

# Erb-B2 beim Mammakarzinom

- Abhängig vom verwendeten Antikörper zeigen immunhistochemisch 2 - 30% aller Mammakarzinome eine Überexpression des erb-B2
- Überexpression ist assoziiert mit
  - Schlechter Prognose bei nodal-positiven Patienten
  - Zyklophosphamid/MTX/5-FU Resistenz
  - Ansprechen auf Anthrazyklin-enthaltende Protokolle
- Chemotherapie kombiniert mit dem humanisierten monoklonalen Antikörper (4D5/trastuzumab) verlängert des Überleben bei erb-B2 überexprimierenden metastasierten Mammakarzinomen

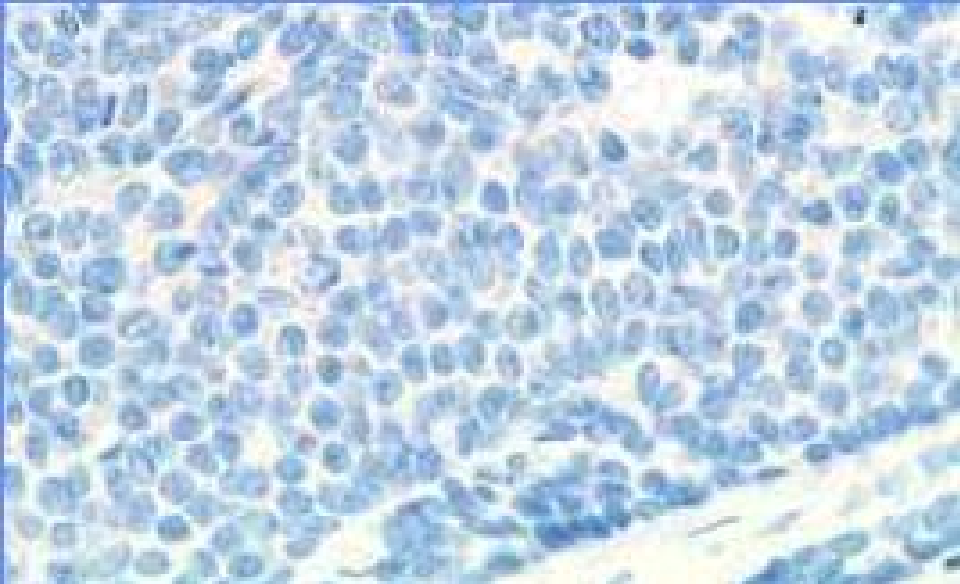
# FDA-approved Tests for erbB-2/ HER-2 in Breast Cancer

Test	Type	Indication
Hercep Test (Dako)	IHC	Suitability for Herceptin
PathVysion (Vysis)	FISH	Assessing prognosis Predicting response to standard chemotherapy
Inform (Oncor/Ventana)	FISH	Assessing prognosis

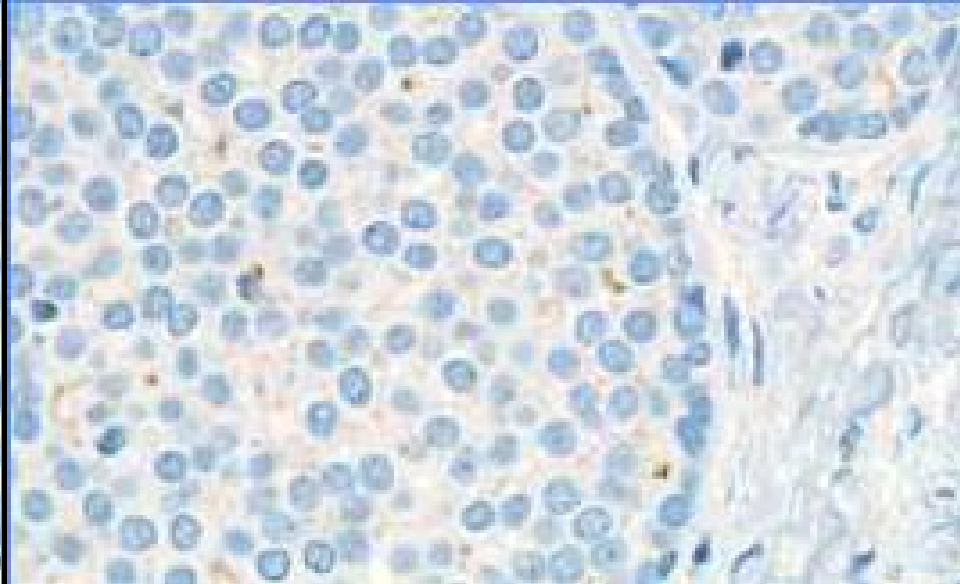
IHC, immunohistochemistry; FISH, fluorescence in situ hybridization

# Recommendations for the interpretation of HercepTest

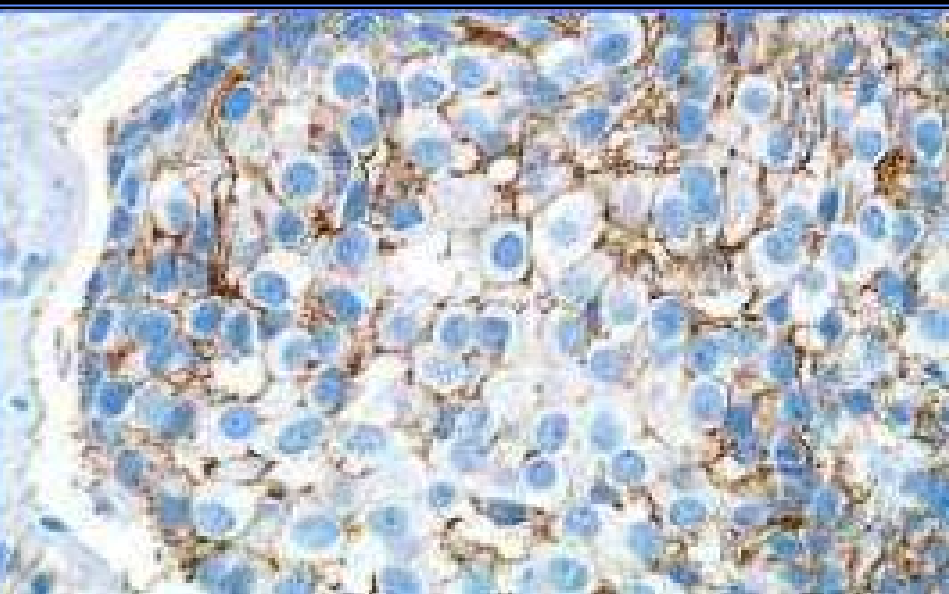
- Include only the invasive part of a tumor in the interpretation.
- Determination of HER2 protein overexpression is based on complete circumferential staining of the membrane, staining intensity and percentage of positive tumor cells.
- Evaluate the tissue section at a low 4x magnification. Look for membrane staining, intensity of staining and the percentage of positive tumor cells.
- Verify integrity of complete circumferential membrane staining with the use of 10x magnification.
- Confirm score using 20x magnification; 40x if necessary
- Evaluate supplied cell line control slide and positive and negative tissue control slides to validate assay performance.



