# Leica HER2 FISH System for BOND™ - Interpretation Guide for Breast Cancer Tissue

- A serial section H&E of the breast tissue specimen should be available for reference to verify the presence of invasive tumor.
- Count the number of HER2 (orange) and CEP17 (green) signals in 20 nuclei.

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- Use the following ratio to calculate the final result:
  Ratio = Total HER2 signals/Total CEP17 signals

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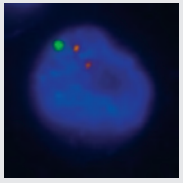
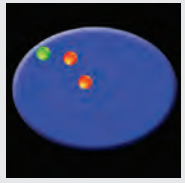
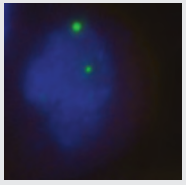
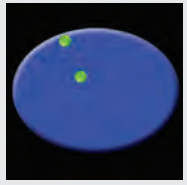
- If the ratio is equivocal (1.80-2.20) count an additional 20 nuclei and recalculate the ratio.

1

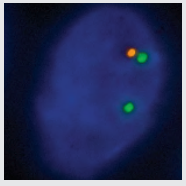
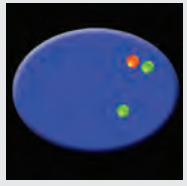
Count as **2 orange signals** and **1 green signal**

2

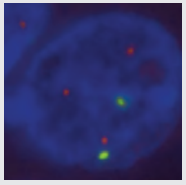
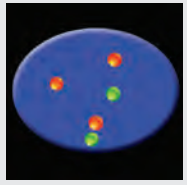
Do not count. Nuclei with no signals or with signals of only 1 colour should not be scored. **Only score those nuclei with 1 or more FISH signals of each colour**

3

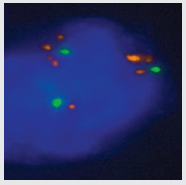
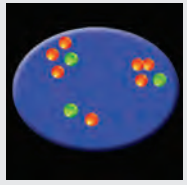
Count as **1 orange signal** and **2 green signals**

4

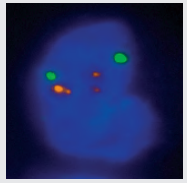
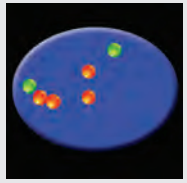
Count as **3 orange signals** and **2 green signals**

5

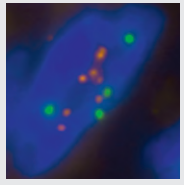
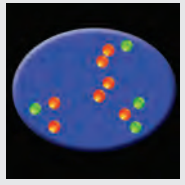
Count as **6 orange signals** and **3 green signals**.
1 orange signal is diffuse

6

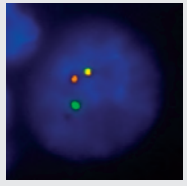
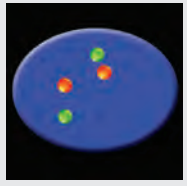
Count as **3 orange signals** and **2 green signals**. 1 orange signal is diffuse. Count 2 signals that are the same size and separated by a distance equal or less than the diameter of the signal, as 1 signal

7

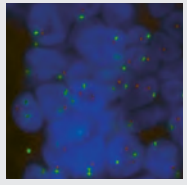
Count as **6 orange signals** and **4 green signals**. 1 orange signal is diffuse

8

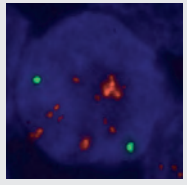
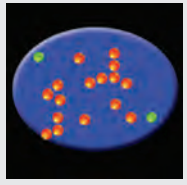
Count as **2 orange signals** and **2 green signals**. 1 orange signal and 1 green signal are overlapping

9




**Do not count.** The nuclei are overlapping. It is too difficult to tell which nuclei the signals are located in

10

Count as **16 orange** signals and **2 green** signals.
Note that the orange count is an approximation

# Leica HER2 FISH Control Slides

Leica BIOSYSTEMS

## Why do we use control slides?

It is recommended that a Leica HER2 FISH Control Slide is included in each test run with the Leica HER2 FISH System to monitor assay performance. Control cell lines do not validate laboratory specimen preparation procedures or replace the requirement for appropriately fixed and processed in-house tissue controls.

