For In Vitro Diagnostic Use. FDA 510(k) cleared as an aid in identifying patients eligible for treatment with anti-hormonal or aromatase inhibitor therapies as well as an aid in the prognosis and management of breast cancer.
Table of Contents

Introduction 2
ER/PR pharmDx™ Overview 3
The ER/PR pharmDx™ Kit 4
ER/PR Expression Rates 5
Concordance Studies 6
- Concordance of Immunohistochemistry (IHC) to Ligand-Binding Assay
- ER/PR pharmDx™ Concordance to the Allred Procedure
- Determination of the Cut-off IHC Score
ER/PR Expression in Normal Tissue 9
ER/PR pharmDx™ Training Checklist 10
Quality Control 11
Control Slides for ER/PR pharmDx™ Validation 12
Examples of Acceptable and Unacceptable Staining of ER/PR pharmDx™ Control Slides 13
ER/PR pharmDx™ Results: Evaluation and Reporting 14
ER/PR pharmDx™ Scoring System 15
Image Guide for Allred Scoring for ER/PR pharmDx™ 16
- Estrogen Receptor
- Progesterone Receptor
Additional Images 18
- Estrogen Receptor
- Progesterone Receptor
Additional Specimens Stained with ER/PR pharmDx™ 20
Artifacts and Various Factors Affecting Staining 21
- Epitope Retrieval Artifacts
- Background Staining
ER/PR pharmDx™ Pathology Report Form 22
References 23
Introduction

Welcome to the ER/PR pharmDx™ Interpretation Manual

This guide for pathologists includes key technical histological staining and interpretation tips applicable when using the ER/PR pharmDx™ kit. Utilization of the suggestions that follow will ensure that your laboratory achieves the quality results expected from ER/PR pharmDx™.

The ER/PR pharmDx™ Interpretation Manual objectives are simple:

- To ensure that the ER/PR pharmDx™ assay is being performed consistent with Dako recommendations for optimal results.
- To encourage reproducible results by introducing a standard approach to staining and interpretation.
- To provide pathologists with a tool to allow consistent interpretation of ER/PR pharmDx™ kit results to appropriately guide patient management for breast cancer therapy.
- To facilitate troubleshooting of the ER/PR pharmDx™ Kit, if problems occur.
ER/PR pharmDx™ Overview

The ER/PR pharmDx™ Kit is a semi-quantitative immunohistochemical (IHC) assay to identify estrogen receptor (ER) and progesterone receptor (PR) expression in normal and neoplastic tissues, formalin-fixed and paraffin-embedded for histological evaluation. ER/PR pharmDx™ specifically detects the ER alpha protein as well as the PR protein located in the nuclei of ER and PR-expressing cells, respectively. Investigations into the biological mechanisms for breast cancer have found that the growth rate is dependent on the presence of estrogen or progesterone or both in most breast cancers. Thus, estrogen receptor and progesterone receptor status in breast cancer is considered to be a validated prognostic and predictive factor for patient management for anti-hormonal therapy.1-5

ER/PR pharmDx™ is indicated as an aid in identifying patients eligible for treatment with anti-hormonal or aromatase inhibitor therapies, as well as an aid in the prognosis and management of breast cancer.

ER/PR pharmDx™ Provides the Basis for Reliable ER and PR Assessment

- FDA 510(k) cleared, standard, reproducible assay.
- New, highly specific ER antibody cocktail and PR antibody with demonstrated sensitivity and specificity.
- Optimized protocol with clinically validated scoring system for the determination of ER/PR status applicable in the management of breast cancer patients.5-9
- Concordance demonstrated between ER/PR pharmDx™ and an established reference method with positive/negative cut-off IHC score calibrated using samples with known biochemical and clinical response data.10
- Verified cut-off IHC score for positivity for ER/PR pharmDx™.
- FDA clearance and confidence in test sensitivity and specificity lessens the burden of extensive validation by laboratory staff.
The ER/PR pharmDx™ Kit

