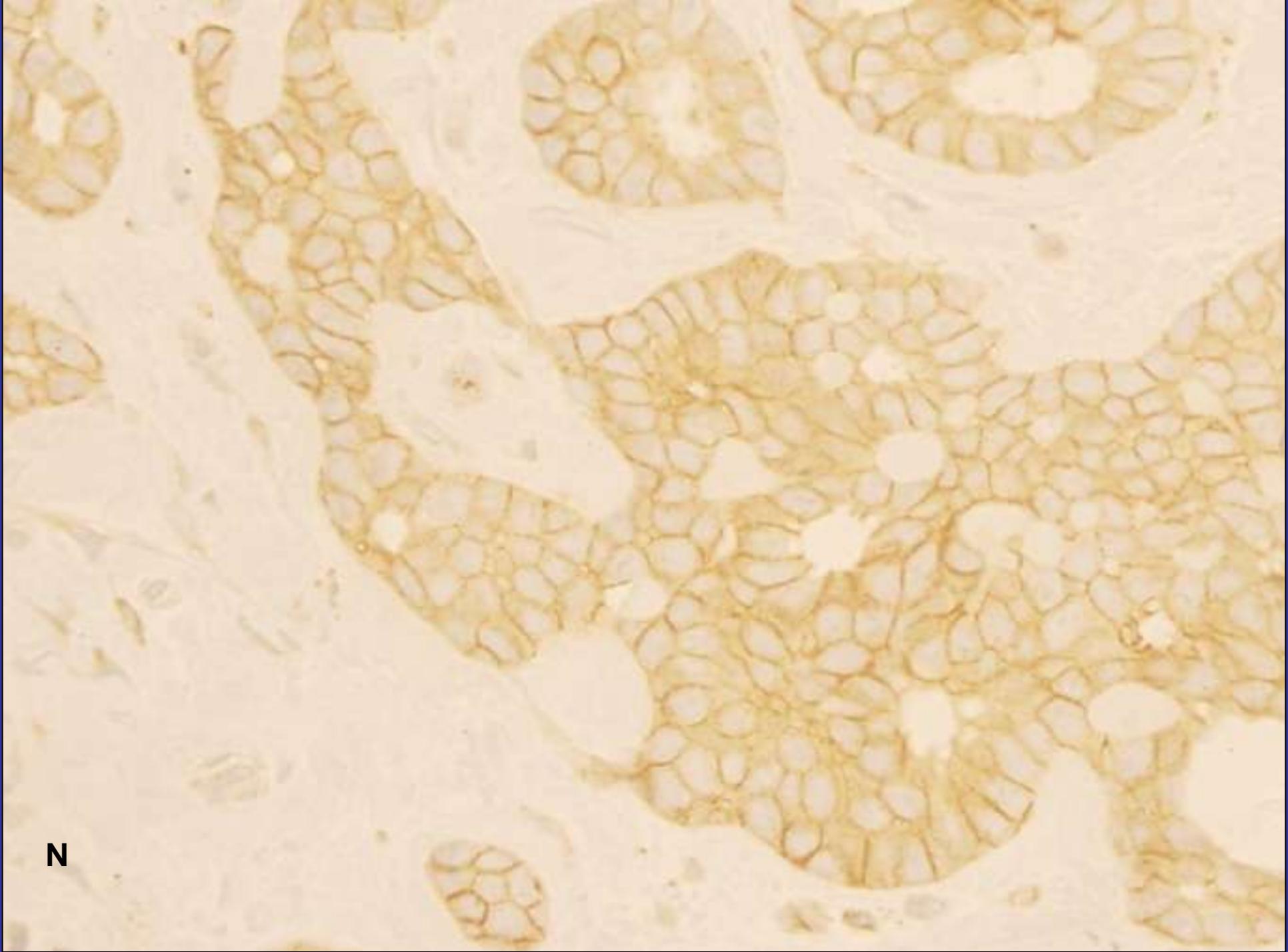
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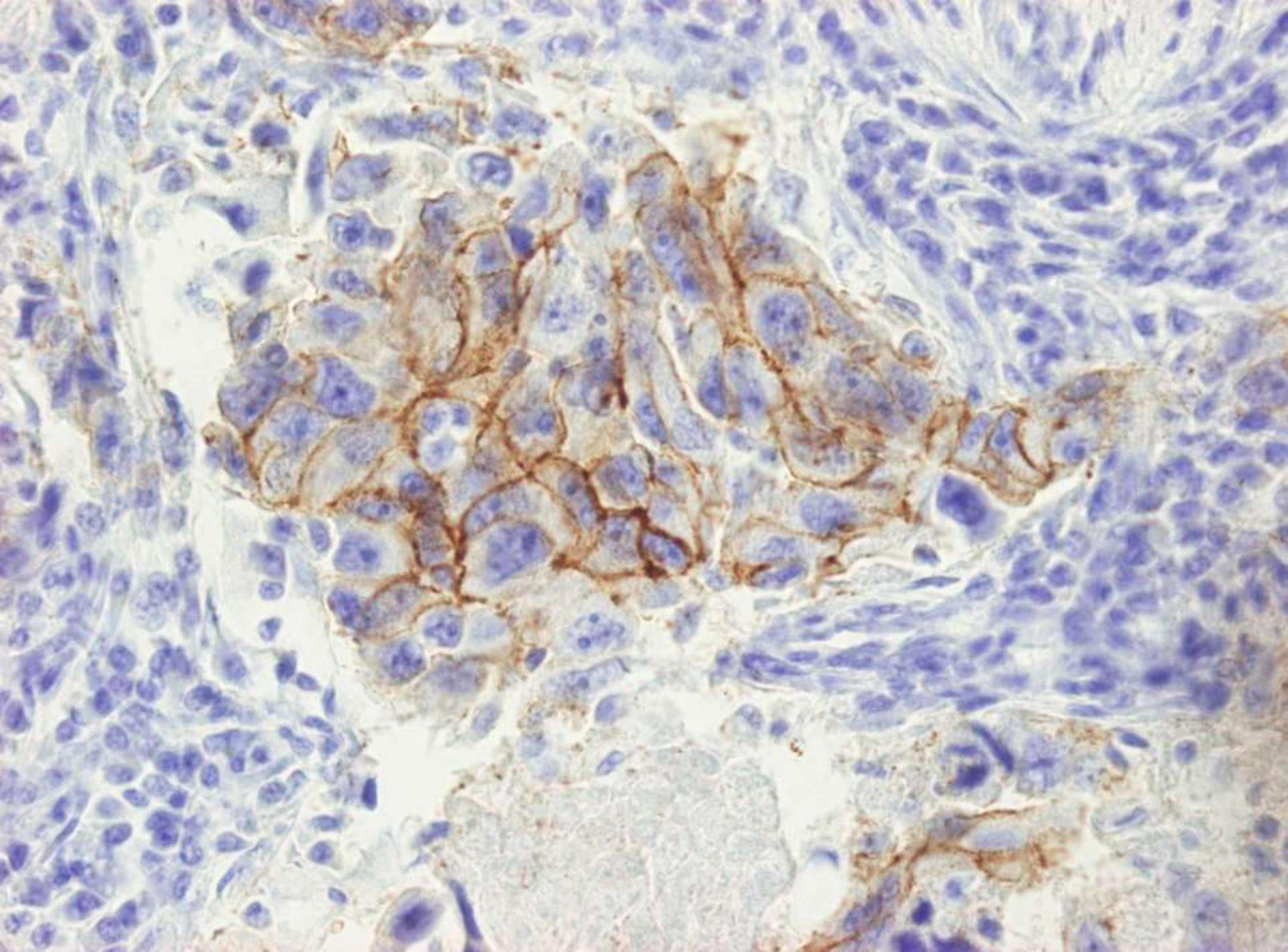