The acceptance criteria and representative images for the Leica HER2 FISH Control Slides are demonstrated in the table to the right.

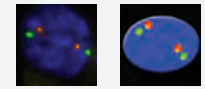
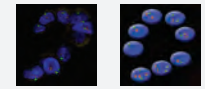
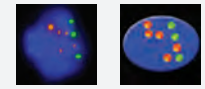
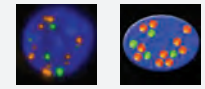
## Results should be reported as follows:

If the ratio is <2, HER2 gene amplification was not observed. The result is negative.

If the ratio is ≥2, HER2 gene amplification was observed. The result is positive.

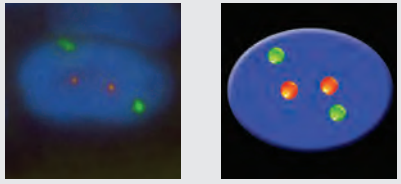
A ratio at or near the cut-off (1.80 - 2.20) should be interpreted with caution.

**Acceptance Criteria for the Leica HER2 FISH Control Slides**

| Cell Line | Leica Bond™ Oracle HER2 IHC System Profile | | Leica HER2 FISH System HER2/CEP17 Acceptance Criteria |
|---|---|---|---|
| MDA-MB-231 | 0 |  | HER2 amplification is not observed |
| MDA-MB-175 | 1+ |  | HER2 amplification is not observed |
| MDA-MB-453 | 2+ |  | HER2/CEP17 gene ratio should be between 1.5 - 2.5 |
| SKBr-3 | 3+ |  | HER2 amplification is observed |

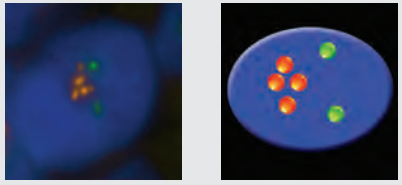


# Leica HER2 FISH - Interpretation of Breast Specimens



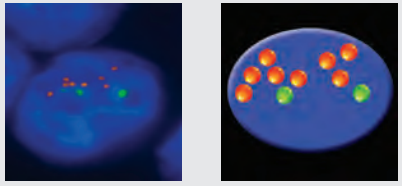
**Invasive tumor - Non-amplified**

Non-overlapping nuclei
Count HER2 and CEP17 signals
Calculate the ratio
Result - ratio <2.0 HER2 gene amplification was not observed



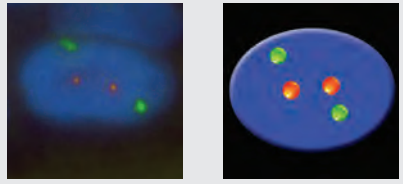
**Invasive tumor - Equivocal**

Non-overlapping nuclei
Count HER2 and CEP17 signals
Calculate the ratio
Ratio is between 1.80 and 2.20. Result is equivocal.
Count a further 20 nuclei and recalculate the ratio
Result - equivocal. Ratio is between 1.80 and 2.20



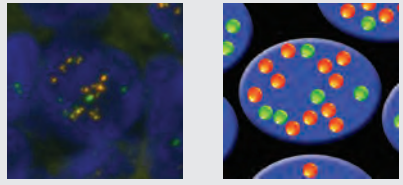
**Invasive tumor - Amplified**

Non-overlapping nuclei
Count HER2 and CEP17 signals
Calculate the ratio
Result - ratio ≥2.0 HER2 gene amplification was observed



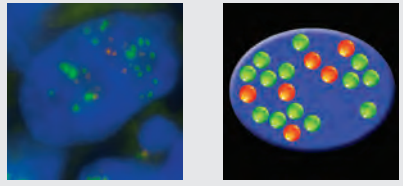
**Normal Epithelium**

Normal breast epithelium should demonstrate a normal ratio of HER2/CEP17



**Heterogeneity**

Some tumors may be heterogeneous with clusters or scattered amplified nuclei within non-amplified areas of tumor



**Polysomy**

Polysomy or multiple copies of chromosome 17, correlates with multiple copies of the HER2 gene, but not necessarily with HER2 amplification