510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k081286

B. Purpose for Submission:
New device

C. Measurand:
Estrogen Receptor on formalin-fixed paraffin-embedded breast cancer specimens

D. Type of Test:
Semi-quantitative, immunohistochemical

E. Applicant:
Dako North America, Inc

F. Proprietary and Established Names:
Dako Monoclonal Rabbit Anti-Human Estrogen Receptor, Clone SP1

G. Regulatory Information:

1. Regulation section:
   21 CFR 864.1860, Immunohistochemistry reagents and kits

2. Classification:
   Class II

3. Product code:
   MYA, Immunohistochemistry Antibody Assay, Estrogen Receptor

4. Panel:
   Pathology 88

H. Intended Use:

1. Intended use(s):
   Monoclonal Rabbit Anti-Human Estrogen Receptor α (ER α) antibody, Clone SP1, may be used in the semi-quantitative detection of human estrogen receptor in formalin-fixed, paraffin-embedded tissue sections of human breast cancer by immunohistochemistry. The information gained by this assay can aid in assessing the likelihood of response to therapy as well as in the prognosis and management of breast cancer patients.
   Clinical interpretation of any positive staining or its absence should be complemented by morphological and histological studies with proper controls. Evaluations should be within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

2. Indication(s) for use:
   Not applicable.

3. Special conditions for use statement(s):
   For prescription use only.

4. Special instrument requirements:
   Dako Autostainer

I. Device Description:
Dako Monoclonal Rabbit Anti-Human Estrogen Receptor α antibody, Clone SP1, is a semi-quantitative immunohistochemical (IHC) assay kit to identify estrogen receptor (ER) expression in normal and neoplastic tissues routinely processed and paraffin-

embedded. The monoclonal rabbit antibody is provided in concentrated liquid form as cell culture supernatant and contains 0.015mol/L sodium azide.

J. Substantial Equivalence Information:
1. Predicate device name(s):
   Dako ER/PR pharmDx™ Kit-Monoclonal Mouse ER 1D5/ER-2-123 reagent only
2. Predicate 510(k) number(s):
   k042884
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Antibody type	Monoclonal	Same
Intended Use	Semi-quantitative detection of ER	Same
Technology	Immunohistochemistry	Same

Differences		
Item	Device	Predicate
Clone (ER)	1D5/ER-2-123	SP1

K. Standard/Guidance Document Referenced (if applicable):
Guidance for Submission of Immunohistochemistry Applications to the FDA; Final

L. Test Principle:
Monoclonal Rabbit Anti-Human Estrogen Receptor α (ERα) antibody, Clone SP1, may be used in the semi-quantitative detection of human estrogen receptor in tissue sections of human breast cancer by immunohistochemistry. The information gained by this assay can aid in assessing the likelihood of response to therapy as well as in the prognosis and management of breast cancer patients. Steroid receptors exhibit a high affinity and specificity for their ligands. The human estrogen receptor (ER) is a dimeric protein of 65 kDa located primarily in the nucleus and belongs to a class of trans-acting proteins which stimulate transcription by binding to specific DNA elements, also known as hormone response elements. Through binding estrogen, the ER is induced to stimulate gene transcription, hence is also known as an inducible enhancer factor.

Immunohistochemical staining is performed on routinely processed, paraffin-embedded specimens. Immunohistochemistry is a well established, widely accepted laboratory methodology. The assay detects the presence of Estrogen receptor (ER) through first, the binding of an antibody to the ER antigen and second, visualization of the bound primary antibody through a reagent based on dextran technology. This reagent consists of both secondary goat anti-rabbit antibody molecules and horseradish peroxidase molecules linked to a common dextran polymer backbone, thus eliminating the need for sequential application of link antibody and peroxidase conjugate. The enzymatic conversion of the subsequently added chromogen results in formation of a visible reaction product at the antigen site. The specimen is then counterstained and coverslipped. Results are interpreted using a light microscope.

M. Performance Characteristics (if/when applicable):
1. Analytical performance:
   a. *Precision/Reproducibility:*
   Precision:
   Serial sections from each of three different formalin-fixed paraffin embedded blocks of breast carcinoma were collected for testing from the Dako tissue bank. Test slides from the same specimen must not display staining intensity deviation of ± 0.5 grade for a total of 1 grade variation on a 0-4 scale.

   Intra-run Precision: Three slides from each tissue block were stained with Monoclonal Rabbit Anti-Human ERα Clone SP1 by three analysts. Concurrently one slide from each block was stained with a negative control reagent.

   Inter-run Precision: One slide from each tissue block was stained on two additional days by one analyst. Concurrently, one slide from each tissue block was stained with a negative control reagent.

   The precision experiment results met the acceptance criteria described above. Additionally, the estimated percent of tumor cells stained was evaluated and all results were in agreement.