**HercepTest-Score 0**



**HercepTest-Score 1**



**HercepTest-Score 2**



**HercepTest-Score 3**

# DAKO HercepTest: Auswertungsrichtlinien

## 0 Negativ

Keine Färbung oder Membranfärbung in weniger als 10% der Tumorzellen

## 1+ Negativ

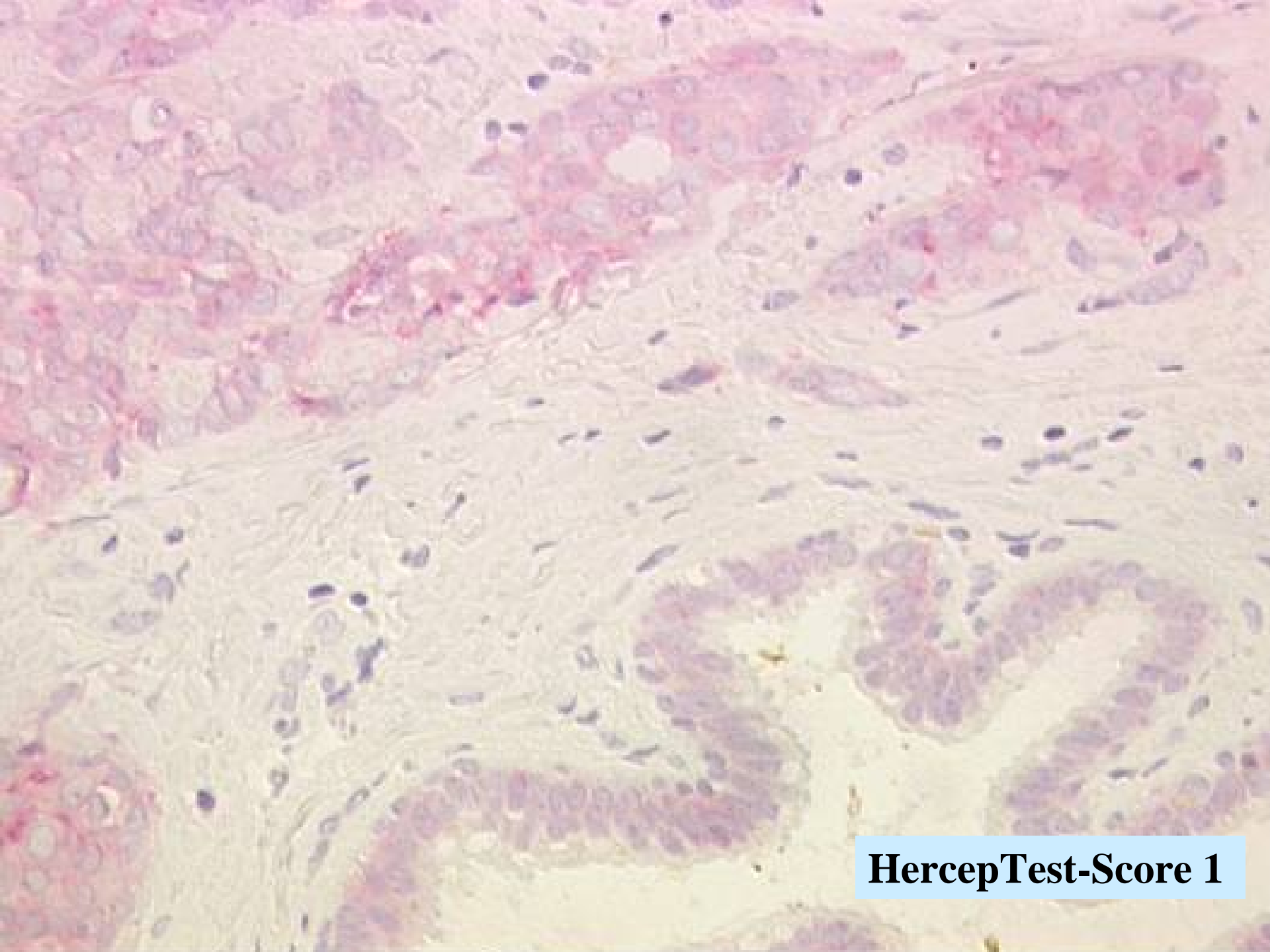
Sehr schwache / kaum wahrnehmbare Membranfärbung in mehr als 10% der Tumorzellen. Es ist jeweils nur ein Teil der Zellmembran gefärbt