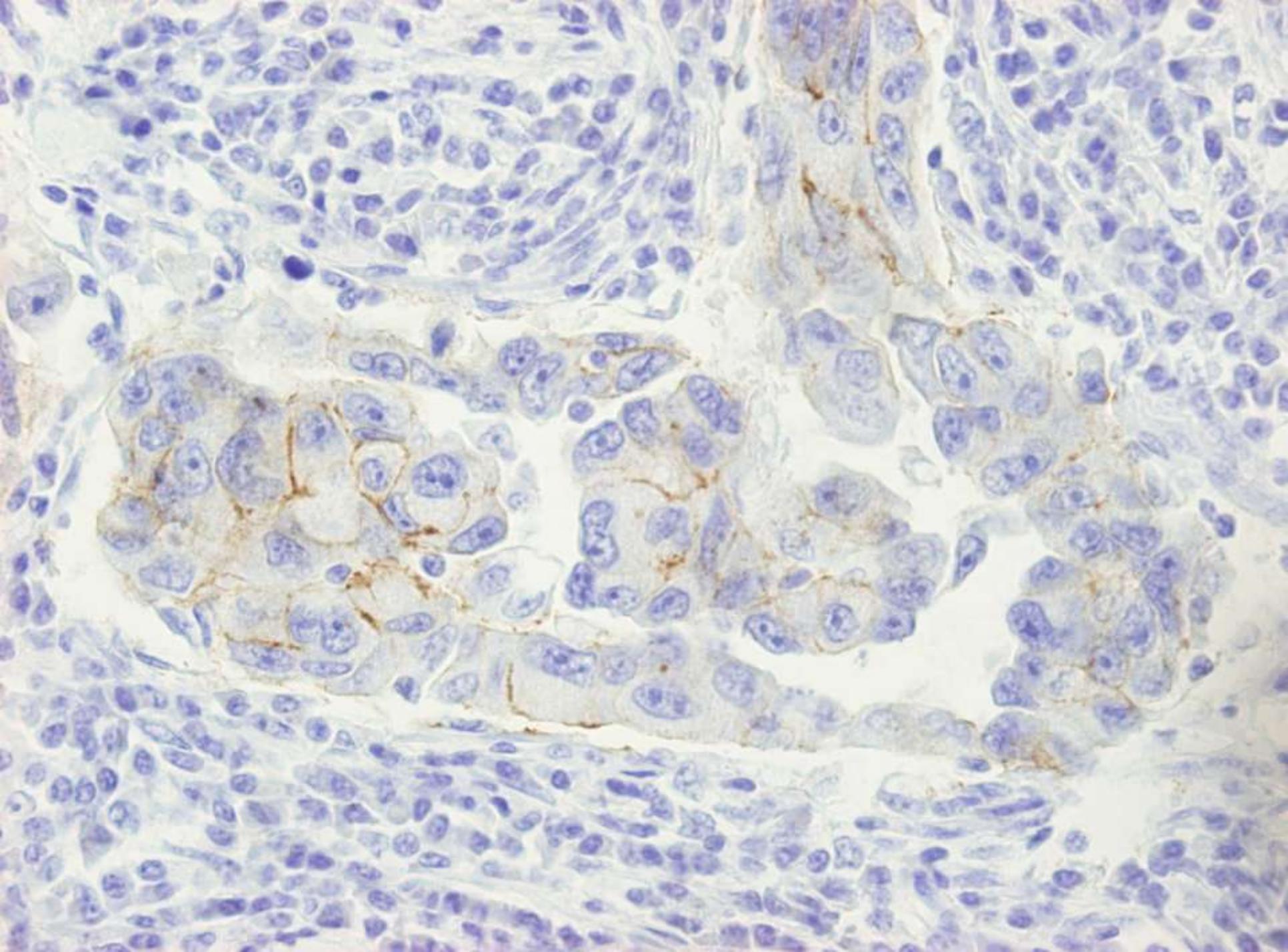
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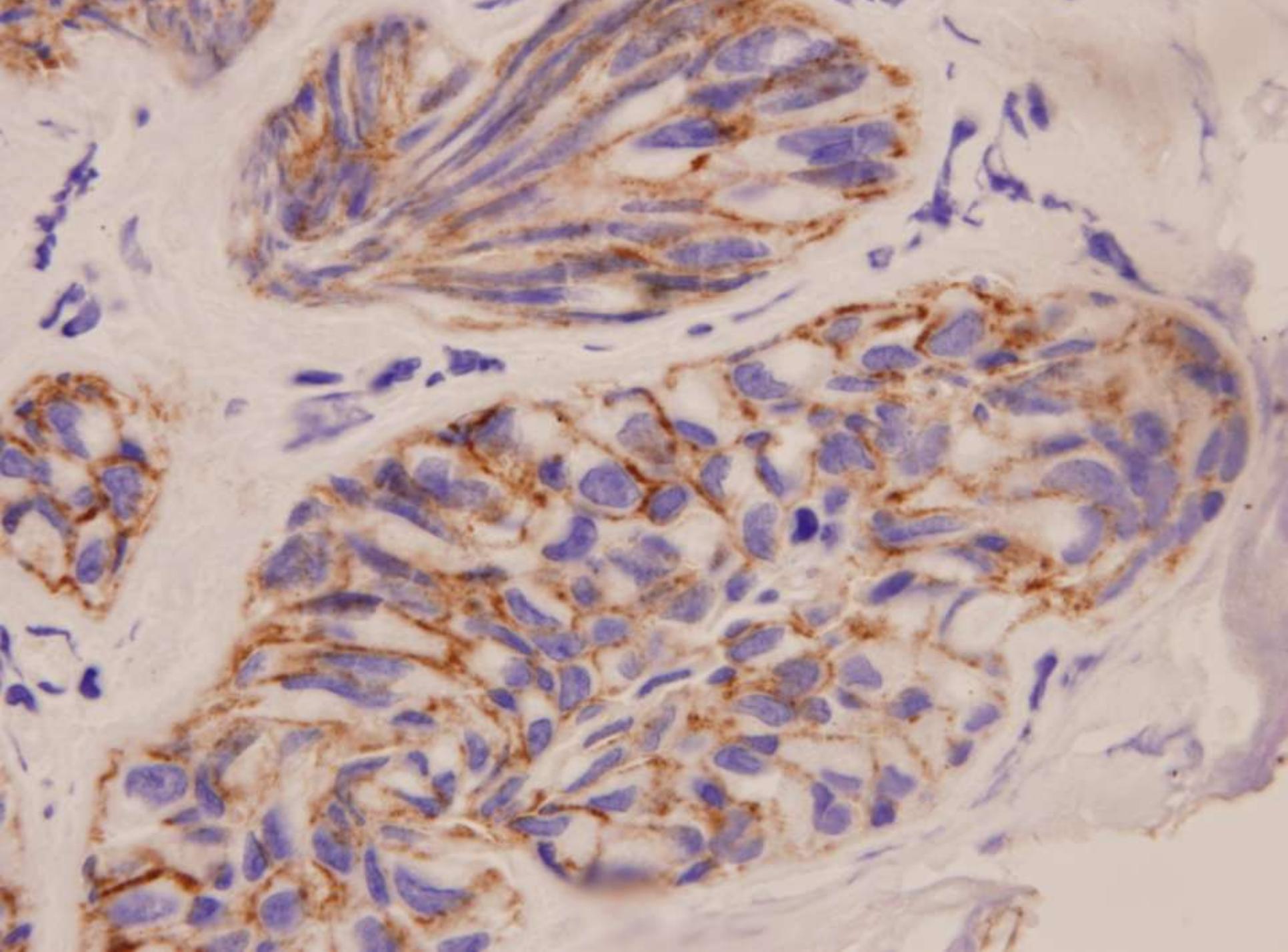


