HER2 CISH pharmDx™ Kit Interpretation Guide – Breast Cancer

FROM CERTAINTY COMES TRUST
**HER2 CISH pharmDx™ Kit**

*HER2 CISH pharmDx™ Kit* is intended for dual-color chromogenic visualization of signals achieved with directly labeled in situ hybridization probes targeting the *HER2* gene as well as centromeric region of chromosome 17. The kit is designed to quantitatively determine *HER2* gene status in formalin-fixed, paraffin-embedded breast cancer tissue specimens.

Red and blue chromogenic signals are generated on the same tissue section for evaluation under bright field microscopy. The CISH procedure can be performed automated using Dako Autostainer instruments.

*HER2 CISH pharmDx™ Kit* is indicated as an aid in the assessment of patients for whom Herceptin™ (trastuzumab) treatment is being considered. Results from the *HER2 CISH pharmDx™ Kit* are intended for use as an adjunct to the clinicopathologic information currently used for estimating prognosis in stage II, node-positive breast cancer patients.

**Quality Control**

- Signals must be clear, well balanced in intensity, distinct and easy to evaluate
- Normal cells within the sample allow for an internal control of the staining run
  - Normal cells should have 1-2 clearly visible blue signals and 1-2 clearly visible red signals
  - Failure to detect signals in normal cells indicates assay failure, and results should be considered invalid
  - Numeric evaluation of normal cells should give a result corresponding to the expected value for normal diploid cells (1:1)

**Scoring Guide**

**Assessable tissue**

- Score only invasive component of carcinoma
- Avoid necrotic areas and areas where the nuclear borders are ambiguous
- Disregard nuclei with weak signal intensity and non-specific staining or high background staining

**Assessment of HER2 CISH**

- Scan the slide to account for possible heterogeneity
- Select distinct tumor areas for assessment
- Begin analysis in upper left quadrant of selected area. Scan from left to right, counting signals in each tumor nucleus
Signal enumeration

- If a signal appears to have more than one center of origin, and hence has a shape that differs significantly from a circular dot, it should be counted as two.

- In nuclei with high levels of HER2 gene amplification, the HER2 signals may be positioned very close to each other forming a cluster of signals. In these cases the number of HER2 signals cannot be counted, but must be estimated. Special attention must be paid to the blue signals, as clusters of HER2 signals can cover the blue signals making them hard to see.

- Nuclei exhibiting signals of only one color should not be scored.

- Do not score nuclei demonstrating over- or under digestion.

- Adjust microscope focus to locate all signals in individual nuclei.

- In case of doubt, do not include the nuclei in the evaluation.

Record Counts

- Count HER2 (red) and CEN-17 (blue) signals in 20 nuclei in representative tumor areas.

- Calculate the HER2:CEN-17 ratio by dividing the total number of HER2 signals by the total number of CEN-17 signals.

- Results at or near the cut-off (1.8 – 2.2) should be interpreted with caution. In those cases, count an additional 20 nuclei and recalculate the ratio for the 40 nuclei.

<table>
<thead>
<tr>
<th>Ratio of HER2/CEN-17 signals</th>
<th>HER2 Gene status</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2.0</td>
<td>Non-amplified</td>
<td>Negative</td>
</tr>
<tr>
<td>≥ 2.0</td>
<td>Amplified</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Breast carcinoma stained with HER2 CISH pharmDx™ Kit

<table>
<thead>
<tr>
<th>Non-amplified result</th>
<th>Red/blue ratio &lt; 2</th>
</tr>
</thead>
</table>

Breast carcinoma stained with HER2 CISH pharmDx™ Kit

<table>
<thead>
<tr>
<th>Amplified result</th>
<th>Red/blue ratio ≥ 2</th>
</tr>
</thead>
</table>

Microscope Recommendations

Use a microscope objective of sufficient quality and magnification to allow for optimal scoring of specimens. Adjust light intensity to allow for easy separation of blue and red color. Focus up and down to find all of the signals in the individual nucleus. We recommend that the microscope include 40x and 60x objectives.
**HER2 CISH pharmDx™ Kit, Code SK109**

