For In Vitro Diagnostic Use

FDA approved as an aid in the assessment of patients for whom Herceptin™ (trastuzumab) treatment is being considered.
**HER2 FISH pharmDx™ Kit**

HER2 FISH pharmDx™ Kit is a direct fluorescence in situ hybridization (FISH) assay designed to quantitatively determine HER2 gene amplification in formalin-fixed, paraffin-embedded (FFPE) breast cancer tissue specimens.

It is FDA approved as an aid in the assessment of patients for whom Herceptin™ (trastuzumab) treatment is being considered.

Results from HER2 FISH 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.

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**Scoring Guide**

**Assessable tissue**

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

**Signal location**

- Locate tumor on H&E-stained slide. Evaluate the same area on the FISH-stained slide
- Scan several areas of tumor cells 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
- Adjust microscope focus to locate all signals in the individual nuclei

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*Scan path for signal counting*
Signal enumeration

- Count HER2(red) and CEN-17(green) signals in 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
- Two signals of the same size, separated by a distance equal to or less than the diameter of one signal, are counted as one signal
- Nuclei with high levels of HER2 gene amplification (red) may exhibit formation of signal clusters. Estimate the HER2 signal number. HER2 clusters may obscure CEN-17 (green) signals.
  You can check this using a specific FITC filter.
- Nuclei exhibiting signals of only one color should not be scored
- Do not score nuclei demonstrating overdigestion

<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</td>
<td>Non-Amplified</td>
<td>Negative</td>
</tr>
<tr>
<td>≥ 2</td>
<td>Amplified</td>
<td>Positive</td>
</tr>
</tbody>
</table>

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.

Filter Recommendation

Filters are individually designed for specific fluorochromes and for each microscope.

For the interpretation of HER2 FISH pharmDx™ staining, the following combination of filters is recommended:

- A specific DAPI filter
- A high-quality Texas Red/FITC double filter (or specific Texas Red and FITC single filters)

<table>
<thead>
<tr>
<th>Fluorochrome</th>
<th>Excitation Wavelength</th>
<th>Emission Wavelength</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC</td>
<td>495 nm</td>
<td>520 nm</td>
</tr>
<tr>
<td>Texas Red</td>
<td>596 nm</td>
<td>615 nm</td>
</tr>
</tbody>
</table>
Counting Guide

One green signal (split) indicates the presence of one copy of chromosome 17. One red signal indicates the presence of one copy of the \textit{HER2} gene.

The ratio of \textit{HER2} to CEN-17 is 1/1 = 1; non-amplified.

Three green signals (one out of focus) indicate the presence of three copies of chromosome 17. Three red signals indicate the presence of three copies of the \textit{HER2} gene.

The ratio of \textit{HER2} to CEN-17 is 3/3 = 1; non-amplified.

Two green signals indicate the presence of two copies of chromosome 17. Three red signals (two split signals) indicate the presence of three copies of the \textit{HER2} gene.

The ratio of \textit{HER2} to CEN-17 is 3/2 = 1.5; non-amplified.

One green signal indicates the presence of one copy of chromosome 17. Five red signals indicate the presence of five copies of the \textit{HER2} gene.

The ratio of \textit{HER2} to CEN-17 is 5/1 = 5; amplified.

Three green signals indicate the presence of three copies of chromosome 17. Approximately 12 red signals indicate the presence of 12 copies of the \textit{HER2} gene (cluster estimation).

The ratio of \textit{HER2} to CEN-17 is 12/3 = 4; amplified.

Do not score (nuclei are overlapping, not all areas of nuclei are visible).

Do not score nuclei with signals of only one color (two green signals).

Do not score (overdigested nuclei).

Cluster of red signals hiding green signals. Check the green signals with a specific FITC filter, or do not score.

\*Two signals of the same size, separated by a distance equal to or less than the diameter of one signal, are counted as one signal.
Scoring Sheet

*HER2 FISH pharmDx™ Kit*

Day 1 of Run: _____________________

Staining Run Log ID: ____________________________

Specimen ID: ______________________________

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.

Results at or near the cut-off (1.8–2.2) should be interpreted with caution.

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>HER2 Score (Red)</th>
<th>CEN-17 Score (Green)</th>
<th>Nucleus</th>
<th>HER2 Score (Red)</th>
<th>CEN-17 Score (Green)</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>
<td></td>
<td></td>
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<tr>
<td>3</td>
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<td>6</td>
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<td>7</td>
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<td>17</td>
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<td>8</td>
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<td>18</td>
<td></td>
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<tr>
<td>9</td>
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<td>19</td>
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<tr>
<td>10</td>
<td></td>
<td></td>
<td>20</td>
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<td></td>
</tr>
<tr>
<td>Total (1-10)</td>
<td></td>
<td></td>
<td>Total (11-20)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( \text{HER2 FISH} \) | \( \text{HER2} \) | \( \text{CEN-17} \) | \( \text{HER2/CEN-17 ratio} \)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL SCORE (1-20)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( \Box \) Ratio < 2: HER2 gene amplification was not observed

\( \Box \) Ratio ≥ 2: HER2 gene amplification was observed

Technician signature,____________________________________________________ Date _____________________

Pathologist signature,____________________________________________________ Date _____________________
HER2 FISH pharmDx™ Complete Kit Includes

- Pre-Treatment Solution (20x concentrated)
- Pepsin, Ready-to-Use
- HER2/CEN-17 Probe Mix
- Stringent Wash Buffer (20x concentrated)
- Fluorescence Mounting Medium, containing DAPI
- Wash Buffer (20x concentrated)
- Coverslip Sealant

The HER2 FISH pharmDx™ Kit contains materials necessary to perform 20 consistent, reproducible assays (22 x 22 mm target area) within a maximum of 10 individual staining runs.

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Size</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2 FISH pharmDx™ Kit</td>
<td>20 tests</td>
<td>K5331</td>
</tr>
<tr>
<td>HercepTest™ (for manual use)</td>
<td>35 tests</td>
<td>K5204</td>
</tr>
<tr>
<td>HercepTest™ for the Dako Autostainer/Autostainer Plus</td>
<td>50 tests</td>
<td>K5207</td>
</tr>
<tr>
<td>HercepTest™ for Automated Link Platforms</td>
<td>50 tests</td>
<td>SK001</td>
</tr>
<tr>
<td>Hybridizer</td>
<td>110V</td>
<td>S2450</td>
</tr>
<tr>
<td>Hybridizer</td>
<td>220V</td>
<td>S2451</td>
</tr>
</tbody>
</table>

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