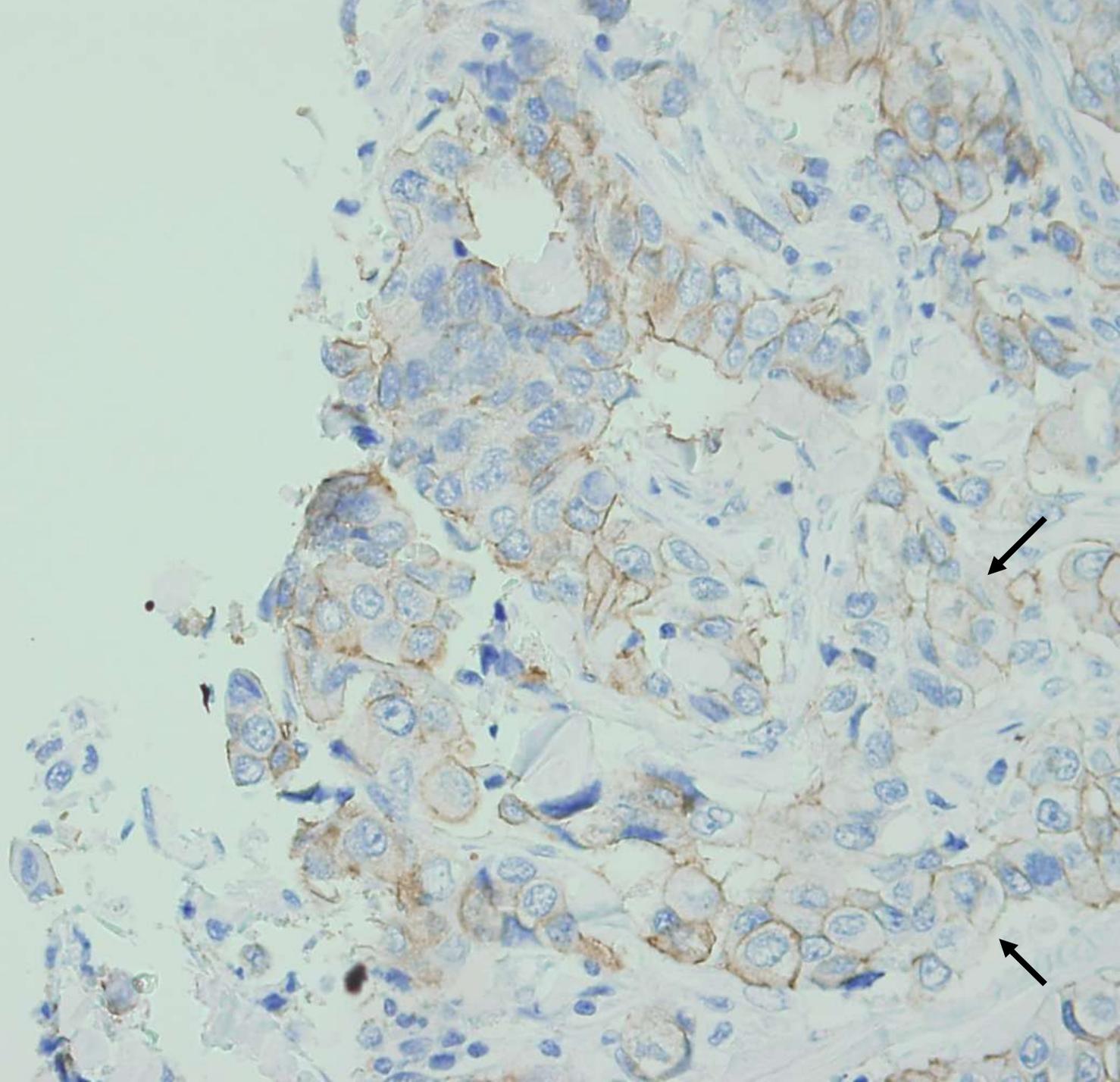


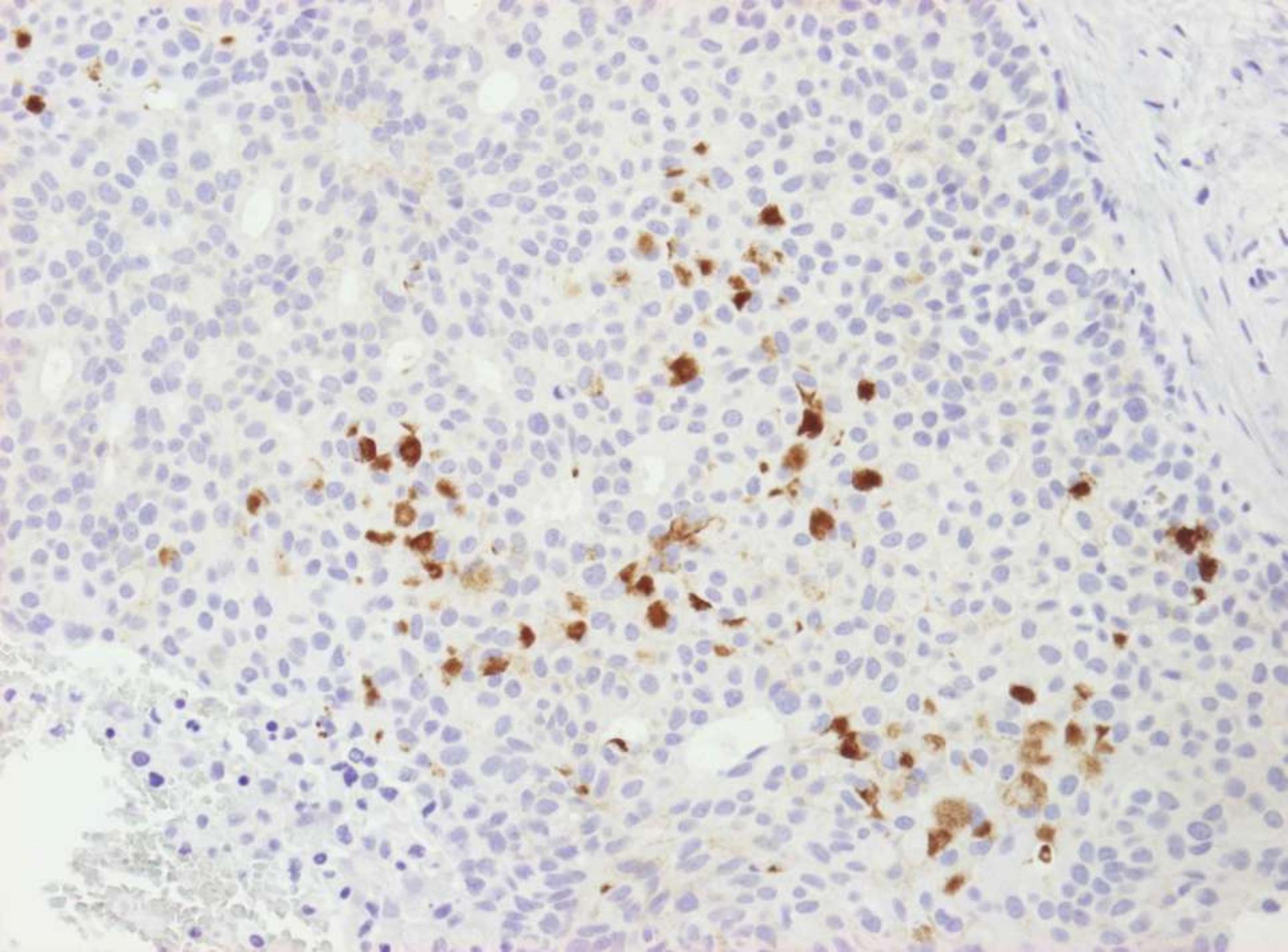
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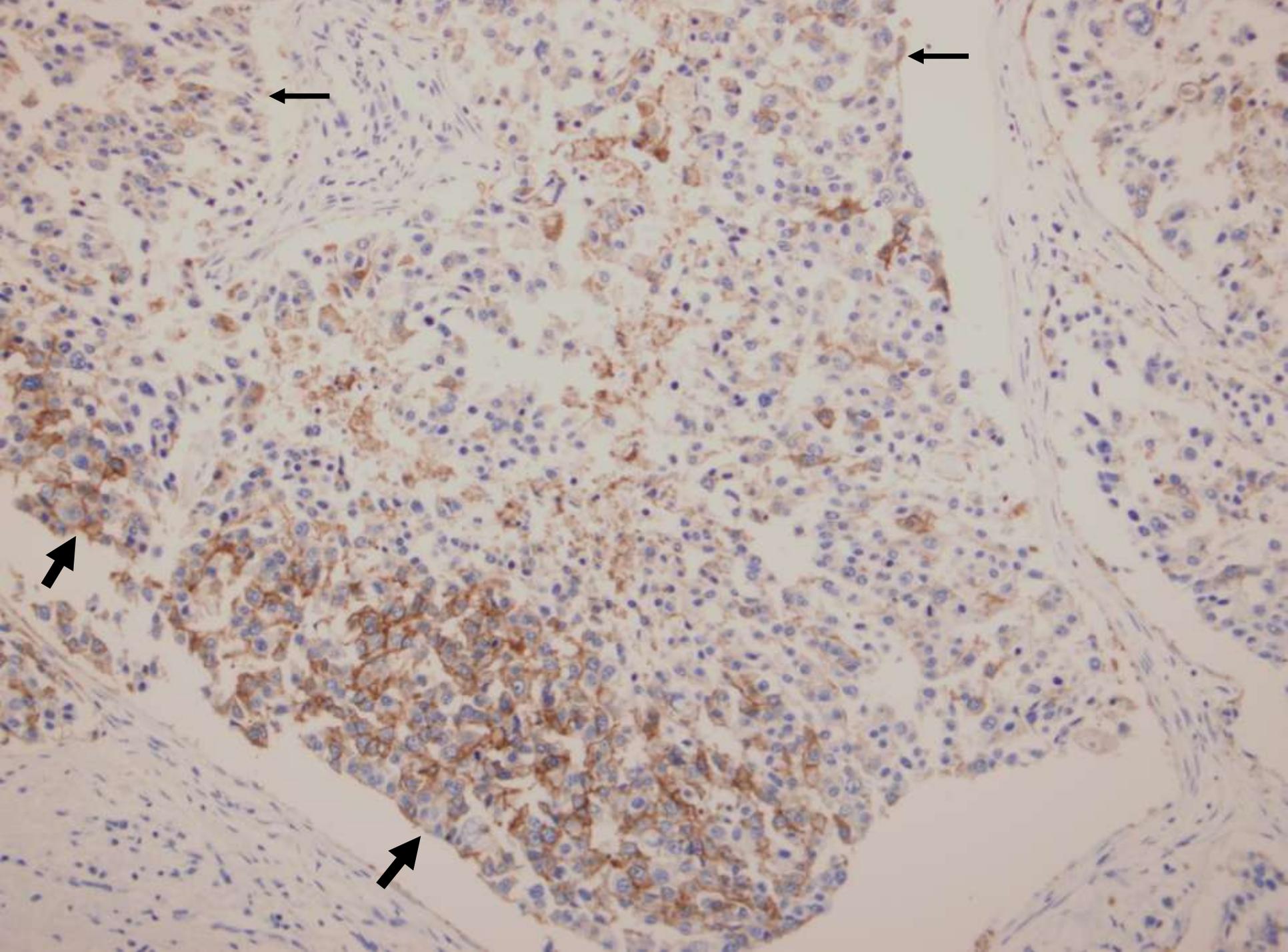