   Reproducibility:
   Fifteen breast carcinoma specimens (5 Negative, 1 Low Positive, and 9 Positive) were stained with Dako Monoclonal Rabbit Anti-Human ERα, Clone SP1 and scored at 3 sites. Between each site comparison pair, 100% (95% C.I. 78.2-100%) overall agreement was obtained.
   b. *Linearity/assay reportable range:*
   Not applicable.
   c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
   Positive and negative controls should be performed with each staining run. The pathologist is responsible for assuring that the assay is performing properly.
   d. *Detection limit:*
   Not applicable.
   e. *Analytical specificity:*
   A total of 87 formalin-fixed and paraffin-embedded tissues covering a wide range of normal human tissue types were tested with the ER antibody. The antibody demonstrated negative immunoreactivity with most tissues. Positive immunoreactivity was noted with some normal tissues which are typically positive, like uterus, ovary and ductal epithelial cells of the breast.
   f. *Assay cut-off:*
   A positive staining result is defined as more than 1% of tumor cells with stained nuclei of any intensity.
2. Comparison studies:

a. *Method comparison with predicate device:*
Estrogen receptor status was evaluated at two sites on a total of 228 cases using formalin-fixed paraffin-embedded cases of breast carcinoma. ERα, SP 1 testing was performed with EnVision™ FLEX and scored with positive staining result defined as more than 1% of tumor cells with stained nuclei of any intensity. ER 1D5/2-123 was performed using the ER/PR pharmDx™ Kit and scored using the Allred scoring guideline. Method comparison data are presented below:

Study Site 1:

		ER Component of ER/PR pharmDx	
		Positive	Negative
ER Clone SP1	Positive	69	6
	Negative	0	34
	Total	69	40

Positive Percent Agreement = 69/69 = 100% (95% CI: 94.8-100%)
Negative Percent Agreement = 34/40 = 85% (95% CI: 70.2-94.3%)
Total Percent Agreement = 103/109 = 94.5% (95% CI: 88.4-97.95%)

Study Site 2:

		ER Component of ER/PR pharmDx	
		Positive	Negative
ER Clone SP1	Positive	27	1
	Negative	7	84
	Total	34	85

Positive Percent Agreement = 27/34 = 79.4% (95% CI: 62.1-91.3%)
Negative Percent Agreement = 84/85 = 98.8% (95% CI: 93.6-99.9%)
Total Percent Agreement = 111/119 = 93.3% (95% CI: 87.2-97.1%)

Clone SP1 has been noted in the literature to have a higher sensitivity than Clone 1D5.

b. *Matrix comparison:*
Not applicable.
3. Clinical studies:
a. *Clinical Sensitivity:*
Not applicable.
b. *Clinical specificity:*
Not applicable.
c. Other clinical supportive data (when a. and b. are not applicable):
A study published in the Journal of Clinical Oncology was used to establish clinical performance of Dako Monoclonal Rabbit Anti-Human Estrogen Receptor α (ER α) antibody, Clone SP1. Estrogen receptor status was evaluated in 4,150 breast cancer patient samples on formalin-fixed, paraffin-embedded tissue samples with anti-ERα, Clone SP1 and anti-ER α, Clone

1D5. The rate of positivity for SP1 was 69.5% compared to 63.1% for 1D5. For SP1 the weighted estimate for ten-year Breast Cancer Specific Survival (BCSS) for $\geq$ 1% nuclear staining in tumor cells is 76.5% (95% C.I.: 73.9-79.0%) and for < 1% nuclear staining in tumor cells is 65% (95% C.I.: 62.68%).[1]

A bridging study was performed to establish that there was no difference between the immunostaining protocol cited in the publication and the protocol used to stain Dako anti-ERα, Clone SP1. Twenty-seven formalin-fixed paraffin embedded breast carcinoma tissue specimens with percent tumor cells stained ranging from 1% to 100% were tested with both protocols. Results showed 100% agreement between the two protocols and the published study can therefore be used to support the clinical performance of the Dako anti-ERα, Clone SP1 (see below):

| | | Protocol 2 | |
		Positive	Negative
Protocol 1	Positive	23	0
	Negative	0	4
	Total	23	4

Total Percent Agreement: 27/27 = 100% (95% CI = 87.2-100%)

[1]Cheang MCU, Treaba DO, Spears CH, Olivotto IA, Bajdik CD, Chia SK, Goldstein LC, Gelmon KA, Huntsmann D, Gilks CB, Nielson TO, Gown AM. Immunohistochemistry detection using the new rabbit monoclonal antibody SP1 of estrogen receptor in breast cancer is superior to mouse monoclonal antibody 1D5 in predicting survival. J Clin Oncol 2006;24:5637-5644.

4. Clinical cut-off:
A positive result is defined as nuclear staining in $\geq$ 1% of tumor cells.
5. Expected values/Reference range:
Not applicable.

N. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:
The submitted information in this premarket notification is complete and supports a substantial equivalence decision.