## 2+ Schwach positiv

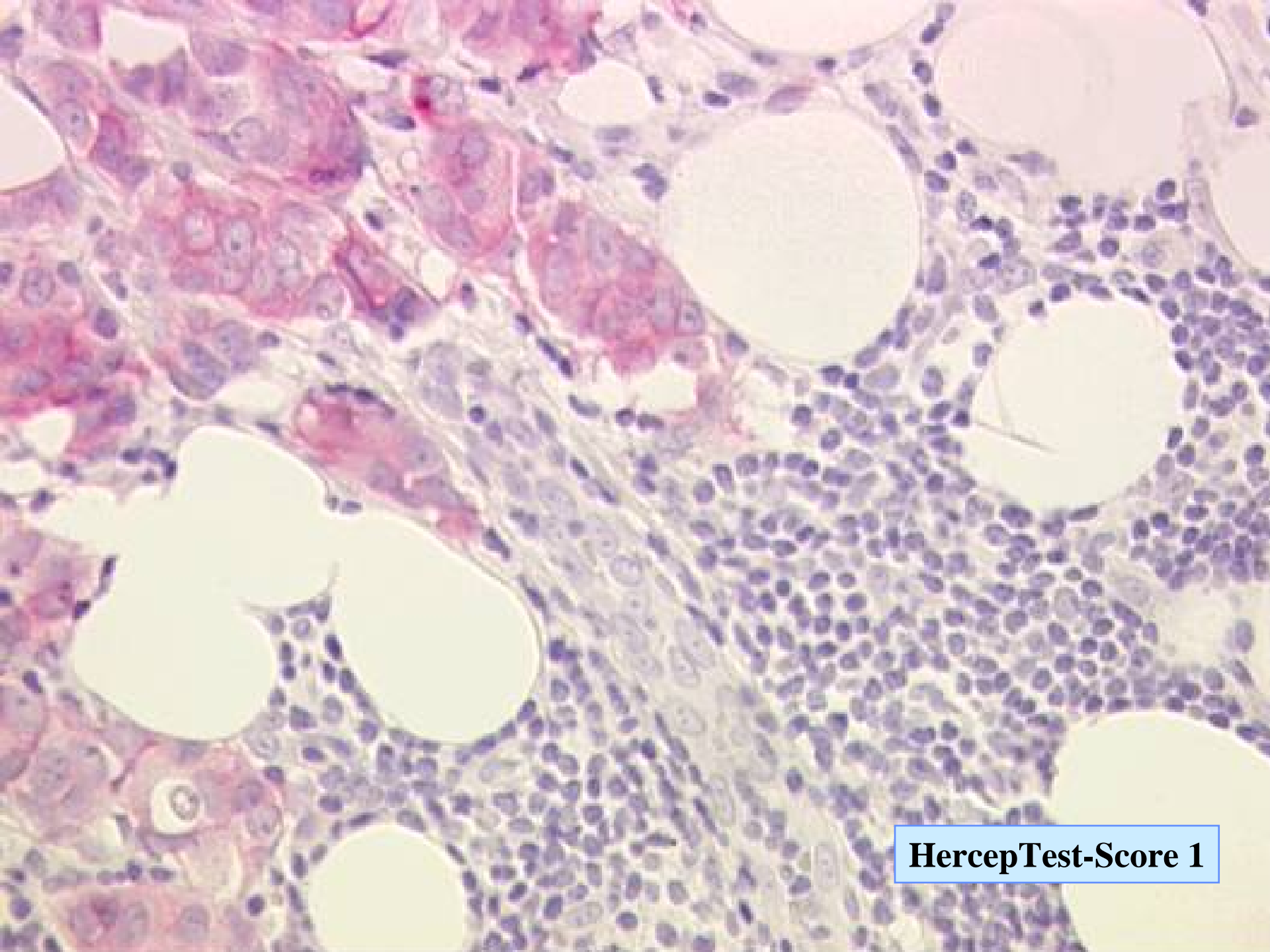
Schwache bis mässig intensive Färbung der gesamten Zellmembran in mehr als 10% der Tumorzellen

## 3+ Stark positiv

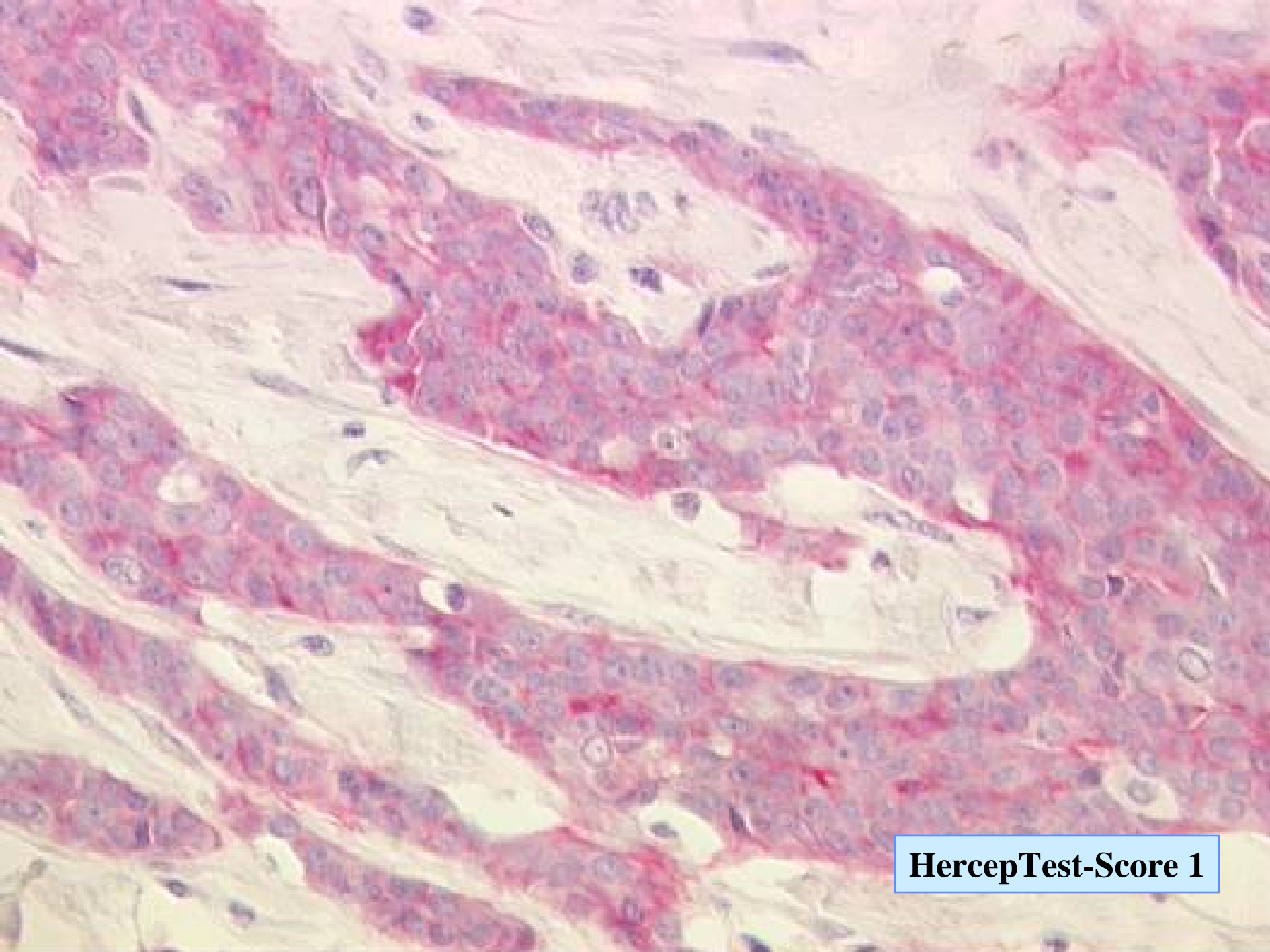
Intensive Färbung der gesamten Zellmembran in mehr als 10% der Tumorzellen