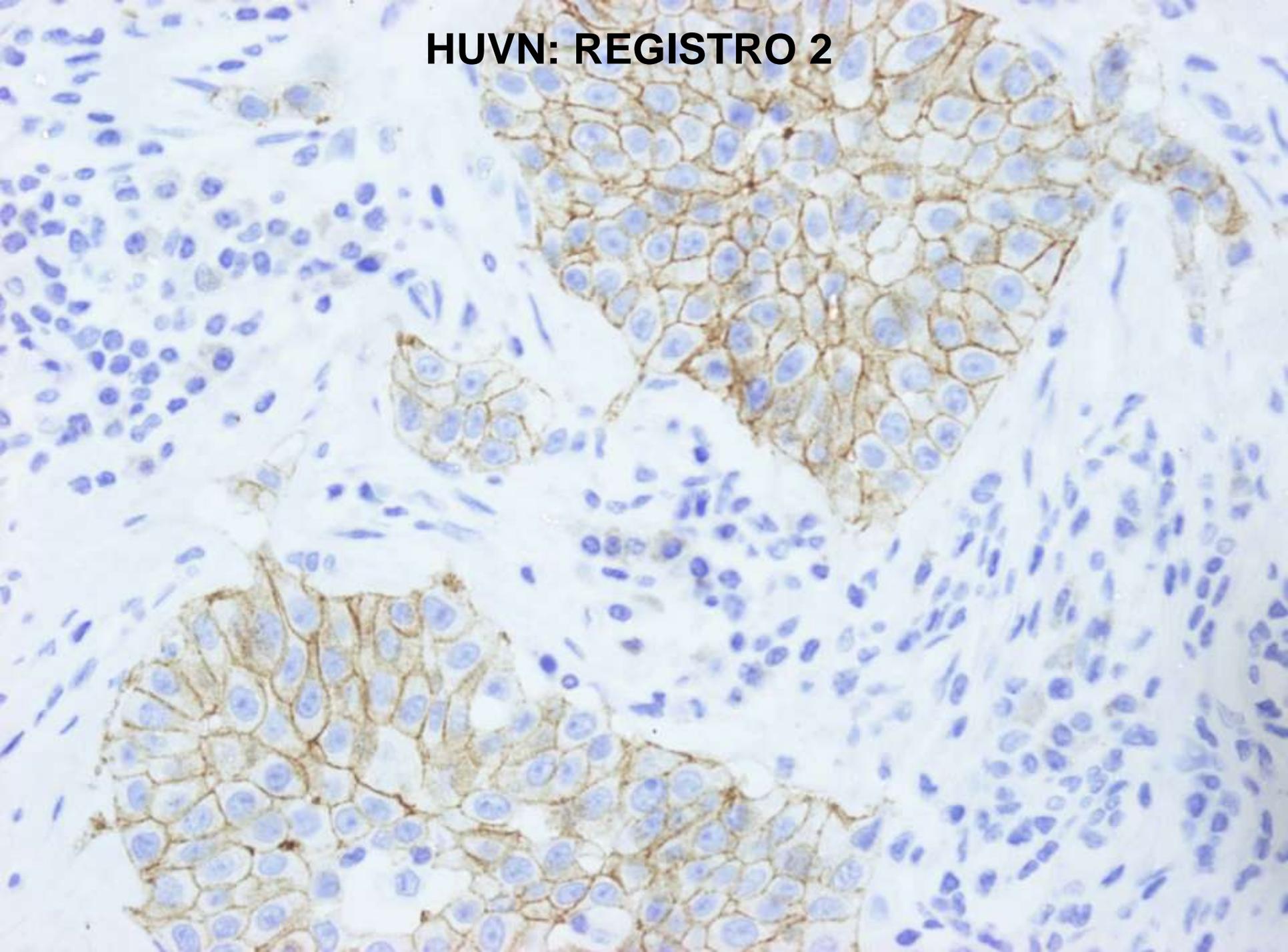




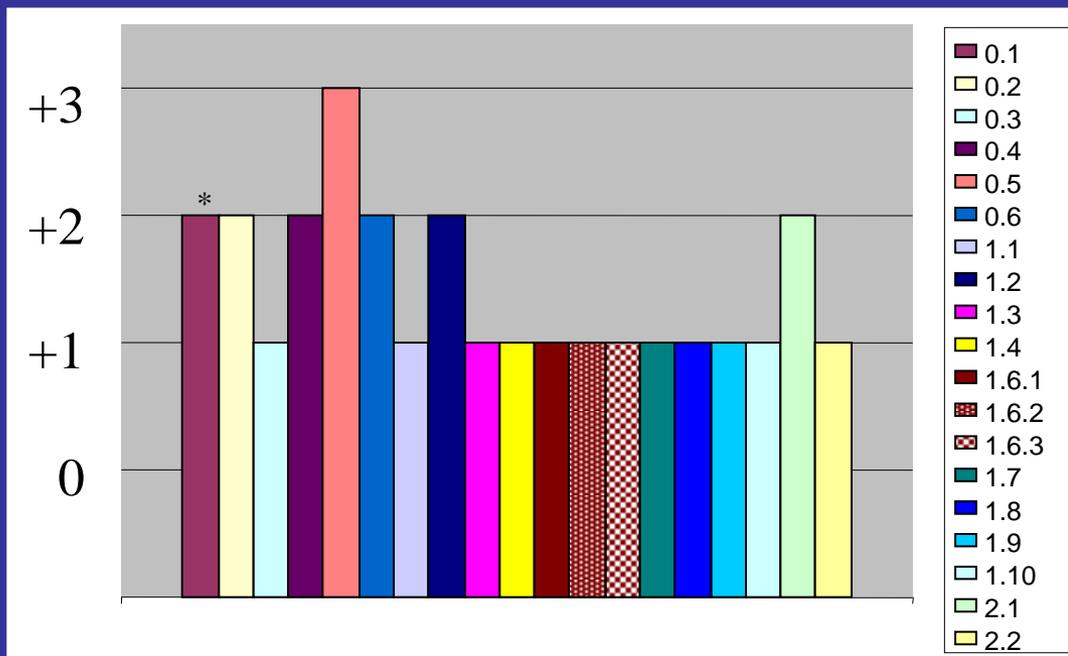




HUVN: REGISTRO 2



REGISTRO 2



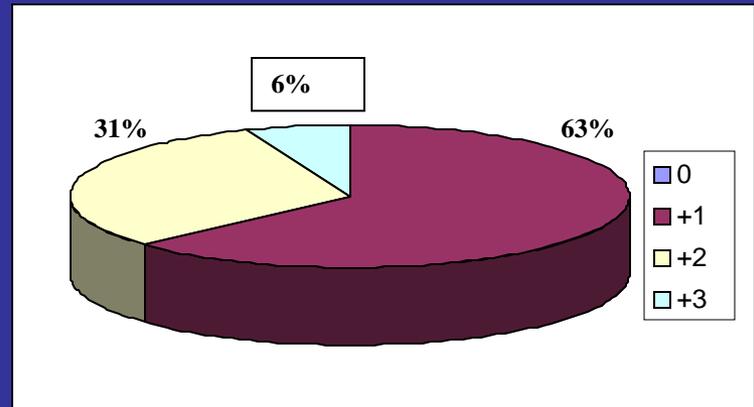
* + 2 CDI/+3 CID

TÉCNICA DE TINCIÓN UTILIZADA: HERCEPTEST

PUNTUACIÓN H.U. VIRGEN DE LAS NIEVES .- +3/0
 infiltrante; +3 in situ

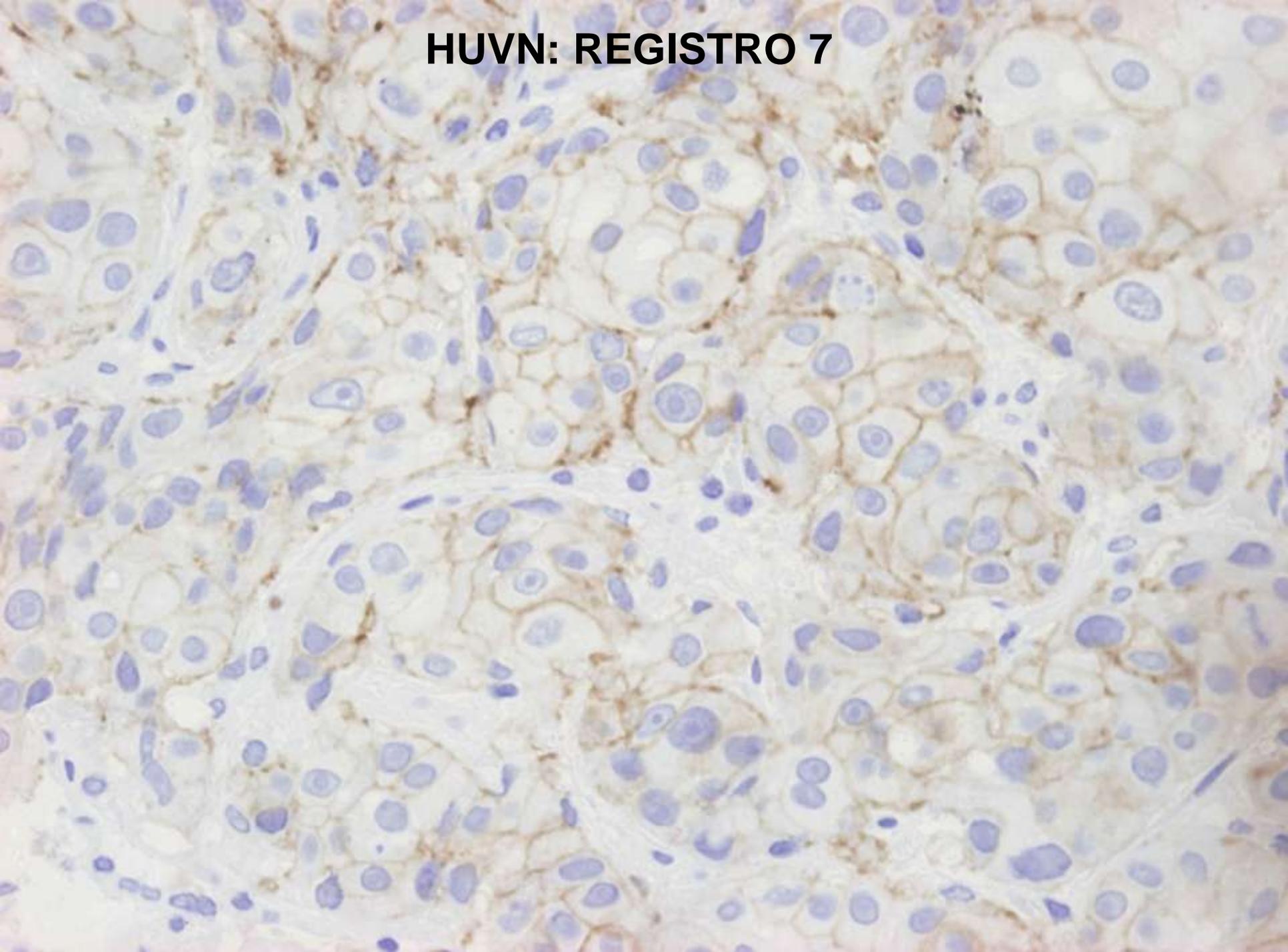
RESULTADO DETERMINACIÓN FISH:
NEGATIVO (1-2 HER-2/centrómero)

A. Evaluación de las tinciones realizadas en el H.U. Virgen de las Nieves

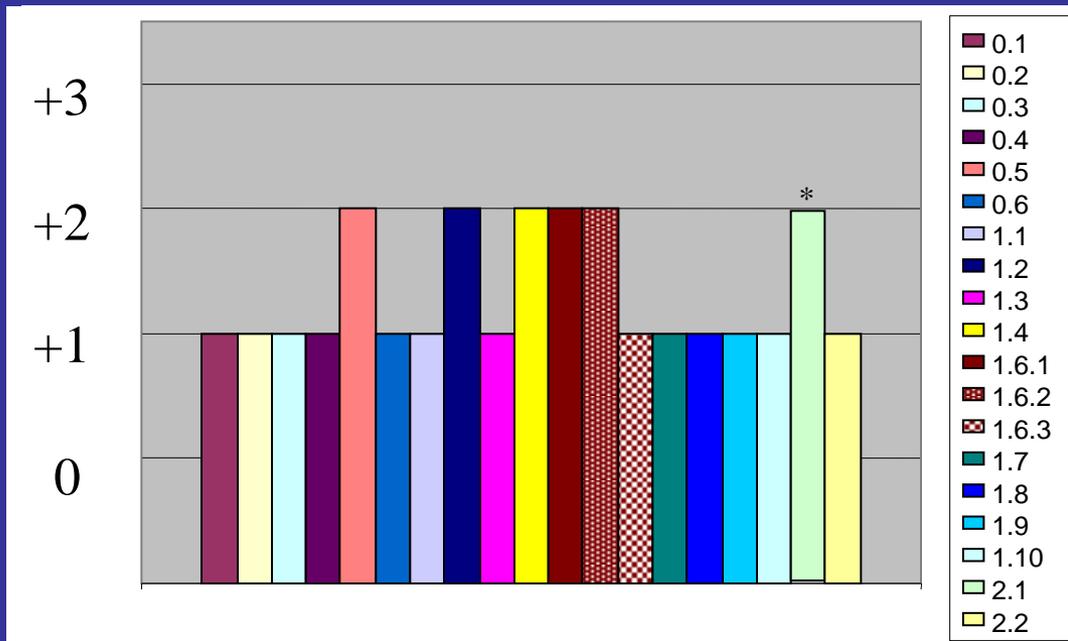


En un recuadro el porcentaje de hospitales que coinciden con el de referencia

HUVN: REGISTRO 7

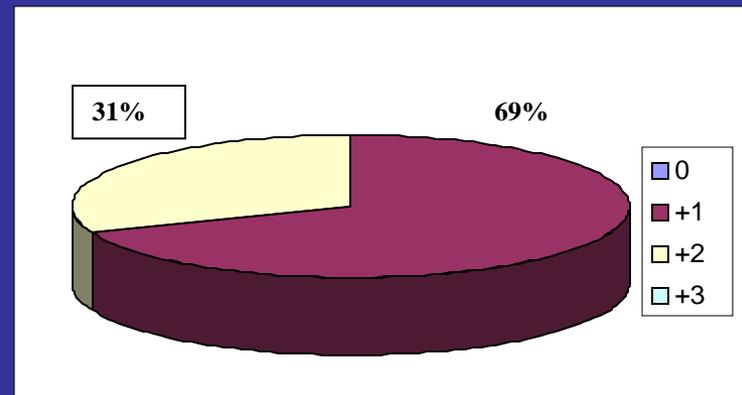


REGISTRO 7



* +2/+3

A. Evaluación de las tinciones realizadas en el H.U. Virgen de las Nieves



En un recuadro el porcentaje de hospitales que coinciden con el de referencia

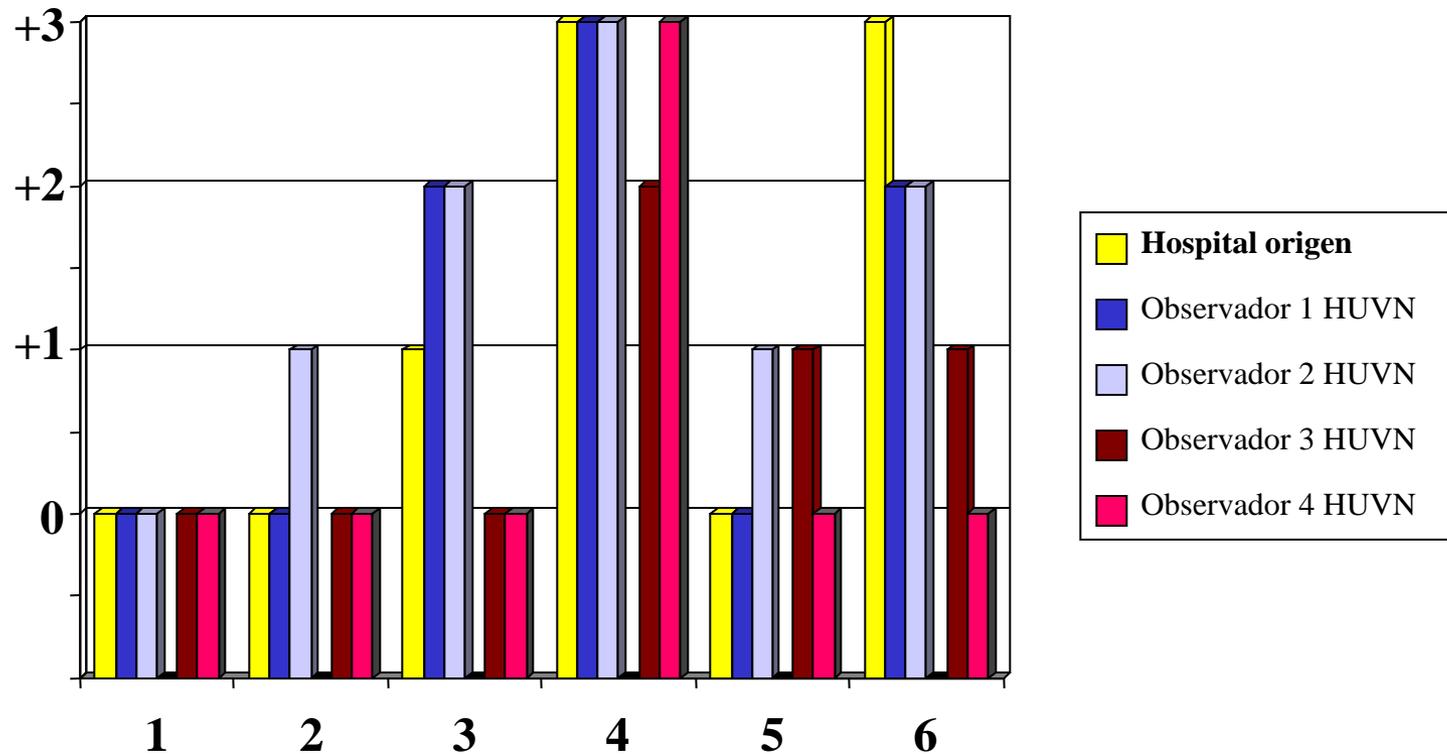
TÉCNICA DE TINCIÓN UTILIZADA: HERCEPTEST