The ER/PR pharmDx™ Kit is a semi-quantitative IHC assay to identify ER and PR expression in normal and neoplastic tissues, formalin-fixed and paraffin-embedded for histological evaluation. ER/PR pharmDx™ specifically detects ER alpha protein as well as the PR protein located in the nuclei of ER and PR-expressing cells, respectively. Following incubation of the primary monoclonal antibody to human ER or PR proteins or the Negative Control Reagent, this validated protocol employs a ready-to-use visualization reagent based on dextran technology. This reagent consists of both secondary goat anti-mouse antibody molecules and horseradish peroxidase molecules linked to a common dextran polymer backbone. Enzymatic conversion of the subsequently added chromogen results in formation of a visible reaction product at the antigen site. The specimens may then be counterstained and coverslipped. Results are interpreted using a light microscope. Control slides containing two formalin-fixed, paraffin-embedded human cell lines are provided for quality control of the kit reagent performance. A minimum of four slides per patient sample is required: one slide for tumor presence, one slide for ER protein evaluation, one slide for PR protein evaluation and one slide for Negative Control Reagent.

Two ER/PR pharmDx™ Kit Configurations are Available

<table>
<thead>
<tr>
<th>Kit Code</th>
<th>Description</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>K4071</td>
<td>ER/PR pharmDx™ Kit for the Dako Autostainer</td>
<td>50</td>
</tr>
<tr>
<td>SK310</td>
<td>ER/PR pharmDx™ Kit for Automated Link Platforms</td>
<td>50</td>
</tr>
</tbody>
</table>

The ER/PR pharmDx™ Kit Includes:
- ER/PR pharmDx™ Mouse Anti-Human ER Antibody Cocktail (ER Mouse Monoclonal Antibody Cocktail (Clones 1D5 and ER-2-123)
- ER/PR pharmDx™ Mouse Anti-Human PR Antibody PR Mouse Monoclonal Antibody (Clone PgR 1294)
- ER/PR pharmDx™ Negative Control Reagent (Negative Control Reagent (Cocktail of Mouse IgG1 and Mouse IgG2a))
- ER/PR pharmDx™ Control Slides
  Each slide contains two pelleted, formalin-fixed, paraffin-embedded cell lines representing negative (0) and moderate levels of ER or PR protein expression (dependent on primary antibody applied to slide).
- ER/PR pharmDx™ Epitope Retrieval Solution (10x)
- ER/PR pharmDx™ Peroxidase-Blocking Reagent
- ER/PR pharmDx™ Visualization Reagent
- ER/PR pharmDx™ DAB+ Substrate-Chromogen
- Wash Buffer (10x)

Materials Required, but not Supplied:
- Calibrated pressure cooker with the capability of reaching and maintaining a temperature of 125 °C for 5 minutes
- Hematoxylin (Code SK308 or S3301)

It is essential that laboratories strictly adhere to utilization of the reagents and protocol specified for use with ER/PR pharmDx™ to ensure consistent, reproducible results. All reagents are formulated specifically for use with this test.
ER/PR Expression Rates

Historical studies have shown that ER/PR status is correlated with untreated outcome, i.e. prognostic for well-differentiated invasive breast cancer, and especially correlated with response to anti-hormonal therapy. As shown in Table 1 below, a phenotype of ER and PR expression offers a more accurate prediction of a patient's response to therapy. Thus, estrogen receptor and progesterone receptor test results in breast cancer specimens are considered to be a validated prognostic and predictive factor for patient management for anti-hormonal therapy.1,5

Table 1. Percent of Incidence and Response Rate of Estrogen Receptor / Progesterone Receptor Expression Phenotypes

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Incidence (%)</th>
<th>Response Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+ / PR+</td>
<td>58</td>
<td>77</td>
</tr>
<tr>
<td>ER+ / PR–</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>ER– / PR+</td>
<td>4</td>
<td>46</td>
</tr>
<tr>
<td>ER– / PR–</td>
<td>15</td>
<td>11</td>
</tr>
</tbody>
</table>

ER, estrogen receptor; PR, progesterone receptor; patients with advanced breast cancer receiving anti-hormonal therapy.6,7

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ER/PR pharmDx™ Interpretation Manual

Figure 1. Kit Procedure

Step 1: Epitope Retrieval in pressure cooker. Incubate 5 minutes at 125 °C.

Step 2: Application of ER/PR pharmDx™ Peroxidase-Blocking Reagent. Incubate 5 minutes.

Step 3: Application of Primary Antibody. Incubate 30 minutes.

Step 4: Application of ER/PR pharmDx™ Visualization Reagent. Incubate 30 minutes.