**Scoring Scheme**

- **Count signals in 20 nuclei**

<table>
<thead>
<tr>
<th>Nucleus No.</th>
<th><strong>HER2 score (red)</strong></th>
<th>CEN-17 score (blue)</th>
<th>Nucleus No.</th>
<th><strong>HER2 score (red)</strong></th>
<th>CEN-17 score (blue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>12</td>
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<td>3</td>
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<td>9</td>
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<td>19</td>
<td></td>
<td></td>
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<tr>
<td>10</td>
<td></td>
<td></td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total (1-10)</strong></td>
<td></td>
<td></td>
<td><strong>Total (11-20)</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For determination of the HER2/CEN-17 ratio, count the number of HER2 signals and the number of CEN-17 signals in the same 20 nuclei and divide the total number of HER2 signals by the total number of CEN-17 signals. If the HER2/CEN-17 ratio is borderline (1.8-2.2), count an additional 20 nuclei and recalculate the ratio for the 40 nuclei. A ratio at or near the cut-off should be interpreted with caution.

<table>
<thead>
<tr>
<th><strong>Total Score (1-20)</strong></th>
<th><strong>HER2</strong></th>
<th><strong>CEN-17</strong></th>
<th><strong>HER2/CEN-17 ratio</strong></th>
</tr>
</thead>
</table>

- ✓ Ratio < 2: HER2 gene amplification was not observed
- ✓ Ratio ≥ 2: HER2 gene amplification was observed

**Date and signature, Technician:** ________________________________

**Date and signature, Pathologist:** ________________________________

Date (day 1) of the run: ___________________________  
Staining Run Log ID: ___________________________  
HER2 CISH pharmDx™ Kit, SK109 Lot: ___________________________  
Specimen ID(s): ___________________________  

**HER2 CISH pharmDx™ Kit, Code SK109 Lot:** ___________________________
Counting Guide

Do not count. Nuclei are overlapping, not all areas of nuclei are visible.

Do not score nuclei with signals of only one color

Count as 3 blue and 21 red signals (cluster estimation)

Count as 2 blue and 4 red signal. Signals that appear to have more than one centre of origin should be counted as two signals

Do not count over- or under digested nuclei.
Missing signals in the centre of nuclei

Count as 4 blue and 4 red signals. Signals that appear to have more than one centre of origin, should be counted as two signals

Count as 2 blue and 4 red signals

Count as 2 blue (1 blue out of focus) and 4 red signals

Cluster of red signals hiding blue signals. Go to a higher level of magnification to confirm presence or absence of blue signals. In case of doubt, do not count
**HER2 CISH pharmDx™ Kit Includes**

- Pre-Treatment Solution (20x concentrated)
- Pepsin, Ready-to-Use
- HER2/CEN-17 Probe Mix
- Stringent Wash Buffer (20x concentrated)
- Wash Buffer 1 (20x concentrated)
- Wash Buffer 2 (10x concentrated)
- Peroxidase Block, Ready-to-use
- CISH Antibody Mix, Ready to use
- Red Substrate Buffer
- Blue Substrate Buffer
- Red Chromogen
- Blue Chromogen
- Tissue-Clear®-Based Mounting Medium
- Coverslip Sealant
- User-Fillable Reagent Bottles

The HER2 CISH pharmDx™ Kit contains materials necessary to perform 20 consistent, reproducible assays (22 x 22 mm target area) within a maximum of 5 individual runs (automated).

**Related Products**

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Size</th>
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<tbody>
<tr>
<td>HER2 FISH pharmDx™ Kit</td>
<td>20 tests</td>
<td>K5331</td>
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<td>HercepTest™ (for manual use)</td>
<td>35 tests</td>
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<td>HercepTest™ for the Dako Autostainer/Autostainer Plus</td>
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<td>HercepTest™ for Automated Link Platforms</td>
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For professional users. For interactive e-learning please use the HER2 CISH pharmDx™ e-learning program designed to supply laboratory technicians, pathologists and scientists with an accurate and fast knowledge of how to achieve optimal results using HER2 CISH pharmDx™ Kit. www.dako.com/e-learning.