PUNTUACIÓN H.U. VIRGEN DE LAS NIEVES .- **+2**

RESULTADO DETERMINACIÓN FISH:
NEGATIVO (1-2 HER-2/centrómero)



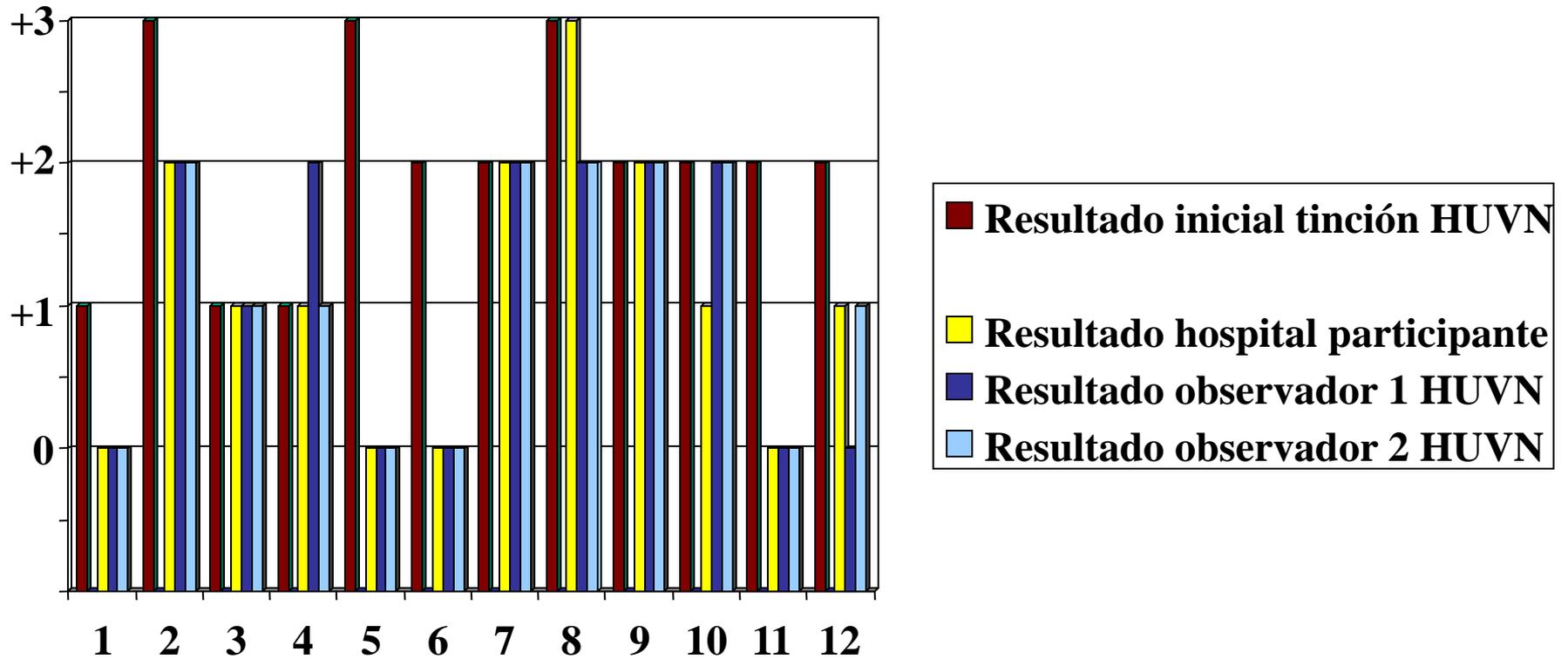
HOSPITAL CON CÓDIGO 0.2



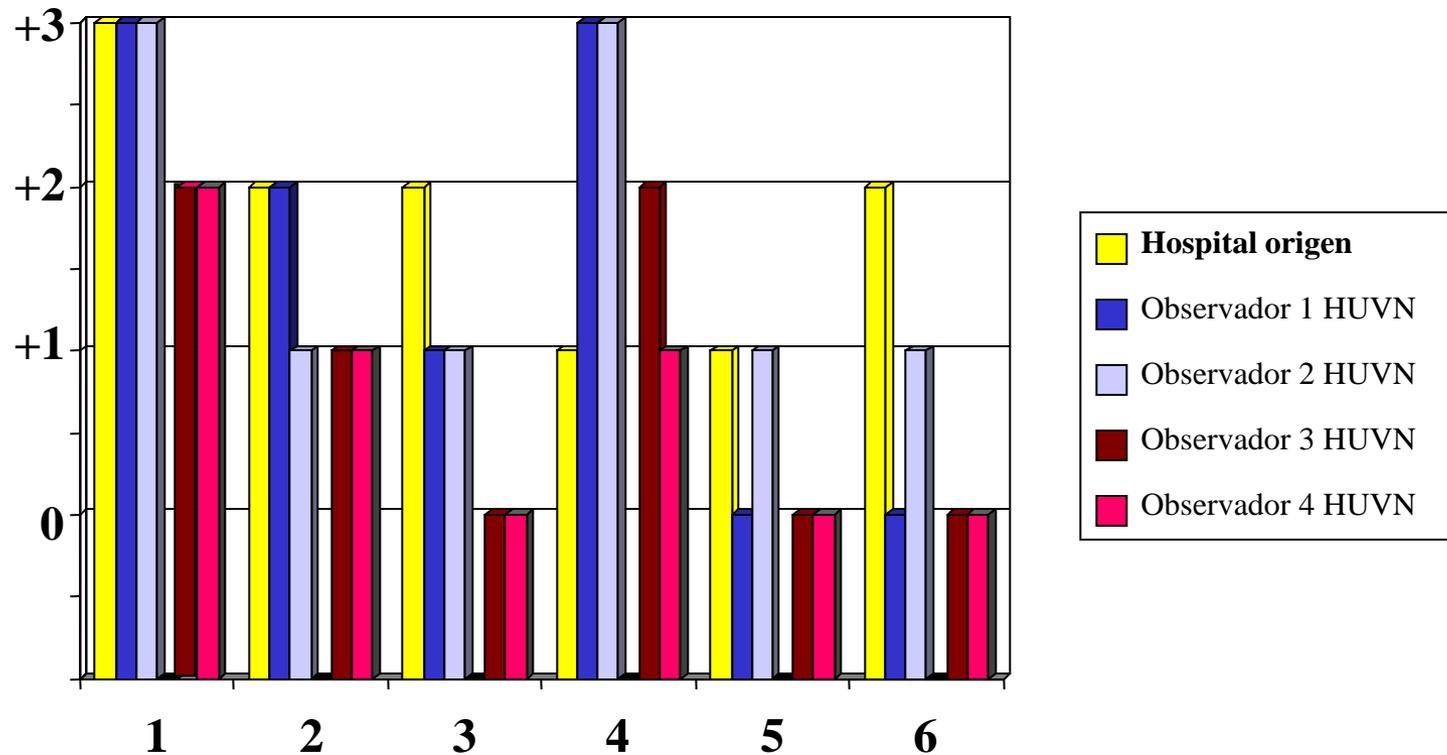
**TÉCNICA HOSPITAL DE ORIGEN: KIT COMERCIAL ZYMED
CON EL ANTICUERPO PRIMARIO TAB250**