**HercepTest-Score 1**



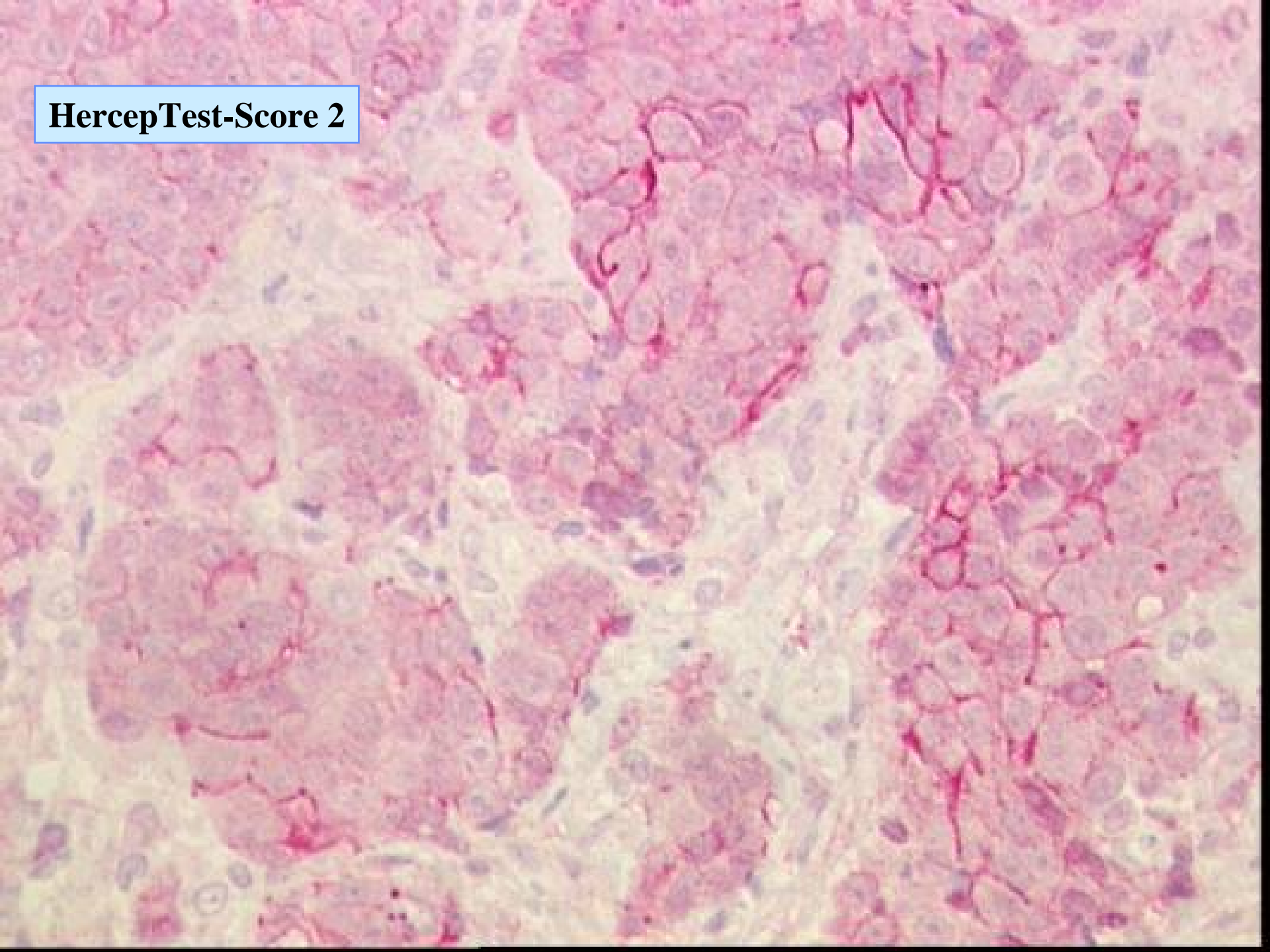
**HercepTest-Score 1**



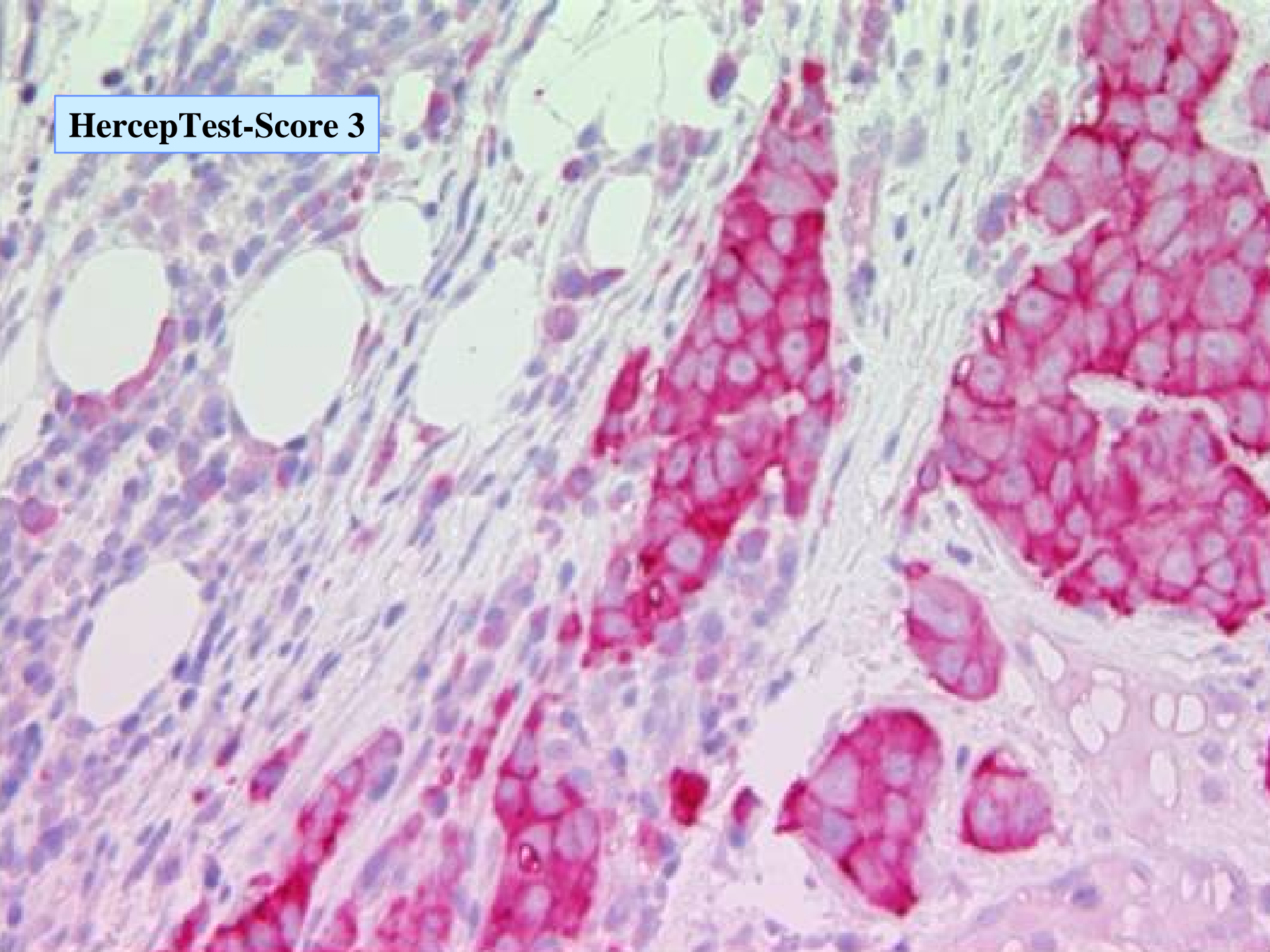
**HercepTest-Score 1**



**HercepTest-Score 2**



**HercepTest-Score 3**



# Immunohistochemische erb-B2 Analytik: Tips

- **Blockauswahl**

Möglichst kompakte, normalgewebetragende  
Gewebsstücke mit gutem Fixationszustand