Step 5: Application of ER/PR pharmDx™ DAB+ Substrate-Chromogen. Incubate 10 minutes.
Concordance Studies

ER/PR pharmDx™ was developed to provide a reproducible test system concordant to a previously validated reference IHC method and utilizes a clinically validated scoring system for the determination of ER/PR status applicable in the management of breast cancer patients.6-9

Concordance of Immunohistochemistry (IHC) to Ligand-Binding Assay

Concordance was performed between a reference IHC method (Allred procedure) and a ligand-binding assay. As shown below, a strong correlation for estrogen and progesterone receptor expression between ligand-binding assay (LBA) and immunohistochemistry (IHC) has been demonstrated and validated (See Figures 2 and 3).6

**Figure 2**
Kaplan-Meier curves of DFS comparing IHC and LBA methods of assessing ER in the subset of patients receiving endocrine therapy. Used with permission of DC Allred, M.D.6

**Figure 3**
Kaplan-Meier curves of DFS comparing IHC and LBA methods of assessing PR in the subset of patients receiving endocrine therapy. Used with permission of DC Allred, M.D.6
ER/PR pharmDx™ Concordance to the Allred Procedure (Reference IHC Method)

A pilot study comparing different assay procedures and antibodies to the Allred procedure was performed on a set of 20 tissues. The assay procedure and antibodies that produced the most similar testing results to the Allred procedure were selected for further testing.\(^\text{10}\)

The validity of the assay procedure and antibody selection/dilution was tested on a set of specimens assembled in tissue arrays. Testing consisted of staining of specimens using the Allred procedure as the reference method, compared to the ER/PR pharmDx™ staining procedure, with all specimens graded and interpreted using the Allred scoring method. Distributions of staining results for positive/negative determination are presented in Tables 2 and 3 for ER and PR, respectively. Concordance to the Allred method for positive/negative hormone receptor result was 99% for both receptors.\(^\text{10}\)

Table 2. Dako ER pharmDx™ Test Results Compared to Allred Procedure ER Test Results

<table>
<thead>
<tr>
<th>Dako ER Test Result</th>
<th>Allred Procedure ER Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>158</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>160</td>
</tr>
</tbody>
</table>

Positive agreement = 158/160 = 0.9875
Negative agreement = 52/52 = 1.0
Concordance was 210/212 = 0.9906. The Kappa statistic was calculated as 0.9748, with a 95% CI of 0.9402-1.0095.

Table 3. Dako PR pharmDx™ Test Results Compared to Allred Procedure PR Test Results

<table>
<thead>
<tr>
<th>Dako PR Test Result</th>
<th>Allred Procedure PR Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>128</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
</tr>
</tbody>
</table>

Positive agreement = 128/130 = 0.9846
Negative agreement = 74/74 = 1.0
Concordance was 202/204 = 0.9902. The Kappa statistic was calculated as 0.9789, with a 95% CI of 0.9498-1.0080.
Determination of Cut-Off IHC Score (Positive/Negative)

The interpretation of results, i.e. definitions of “positive” and “negative,” has been established by calibration to clinical outcome. The Allred score provides a scoring system which incorporates not only the proportion of cells stained, but also the intensity of these cells. On the basis of responses from patients receiving any adjuvant therapy, an optimal cut-off IHC score (>2) for predicting patient improved outcome was established (See Figures 4 and 5).

**Figure 4**
Univariate Disease-Free Survival (DFS) curves for all possible ER IHC scores in patients receiving adjuvant therapy. Reprinted with permission from the American Society of Clinical Oncology.

**Figure 5**
Univariate Disease-Free Survival (DFS) curves for all possible PR IHC scores in patients receiving adjuvant therapy. Reprinted with permission from Modern Pathology, 2004 Macmillian Publishers Ltd.
### ER/PR Expression in Normal Tissue