TÉCNICA H.U. VIRGEN DE LAS NIEVES: HERCEPTEST

HOSPITAL CON CÓDIGO 2.4



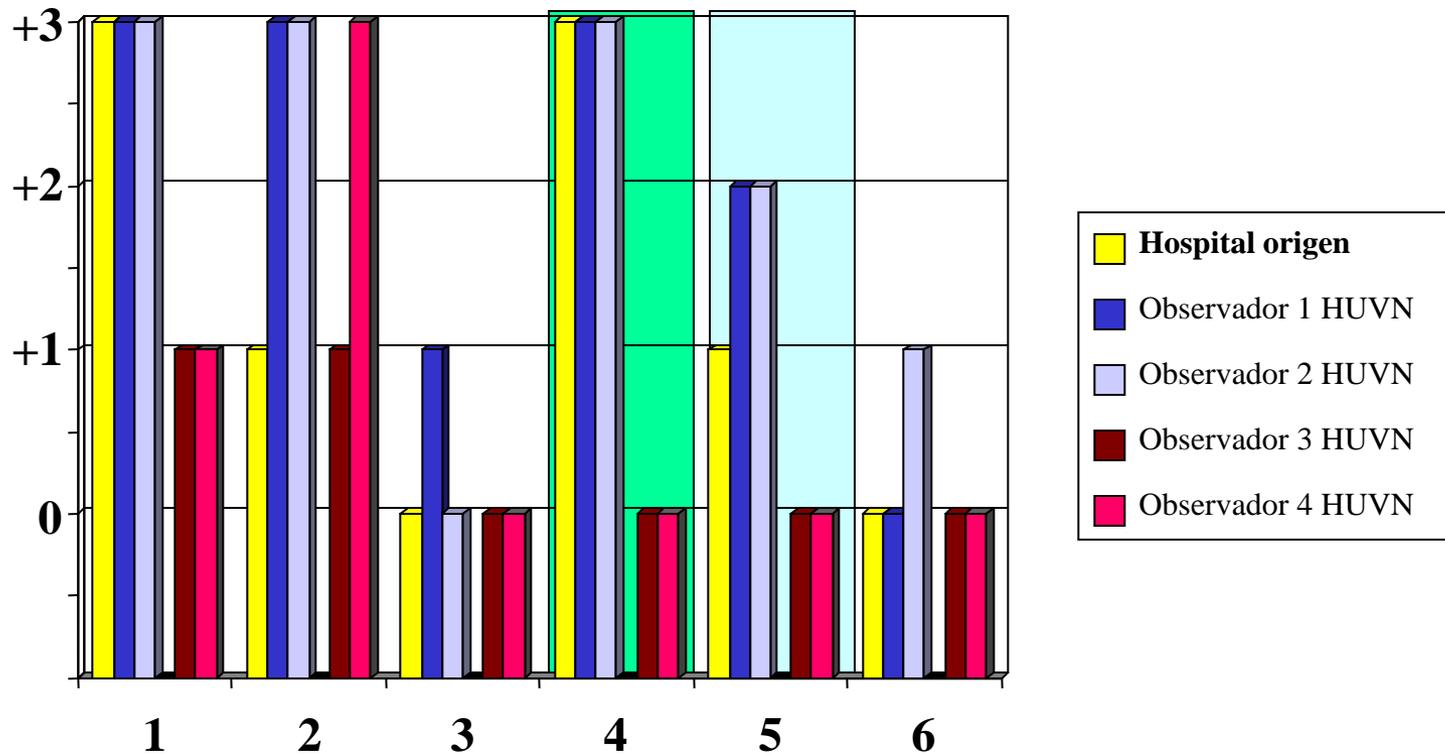
HOSPITAL CON CÓDIGO 0.6



TÉCNICA HOSPITAL DE ORIGEN: HERCEPTEST (DAKOCYTOMATION)SEGÚN INSTRUCCIONES DEL FABRICANTE, UTILIZANDO INMUNOTEÑIDOR DAKO-AUTOSTAINER

TÉCNICA H.U. VIRGEN DE LAS NIEVES: HERCEPTEST

HOSPITAL CON CÓDIGO 1.1



**FISH REGISTRO 4: NEGATIVO
(1-2 HER-2/centrómero)**

**FISH REGISTRO 5: NEGATIVO
(1-2 HER-2/centrómero)**

RESULTADOS EXPRESIÓN

	0	+1	+2	+3
PULMÓN	70% (14/20)	10% (2/20)	5% (1/20)	15% (3/20)
VEJIGA	20% (9/45)	15,5% (7/45)	20% (9/45)	44,5% (20/45)
OVARIO	47,5% (19/40)	25% (10/40)	22,5% (9/40)	5% (2/40)
PRÓSTATA	66,7% (16/24)	8,3% (2/24)	12,5% (3/24)	12,5% (3/24)

VEJIGA

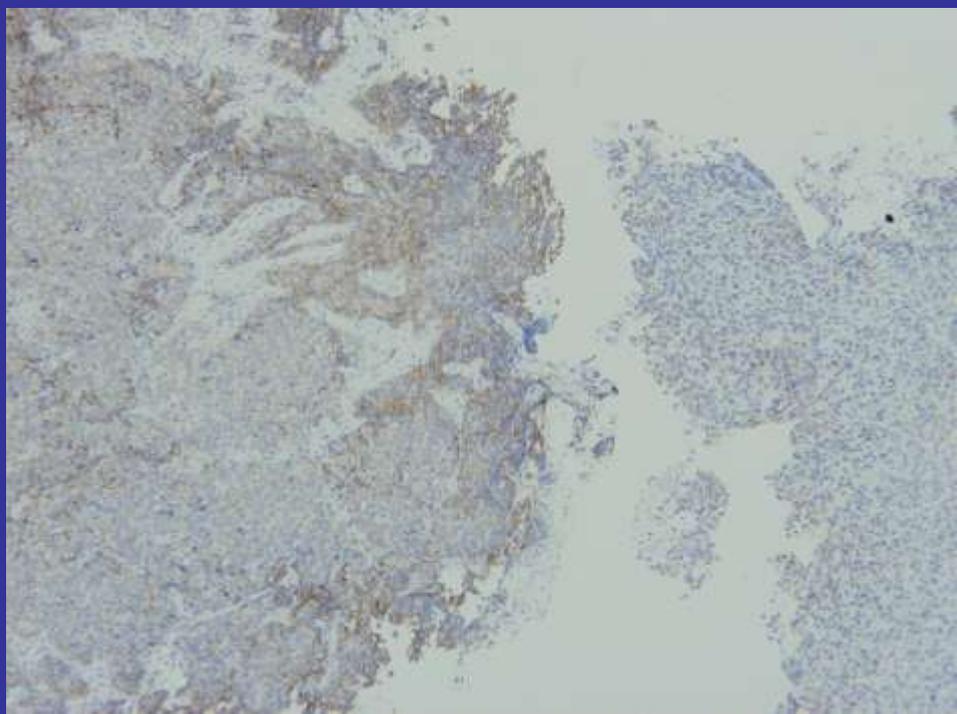
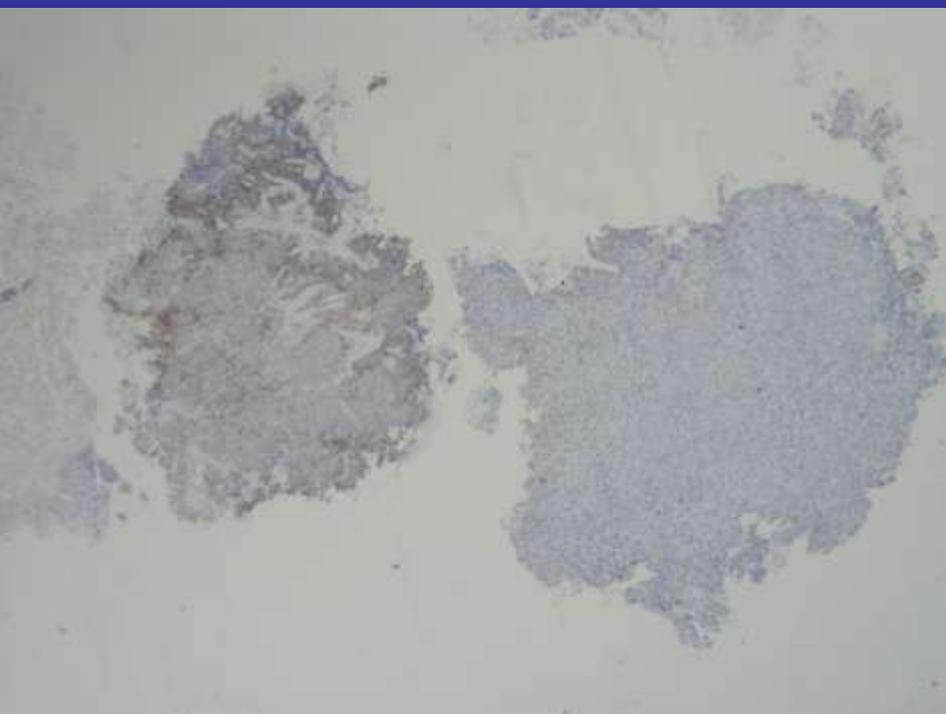
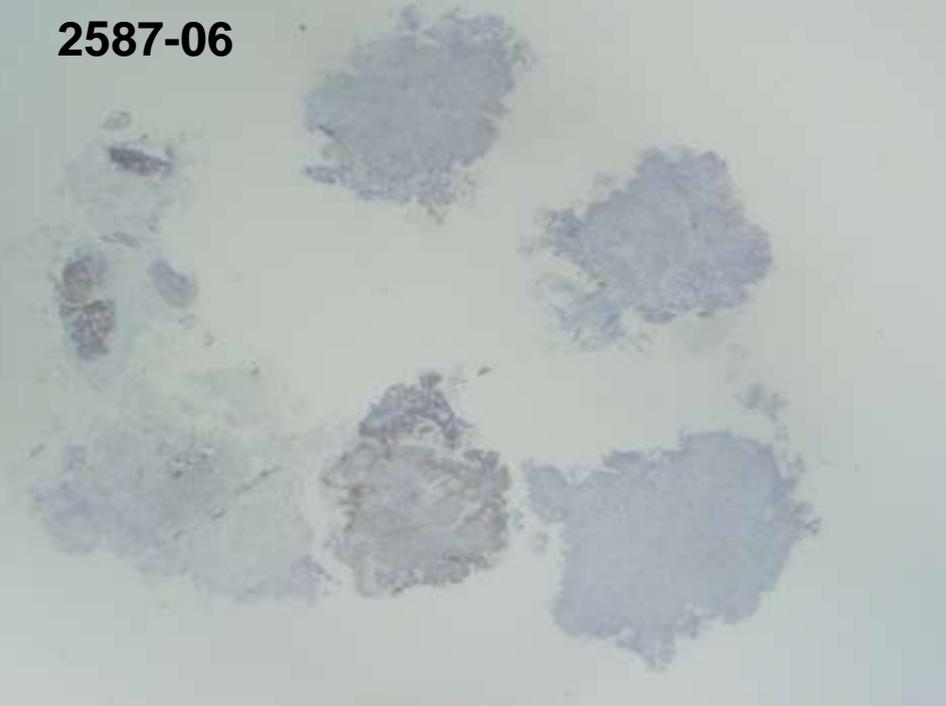
- **17-70% IHQ (+)**