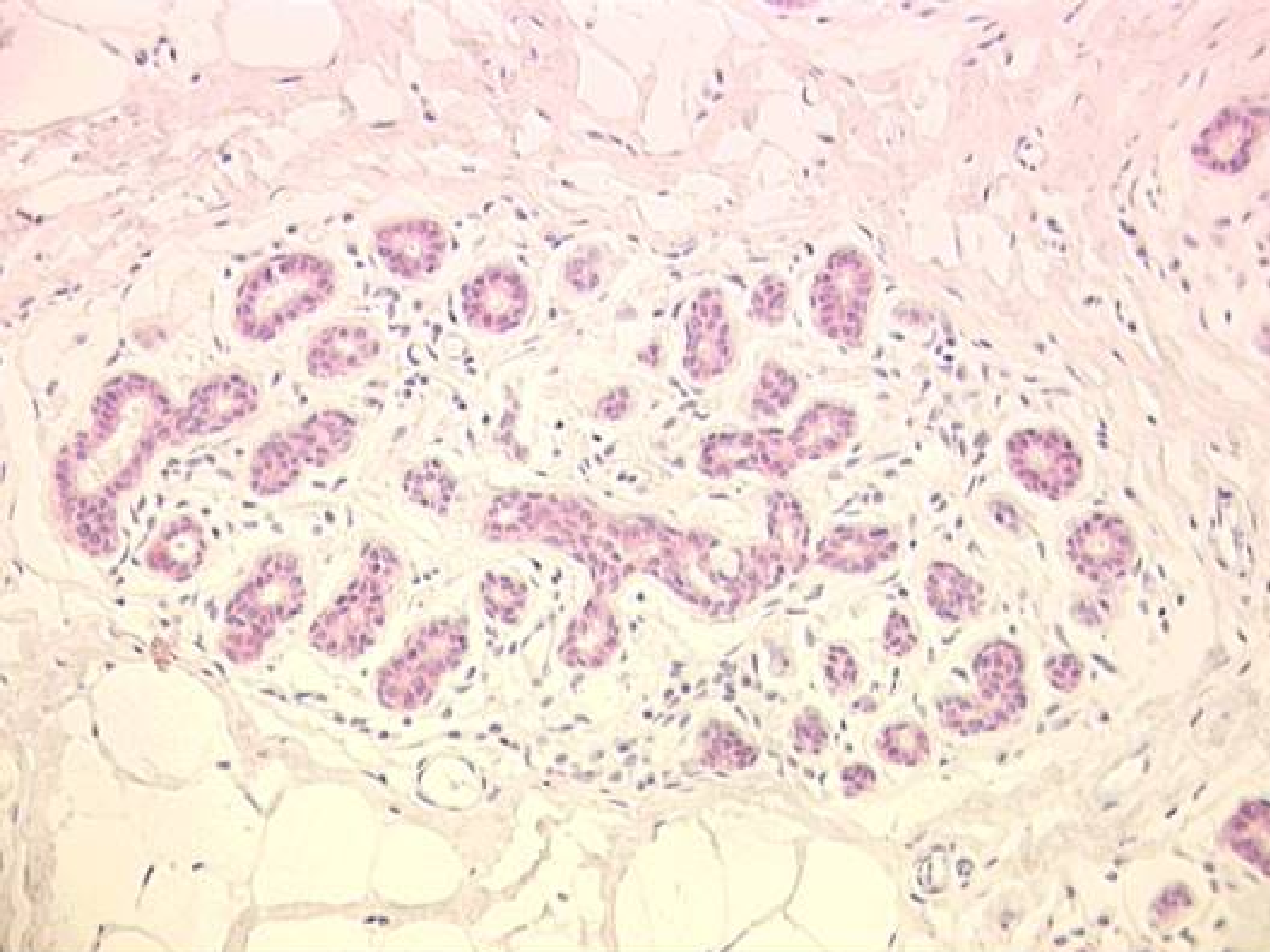
- **Interne Kontrolle**

Genuine Epithelien sollten schwache  
zytoplasmatische Expression zeigen

Intraduktale Karzinomanteile häufig kräftig  
membranös markiert

- **Inter-Assay Kontrolle**

“cell blocks” mit erb-B2 Status definierten Zelllinien-  
pellets mitführen



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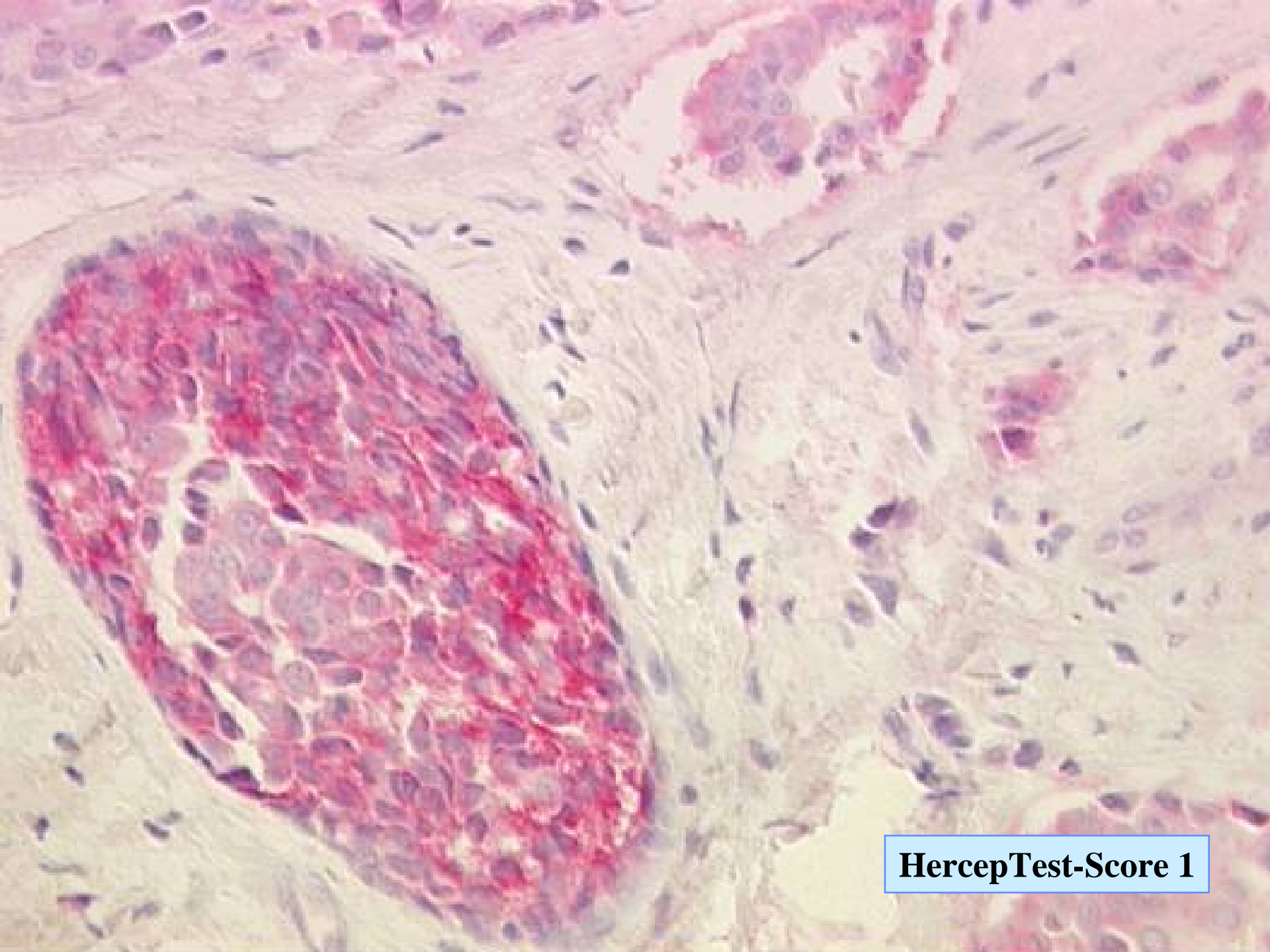
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**HercepTest-Score 1**

# Immunohistochemische erb-B2 Analytik: Tips

- **Normalisierung**

Die tumorzelluläre erb-B2 Expression sollte gegen genuine, nicht-neoplastische Epithelien gewichtet werden.

Wenn nicht-neoplastische Epithelien negativ sind Score anheben.

Wenn nicht-neoplastische Epithelien positiv sind Score absenken.