**Table 4. Evaluation of Normal Tissue Staining by Dako ER/PR pharmDx™**

<table>
<thead>
<tr>
<th>Tissue Type (# Tested)</th>
<th>Tissue Element Stained</th>
<th>ER Staining Intensity</th>
<th>PR Staining Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Bone Marrow (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Breast (3)</td>
<td>Ductal epithelial cells</td>
<td>3,3,3</td>
<td>3,3,3</td>
</tr>
<tr>
<td>Brain/Cerebellum (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Brain/Cerebrum (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Cervix (3)</td>
<td>Basal epithelium</td>
<td>2,3,3</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Stromal cells</td>
<td>1,3,3</td>
<td>2,3,3</td>
</tr>
<tr>
<td>Colon (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Esophagus (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Heart (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Kidney (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Liver (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Lung (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Mesothelial Cells (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Ovary (3)</td>
<td>Surface epithelium</td>
<td>0,0,2</td>
<td>0,3,3</td>
</tr>
<tr>
<td></td>
<td>Stromal cells</td>
<td>None</td>
<td>0,3,3</td>
</tr>
<tr>
<td>Pancreas (3)</td>
<td>Islet cells*</td>
<td>None</td>
<td>1,3,3</td>
</tr>
<tr>
<td>Parathyroid (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Peripheral Nerve (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Pituitary (3)</td>
<td>Pituicytes</td>
<td>0,1,3</td>
<td>1,2,3*</td>
</tr>
<tr>
<td>Prostate (3)</td>
<td>Stromal cells</td>
<td>1,1,2</td>
<td>0,2,2</td>
</tr>
<tr>
<td>Salivary Gland (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Skeletal Muscle (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Skin (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Small Intestine (3)</td>
<td>Muscularis propria</td>
<td>None</td>
<td>0,0,2</td>
</tr>
<tr>
<td>Spleen (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Stomach (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Testis (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Thymus (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Thyroid (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Tonsil (3)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Uterus (3)</td>
<td>Endometrial glands</td>
<td>2,3,3</td>
<td>3,3,3*</td>
</tr>
<tr>
<td></td>
<td>Endometrial stroma</td>
<td>2,2,3</td>
<td>3,3,3</td>
</tr>
<tr>
<td></td>
<td>Myometrium</td>
<td>2,3,3</td>
<td>3,3,3</td>
</tr>
</tbody>
</table>

All slides were graded for intensity only on a 0-3 scale.

Nuclear staining.

* Nuclear and cytoplasmic staining.
**Table 5. ER/PR pharmDx™ Training Checklist**

<table>
<thead>
<tr>
<th>Institution</th>
<th>Trained by</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person Trained/Title</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dako Autostainer or Automated Link Platform Staining Run</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Software Version</td>
<td>Instrument Serial Number</td>
<td></td>
</tr>
</tbody>
</table>

**Companion Products**
- Calibrated pressure cooker
- Hematoxylin (Code SK308 or S3301)

**Dako Autostainer or Automated Link Platform Procedure**
- Control slides and kit stored at 2-8 °C?
- Cell line control slides and all reagents equilibrated to room temperature (20-25 °C) prior to starting assay?
- Tissues formalin-fixed?
- Specimens stained within two months of sectioning when stored at room temperature?
- Clearing solutions changed after 200 slides?
- Deparaffinization and rehydration protocol followed?
- ER/PR pharmDx™ Epitope Retrieval Solution prepared properly?
  - Prepare sufficient quantity of Epitope Retrieval Solution 10x, by diluting 1:10 with reagent-quality water, deionized or distilled water.
- Wash Buffer prepared properly?
  - Prepare sufficient quantity of Wash Buffer 10x, by diluting 1:10 with reagent-quality water, deionized or distilled water.
- Distilled or deionized water (not tap water) used for water washes after last alcohol bath in deparaffinization?
- Appropriate epitope retrieval temperature and incubation time (125 °C for 5 minutes) in a calibrated pressure cooker?
- Progressive hematoxylin counterstain used?

**Dako Autostainer Procedure**
- Slides placed in Wash Buffer for a minimum of 5 minutes before loading onto the Autostainer?
- Appropriate protocol template used?
- Was the Autostainer programming reviewed for accuracy?
- ER/PR pharmDx™ DAB+ Substrate-Chromogen prepared properly?
  - Add 11 drops of DAB+ Chromogen to one vial (11 mL) of DAB+ Substrate Buffer and mix.

**Automated Link Platform Procedure**
- Slides placed in Wash Buffer for a minimum of 5 minutes before loading onto the Automated Link Platform?
- Appropriate protocol template used?
- ER/PR pharmDx™ DAB+ Substrate-Chromogen prepared properly?
  - Add 20 µL of ER/PR pharmDx™ DAB+ Chromogen to each 1 mL of ER/PR pharmDx™ DAB+ Substrate Buffer and mix.