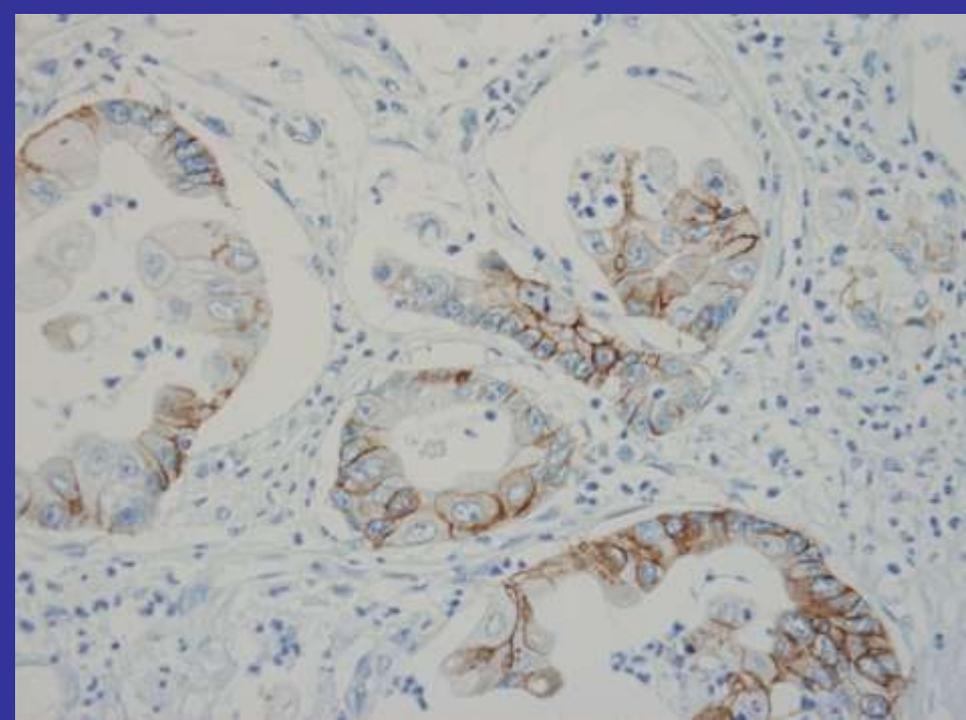
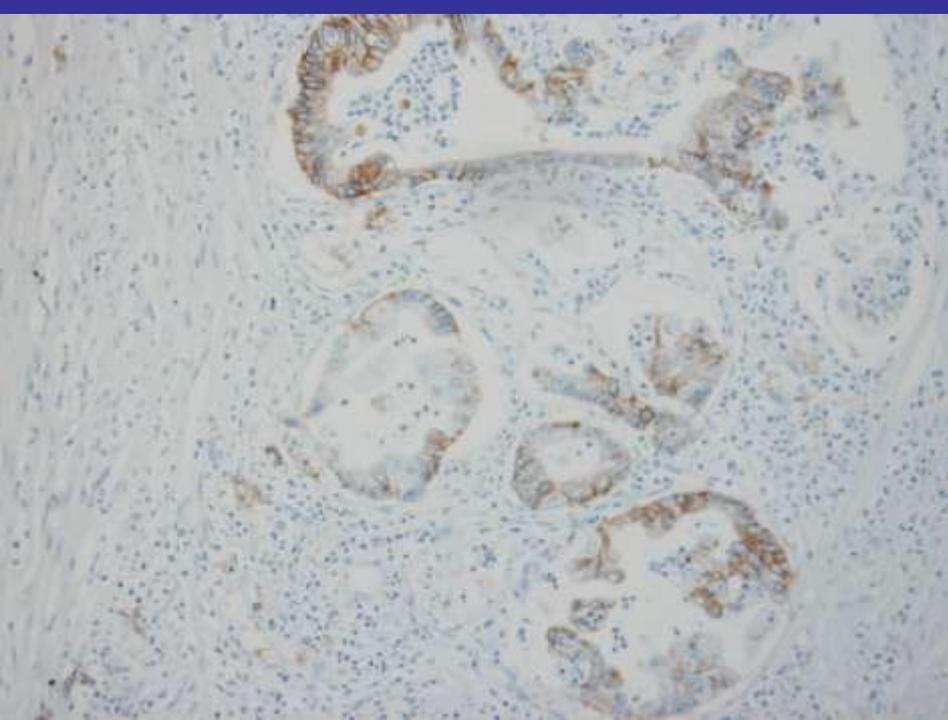
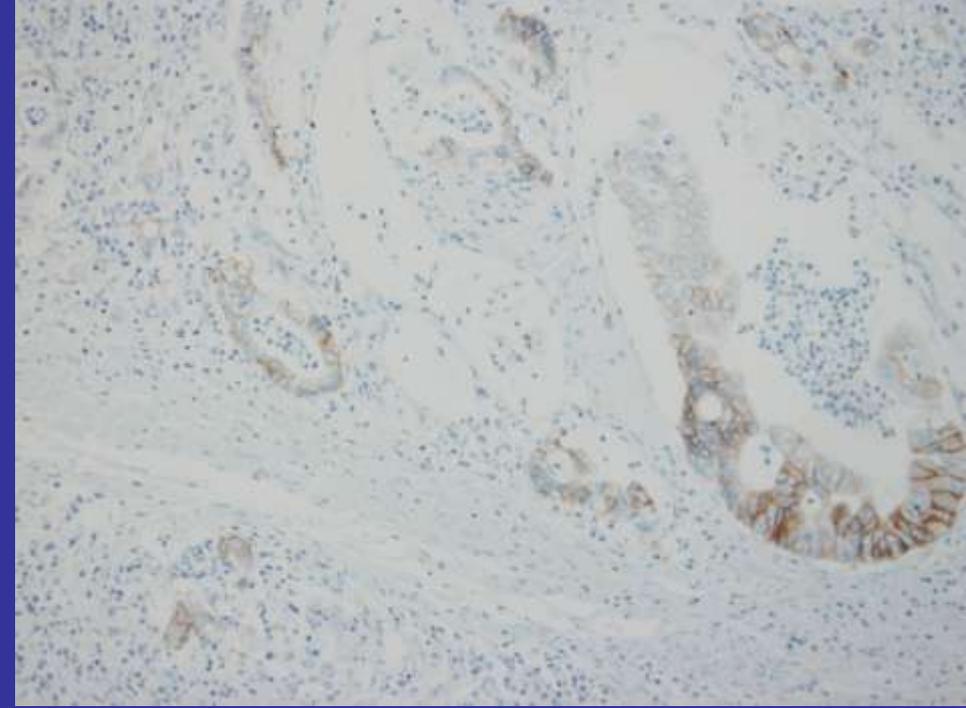
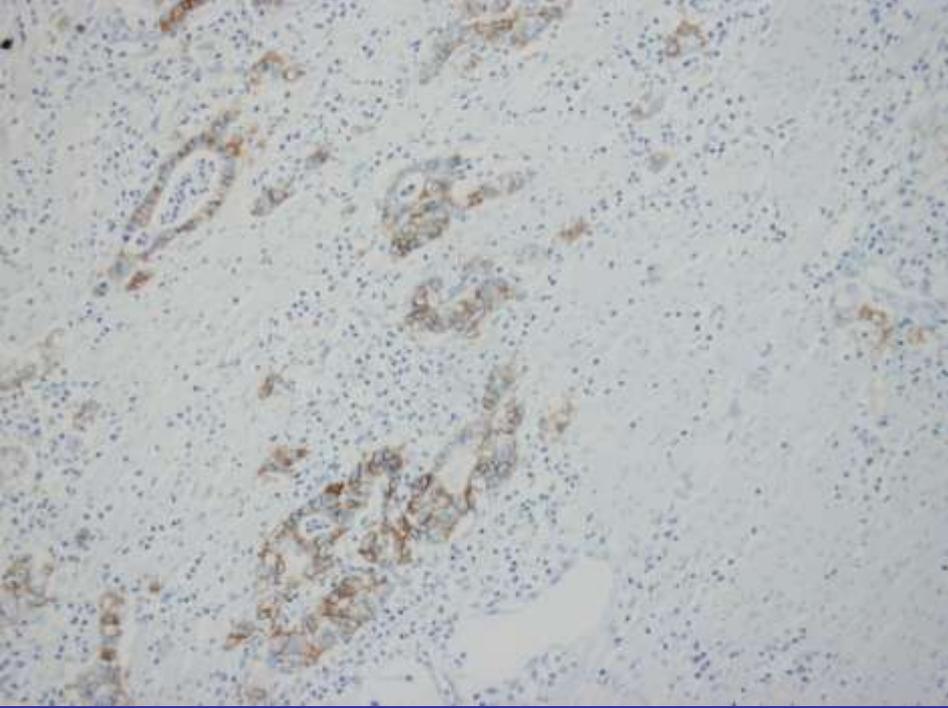
- » **INVASIÓN**
- » **ESTADIO AVANZADO/METÁSTASIS**
- » **GRADO DE DIFERENCIACIÓN**
- » **MAL PRONÓSTICO (p53)**
- » **LESIONES PRECURSORAS (“IN SITU” PLANO) 45%**
- » **CAMBIOS REACTIVOS/DISPLÁSICOS (12%)**
- » **MODIFICACIONES CON BCG (Mitomicina?)**
- » **HER-2/HER-3: MAYOR AGRESIVIDAD**