# Interobserver Reproducibility of Her-2/neu Protein Overexpression in IDC with HercepTest

- 46 cases of IDC evaluated among 5 pathologists
- Complete agreement was achieved in 22/46 (48%) cases (kappa 0.80)
- Discrepancies between negative (0, 1+) and positive (2+, 3+) results occurred in 2 cases (kappa = 0.96)
- Distinguishing weakly (2+) from strongly (3+) positive results showed agreement in only 13/22 (59%) positive cases (kappa 0.38)
- If more than 50% of tumor cells revealing strong complete membrane staining were regarded as strongly positive, agreement would be improved (kappa 0.78)

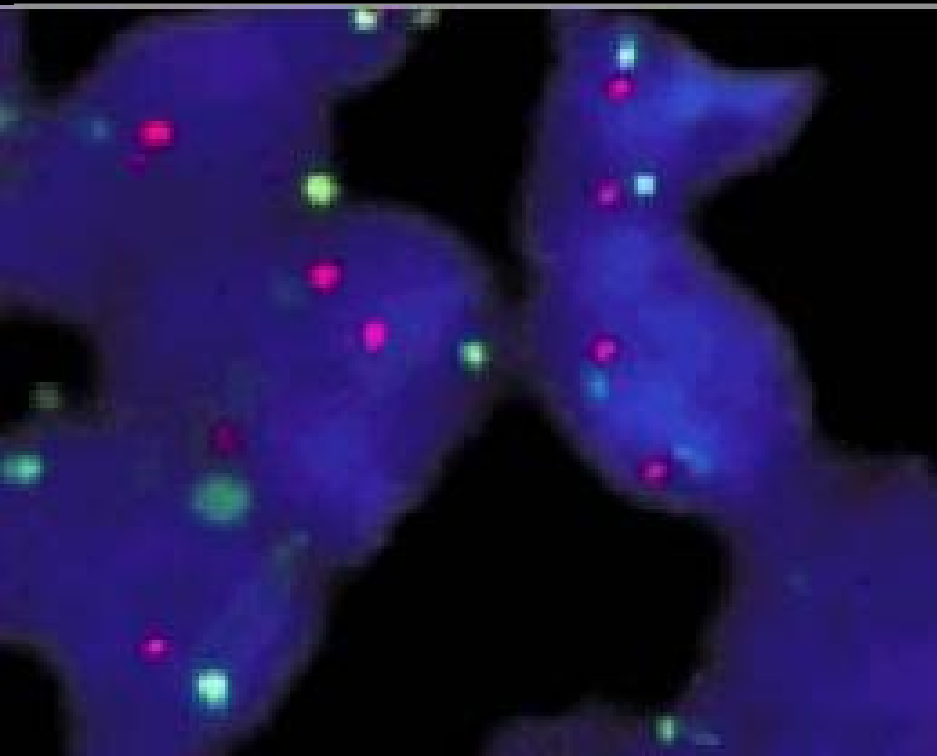


# ISH Assays for erbB-2/ HER-2 in Breast Cancer

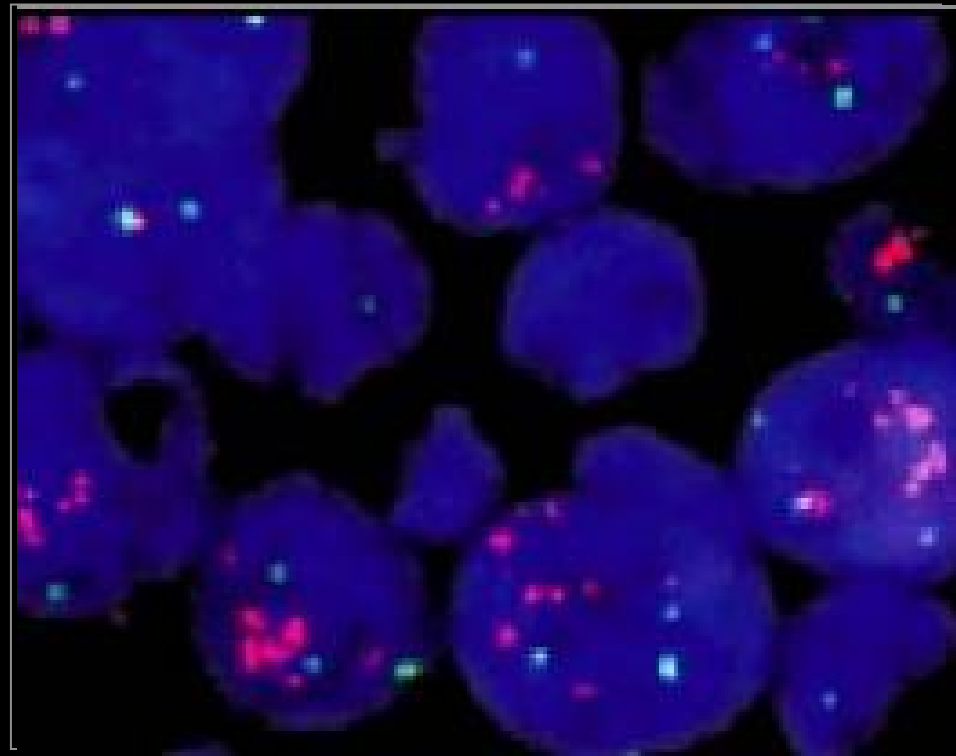
Test	Type	Principle	Supplier
PathVysion	FISH	dual color	Vysis/Abbott
<i>HER2</i> pharmDx	FISH	dual color	DAKO
Inform	FISH	single color	Oncor/Ventana
SPOT-Light	CISH	chromogen	Zymed
GOLDFISH	CARD-ISH	nanogold	Nanoprobes

FISH, fluorescence in situ hybridization; CISH, chromogenic in situ hybridization; CARD, catalyzed reporter deposition

# Dual-color FISH: PathVysion HER-2 Assay



unamplified  
(HER-2/CEP 17 ratio  $<2.0$ )