**Instrumentation / Equipment**
- Is regular preventive maintenance performed on the pressure cooker and the Dako Autostainer or Automated Link Platform?
- Is the pressure cooker properly calibrated?
- Do you have all the necessary equipment and reagents to perform the ER/PR pharmDx™ assay according to protocol?
- If not, specify what is missing in comments below.

If you answered NO to any of the above, you have deviated from protocol and should consult with your Dako Technical Support Representative for assistance.

Additional observations or comments:

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**ER/PR pharmDx™ Interpretation Manual**

10
Quality Control

The first quality control step for interpretation is the evaluation of the ER/PR pharmDx™ Control Slides. Each of the supplied control slides contains two pelleted, formalin-fixed, paraffin-embedded human cell lines: one positive and one negative with ER and PR antibodies. Two control slides should be run in each staining procedure, one incubated with the ER antibody cocktail and one incubated with the PR antibody. The evaluation of the Dako supplied control slides indicates the validity of the staining run. The control slides should not be used to aid in interpretation of patient results. If either of the control cell lines have staining results outside the acceptable criteria, results from all of the test slides stained simultaneously within the same run should be considered invalid and the test should be repeated.

Tissue controls should be fresh biopsy/surgical specimens fixed, processed and embedded as soon as possible in the same manner as the patient sample(s). Positive tissue controls are indicative of correctly prepared tissues and proper staining techniques. One positive tissue control for each set of test conditions should be included in each staining run. Endocervix is recommended as a control tissue that contains both ER and PR expressing cells. The specimens used for the positive tissue controls should give weak positive staining so that subtle changes in the primary antibody sensitivity can be detected. The control slides supplied with this system or specimens processed differently from the patient sample(s) validate reagent performance only and do not verify tissue preparation.

Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents, NOT as an aid in formulating a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate appropriate positive staining, results with the test specimens should be considered invalid and the test should be repeated.

Use a negative control tissue (known to be ER and PR negative) fixed, processed and embedded in the same manner as the patient sample(s) with each staining run to verify the specificity of the primary antibody and to indicate unintended cross-reactivity to cells/cellular components. The variety of different cell types present in most tissue sections offers internal negative control sites. If specific staining occurs in the negative control tissue, results with the patient specimens should be considered invalid and the test should be repeated.
Control Slides for ER/PR pharmDx™ Validation

Estrogen Receptor

![Figure 6](image)

**Figure 6**
CAMA-1 positive cell line control stained with ER from ER/PR pharmDx™ Kit; 40x magnification

![Figure 7](image)

**Figure 7**
HT-29 negative cell line control stained with ER from ER/PR pharmDx™ Kit; 40x magnification

Progesterone Receptor

![Figure 8](image)

**Figure 8**
CAMA-1 positive cell line control stained with PR from ER/PR pharmDx™ Kit; 40x magnification

![Figure 9](image)

**Figure 9**
HT-29 negative cell line control stained with PR from ER/PR pharmDx™ Kit; 40x magnification

**Table 6.** Acceptance Criteria for Positive Cell Line Controls
(Negative cell lines should exhibit no nuclear staining)

<table>
<thead>
<tr>
<th>Intensity Score (based on 0-3 scale)</th>
<th>ER</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-3</td>
<td>2-3</td>
</tr>
</tbody>
</table>
Examples of Acceptable and Unacceptable Staining with ER/PR pharmDx™ Control Slides

Estrogen Receptor (CAMA-1)

Unacceptable staining.
Unacceptable weak staining intensity (too light);
Weak staining may result in false-negative results;
40x magnification

Acceptable staining;
40x magnification

Unacceptable staining.
Unacceptable strong staining intensity (too dark);
Excessively strong staining may result in
false-positive results;
40x magnification

Progesterone Receptor (CAMA-1)

Unacceptable staining.
Unacceptable weak staining intensity (too light);
Weak staining may result in false-negative results;
40x magnification

Acceptable staining;
40x magnification

Unacceptable staining.
Unacceptable strong staining intensity (too dark);
Excessively strong staining may result in
false-positive results;
40x magnification
ER/PR pharmDx™ Results: Evaluation and Reporting

Slide Evaluation Should be Performed by a Pathologist Using a Light Microscope

ER/PR pharmDx™ stains cell nuclei when using anti-ER and anti-PR. The immunostaining pattern in breast cancer is normally heterogeneous. Scoring is based on examination of all tumor cells on the slide.