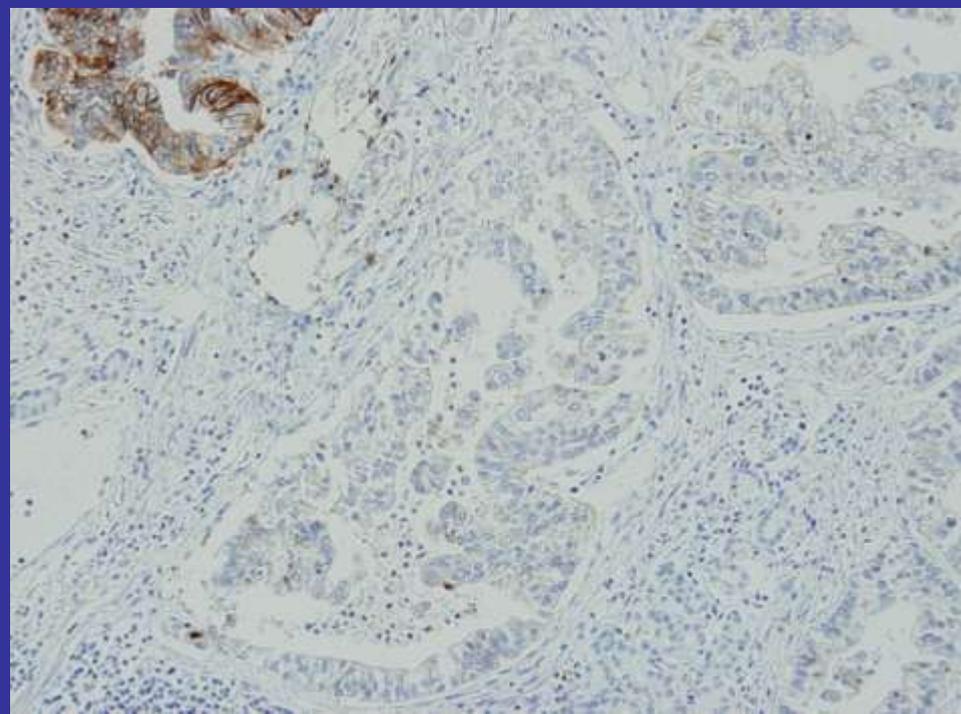
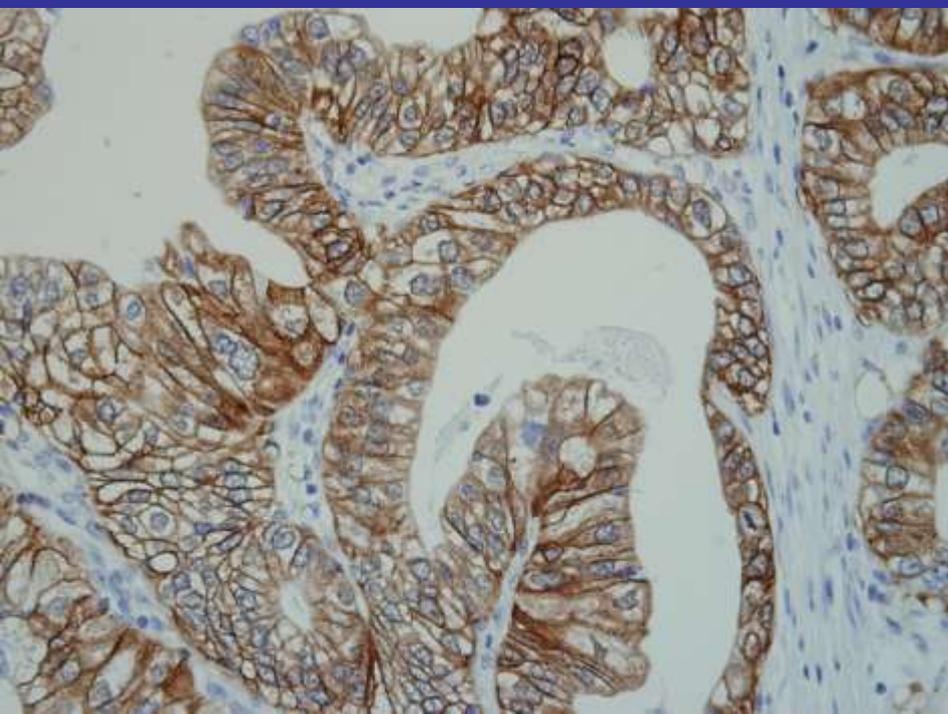
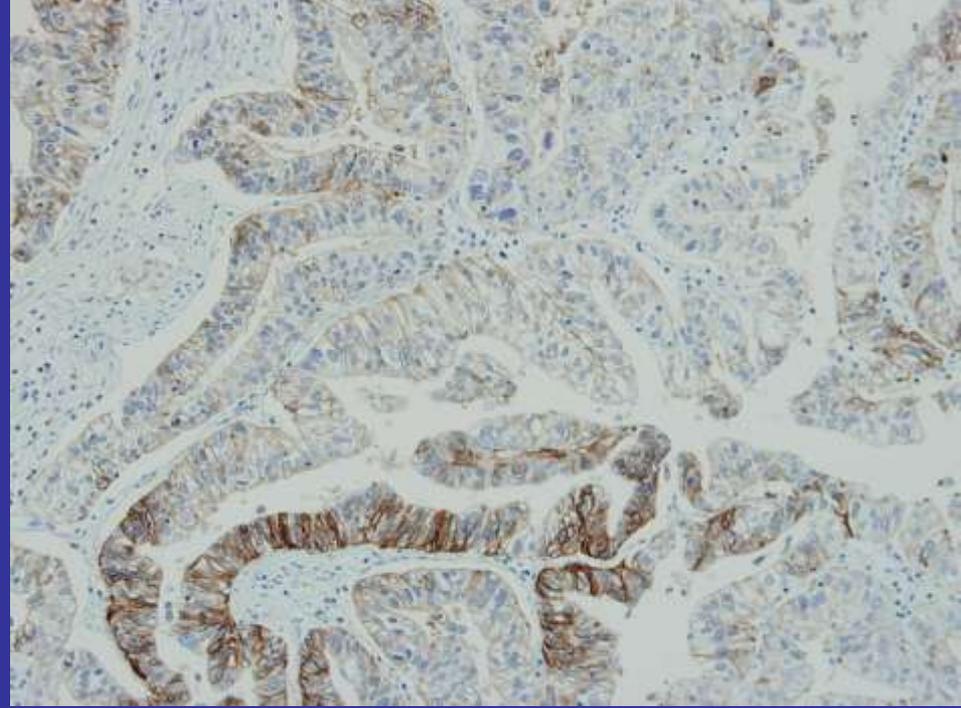
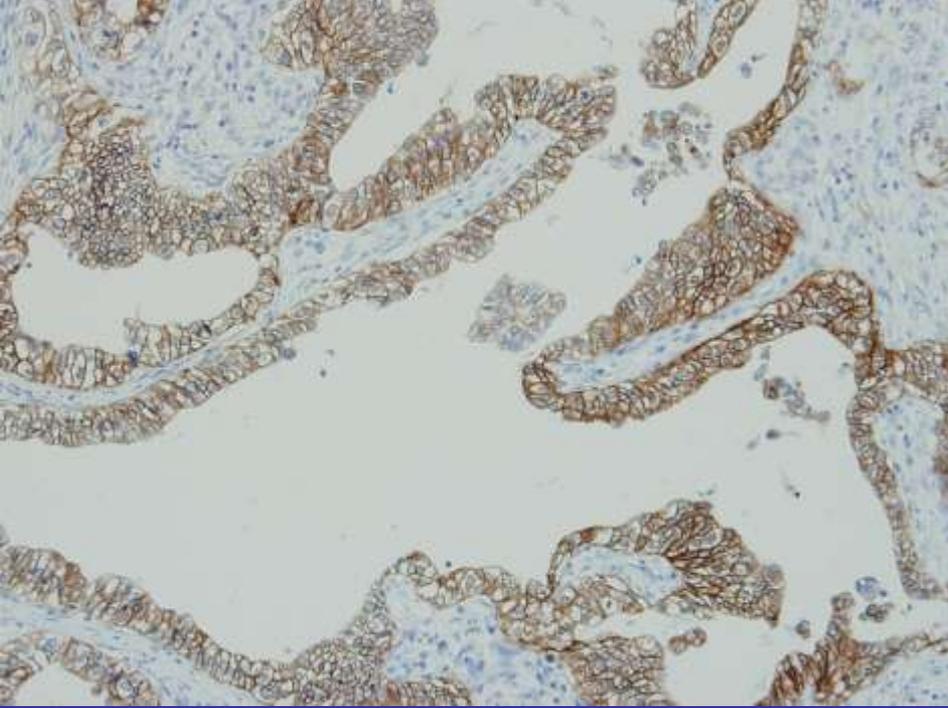
- **FISH**

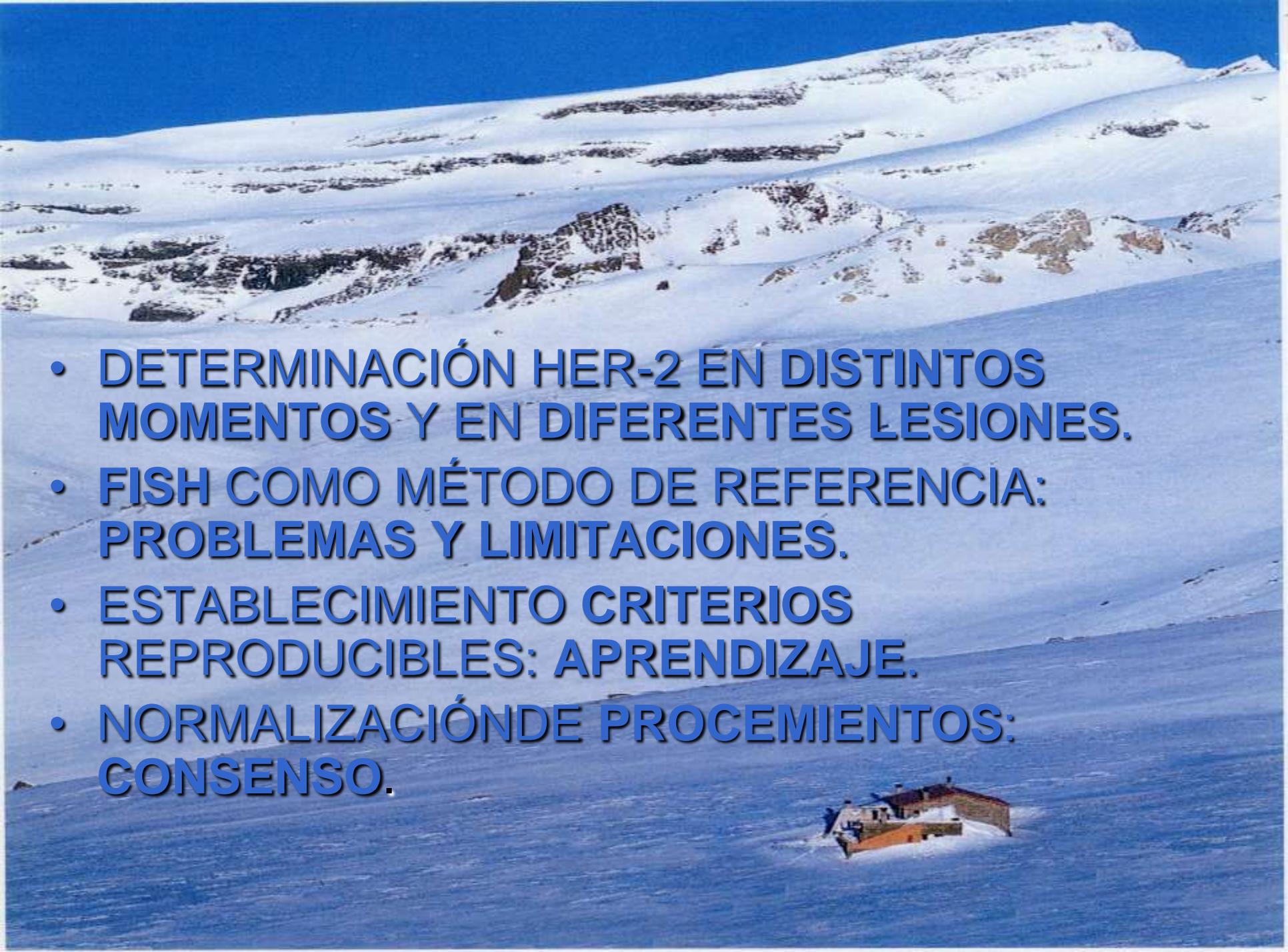
- » **ANEUSOMÍA 17 (70%)**
- » **COPIAS HER-2 (41%)**
- » **RELACIÓN HER-2/CROMOSOMA 17 (4%)**

2587-06







- 
- A photograph of a snowy mountain range under a clear blue sky. In the foreground, a small, rustic cabin with a dark roof and light-colored walls is situated on a snow-covered slope. The cabin has a chimney and a small porch. The snow is bright white, and the sky is a deep, clear blue. The overall scene is serene and cold.
- **DETERMINACIÓN HER-2 EN DISTINTOS MOMENTOS Y EN DIFERENTES LESIONES.**
 - **FISH COMO MÉTODO DE REFERENCIA: PROBLEMAS Y LIMITACIONES.**
 - **ESTABLECIMIENTO CRITERIOS REPRODUCIBLES: APRENDIZAJE.**
 - **NORMALIZACIÓN DE PROCEDIMIENTOS: CONSENSO.**

Reunión de Consenso de Patólogos de Andalucía, Canarias, Extremadura y Portugal sobre Determinación de la Expresión de HER-2 en Cáncer de Mama



GUÍA PRÁCTICA

Dr. Francisco J. Vera-Sempere, Dra. M^a José Calasanz, Dr. Xavier Andreu, Dr. Santiago Ramón y Cajal, Dr. José M^a Corominas, Dr. José Palacios, Dr. Joan Albanell, Dr. Tomás García-Caballero, Dr. Fernando López-Ríos, Dr. José Antonio López y Dr. Ángel Concha.



GRACIAS