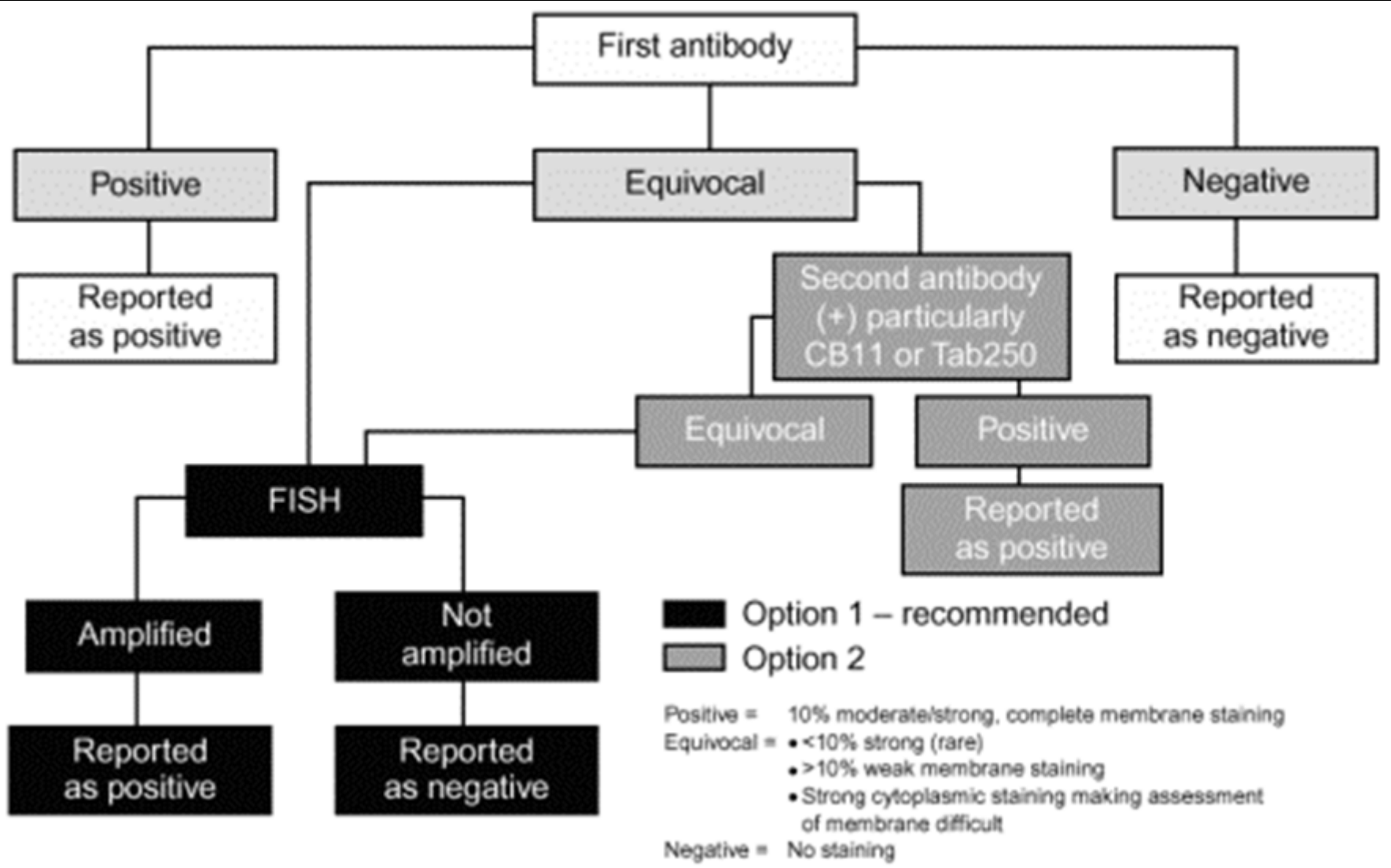
amplified  
(HER-2/CEP 17 ratio  $>2.2$ )

HER-2 genomic probe

CEP 17 17p11.1-q11.1 alpha satellite probe

SpectrumOrange-labeled

SpectrumGreen-labeled



# Real-world performance of erbB-2 testing: NSABP protocol B-31

		Negative cases detected at the central testing facility	
Test used for eligibility	Type of laboratory	HercepTest IHC	PathVysion FISH
HercepTest 3+	small	10/52	12/52
n=80	large	1/28	1/28
Other IHC	small	11/23	9/23
n=24	large	0/1	0/1

small-volume laboratories <99 tests per month

Paik et al., JNCI 2002;94:852

# Issues in Immunohistochemical HER2/erb-B2 Assays

- Selection of antibody
  - Intra- vs. extracellular domain
  - CB11 vs. HercepTest
- Selection of block/control tissue
  - cell blocks
- Variation in antigen-retrieval methods
- Scoring systems:
  - Herceptest Score vs. Non-neoplastic normalized score
- Alternative (to FISH) confirmatory assays needed

# Web-Adressen/URLs (Prefix <http://>)

## **erb-B2 Testverfahren Problematik:**

[www.cap.org/captoday/archive/2002/her2\\_feature.html](http://www.cap.org/captoday/archive/2002/her2_feature.html)

## **FDA Review Dokument:**

[www.fda.gov/ohrms/dockets/ac/01/briefing/3815b1\\_08\\_HER2%20FISH.doc](http://www.fda.gov/ohrms/dockets/ac/01/briefing/3815b1_08_HER2%20FISH.doc)

## **HercepTest Dako:**

[www.dako.com/index/prod\\_introduction/products\\_intro\\_pharmacodiagnosics.htm](http://www.dako.com/index/prod_introduction/products_intro_pharmacodiagnosics.htm)

[www.dakocytomation.com/herceptestquickguide.pdf](http://www.dakocytomation.com/herceptestquickguide.pdf)

## **Qbiogene markierte Zentromer-Sonden:**

[www.qbiogene.com/products/cytogenetics/2G-sat-prob-dc30.shtml](http://www.qbiogene.com/products/cytogenetics/2G-sat-prob-dc30.shtml)

## **VYSIS PathVysion erb-B2 Test:**

[www.vysis.com/PathVysionHER2DNAProbeKit\\_2462.asp?bhcp=1](http://www.vysis.com/PathVysionHER2DNAProbeKit_2462.asp?bhcp=1)

## **Ventana InForm erb-B2 Test:**

[www.ventanamed.com/products/reagents/her2.html](http://www.ventanamed.com/products/reagents/her2.html)