- A Proportion Score (PS) is assigned representing the proportion of tumor cells with positive nuclear staining.
- An Intensity Score (IS) is assigned representing the AVERAGE staining intensity of all positive tumor cells.
- A Total Score (TS) is the sum of PS plus IS (ranging from 0, 2–8). A positive result for both ER and PR is defined as TS ≥ 3, which was validated in numerous large clinical studies.⁵⁻⁸

Figure 16
Alfred Scoring Guidelines for ER/PR pharmDx™
Scoring Guidelines ("Alfred Score") modified and used with the permission of D.C. Allred, M.D.⁶
## ER/PR pharmDx™ Scoring System

### Table 7. Allred Scoring Guidelines

<table>
<thead>
<tr>
<th>PROPORTION SCORE (PS)*</th>
<th>PS OBSERVATION</th>
<th>INTENSITY SCORE (IS)**</th>
<th>IS OBSERVATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>&gt; 0 to 1/100</td>
<td>1</td>
<td>Weak</td>
</tr>
<tr>
<td>2</td>
<td>&gt; 1/100 to 1/10</td>
<td>2</td>
<td>Intermediate</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 1/10 to 1/3</td>
<td>3</td>
<td>Strong</td>
</tr>
<tr>
<td>4</td>
<td>&gt; 1/3 to 2/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>&gt; 2/3 to 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total Score = PS + IS

Each **Proportion Score** encompasses a range represented by a whole number.

<table>
<thead>
<tr>
<th>TOTAL SCORE (TS)***</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>0, 2</td>
<td>Negative</td>
</tr>
<tr>
<td>≥ 3</td>
<td>Positive</td>
</tr>
</tbody>
</table>

* Proportion of tumor cells with positive nuclear staining
** Average intensity of all positive tumor cells
*** Sum of Proportion Score (PS) and Intensity Score (IS)

Figure 17
ER/PR pharmDx™ Positive/Negative Results

![ER/PR Staining of Tumor Cells Diagram](image-url)
Image Guide for Allred Scoring for ER/PR pharmDx™

Estrogen Receptor (40x magnification)

**Figure 18**
Breast cancer (PS 0) + (IS 0) = TS 0 Positive

**Figure 19**
Breast cancer (PS 1) + (IS 1) = TS 2 Positive

**Figure 20**
Breast cancer (PS 2) + (IS 1) = TS 4 Positive

**Figure 21**
Breast cancer (PS 5) + (IS 1) = TS 6 Positive

**Figure 22**
Breast cancer (PS 2) + (IS 2) = TS 7 Positive

**Figure 23**
Breast cancer (PS 5) + (IS 3) = TS 8 Positive

PS = Proportion Score        IS = Intensity Score             TS = Total Score
Progesterone Receptor (40x magnification)

**Figure 24**
Breast cancer (PS 0) + (IS 0) = TS 0  
Negative

**Figure 25**
Breast cancer (PS 1) + (IS 1) = TS 2  
Negative

**Figure 26**
Breast cancer (PS 1) + (IS 2) = TS 3  
Positive

**Figure 27**
Breast cancer (PS 3) + (IS 1) = TS 4  
Positive

**Figure 28**
Breast cancer (PS 4) + (IS 1) = TS 5  
Positive

**Figure 29**
Breast cancer (PS 5) + (IS 3) = TS 8  
Positive

PS = Proportion Score  
IS = Intensity Score  
TS = Total Score
Additional Images

Estrogen Receptor

**Figure 30**
Breast cancer (PS 3) + (IS 3) = TS 6

**Figure 31**
Breast cancer (PS 4) + (IS 2) = TS 6

PS = Proportion Score  IS = Intensity Score  TS = Total Score
Progesterone Receptor

**Figure 32**
Breast cancer (PS 3) + (IS 3) = TS 6

**Figure 33**
Breast cancer (PS 3) + (IS 1) = TS 4

PS = Proportion Score | IS = Intensity Score | TS = Total Score
Additional Specimens Stained with ER/PR pharmDx™

Breast Cancer/Normal Breast Staining

Figure 34  
Breast cancer with normal breast (internal control) stained with ER; (PS 3) + (IS 3) = TS 6; 20x magnification

Figure 35  
Breast cancer with normal breast (internal control) stained with PR; (PS 2) + (IS 1) = TS 3; 20x magnification

Endocervix Staining

Figure 36  
Endocervix stained with ER; 20x magnification

Figure 37  
Endocervix stained with PR; 20x magnification

Nuclear/Cytoplasmic Staining

Figure 38  
Breast cancer stained with ER; Example of nuclear/cytoplasmic staining; (PS 4) + (IS 3) = TS 7; 40x magnification
Artifacts and Various Factors Affecting Staining

Epitope Retrieval Artifacts

ER/PR pharmDx™ includes pre-treatment by means of epitope retrieval in a pressure cooker. Tissue sections may occasionally be harmed by epitope retrieval, causing disruption of cell membranes and overall tissue architecture. Breast tissue commonly contains fat which can be easily disrupted. The use of positively charged slides may improve the adherence of tissue.

Background Staining

Background staining is defined as diffuse, non-specific staining of a specimen. It can be caused by several factors. These factors include, but are not limited to, fixation and processing of the specimen, incomplete removal of paraffin from sections prior to staining, and incomplete rinsing of slides.

Breast Cancer with Adipocytes

Figure 39
Breast cancer stained with ER; Example of breast carcinoma with adipocytes (PS 2) + (IS 2) = TS 4; 40x magnification

Unacceptable Background

Figure 40
Breast cancer stained with ER; Example of unacceptable background staining; Invalid test; 40x magnification
Table 8. ER/PR pharmDx™ Pathology Report Form

<table>
<thead>
<tr>
<th>Patient Name: _____________________________</th>
<th>Collection Date: _____________________________</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ordering Physician: ______________________</td>
<td>Acceptance Date: ______________________________</td>
</tr>
<tr>
<td>Ordering Facility: ________________________</td>
<td>Report Date: _________________________________</td>
</tr>
<tr>
<td>Medical Record #: ________________________</td>
<td>Lab Reference #: ______________________________</td>
</tr>
<tr>
<td>Specimen ID #: __________________________</td>
<td>Patient Gender: _______________________________</td>
</tr>
<tr>
<td>Date of Birth: ___________________________</td>
<td></td>
</tr>
</tbody>
</table>

**Description**

Deparaffinized tissue and appropriate control tissue sections are stained using the FDA 510(k) cleared Dako ER/PR pharmDx™ Immunohistochemistry Kit.

A positive result is based on nuclear staining within the tumor and defined as a Total Score of ≥3 using the Allred Scoring Guidelines for ER/PR pharmDx™.

**Patient Result**

<table>
<thead>
<tr>
<th>POSITIVE</th>
<th>NEGATIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen Receptor</td>
<td></td>
</tr>
<tr>
<td>Progesterone Receptor</td>
<td></td>
</tr>
</tbody>
</table>

ER/PR pharmDx™ is indicated as an aid in identifying patients eligible for treatment with anti-hormonal or aromatase inhibitor therapies, as well as an aid in the prognosis and management of breast cancer.

**Allred Scoring Guidelines**

<table>
<thead>
<tr>
<th>PROPORTION SCORE (PS)*</th>
<th>PS OBSERVATION</th>
<th>INTENSITY SCORE (IS)**</th>
<th>IS OBSERVATION</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>&gt; 0 to 1/100</td>
<td>1</td>
<td>Weak</td>
</tr>
<tr>
<td>2</td>
<td>&gt; 1/100 to 1/10</td>
<td>2</td>
<td>Intermediate</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 1/10 to 1</td>
<td>3</td>
<td>Strong</td>
</tr>
<tr>
<td>4</td>
<td>&gt; 1 to 1/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>&gt; 1 to 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total Score = PS + IS

Each Proportion Score encompasses a range represented by a whole number.

<table>
<thead>
<tr>
<th>TOTAL SCORE (TS)***</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>0, 2</td>
<td>Negative</td>
</tr>
<tr>
<td>≥ 3</td>
<td>Positive</td>
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</tbody>
</table>

* Proportion of tumor cells with positive nuclear staining

** Average intensity of all positive tumor cells

*** Sum of Proportion Score (PS) and Intensity Score (IS)
References


<table>
<thead>
<tr>
<th>Country</th>
<th>Phone Number</th>
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</thead>
<tbody>
<tr>
<td>Australia</td>
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</tr>
<tr>
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<td>+43 1 408 4334 50</td>
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