510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K170028

B. Purpose for Submission:

Addition of the Dako Omnis staining platform to the FLEX Monoclonal Rabbit Anti-Human Estrogen Receptor α, Clone EP1, Ready-to-Use Primary Antibody

C. Measurand:

Human Estrogen Receptor α protein

D. Type of Test:

Immunohistochemistry

E. Applicant:

Dako North America, Inc.

F. Proprietary and Established Names:

FLEX Monoclonal Rabbit Anti-Human Estrogen Receptor α, Clone EP1, Ready-to-Use (Dako Omnis)

G. Regulatory Information:

1. Regulation section:

   21 CFR 864.1860

2. Classification:

   Class II

3. Product code:

   MYA

4. Panel:
   Pathology (88)

H. Intended Use:

1. Intended use(s):

   For in vitro diagnostic use.
   FLEX Monoclonal Rabbit Anti-Human Estrogen Receptor α, Clone EP1, Ready-to-Use (Dako Omnis), is intended for use in immunohistochemistry with the EnVision FLEX visualization system together with the Dako Omnis instrument to qualitatively detect human estrogen receptor in formalin-fixed, paraffin-embedded tissue sections of human breast cancer. The antibody binds estrogen receptor α expressing cells and is useful in the assessment of estrogen receptor status in human breast carcinomas.

   The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using non-immunologic histochemical stains.

2. Indication(s) for use:

   Same as in intended use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Dako Omnis Autostainer

   EnVision FLEX High pH visualization system

I. Device Description:

The FLEX Monoclonal Rabbit Anti-Human Estrogen Receptor α, Clone EP1, Ready-to-Use (Dako Omnis) primary antibody is a rabbit anti-human monoclonal antibody which is available in a ready-to-use (RTU) format and is optimized for use with the Dako Omnis automated stainer with the EnVision FLEX, High pH visualization kit. The antibody is provided in a pre-diluted liquid form in a buffer containing stabilizing protein and 0.015 mol/L sodium azide. The antibody target concentration is 14.8 µg/mL.

The Dako Omnis is intended for fully automated slide-based staining of formalin-fixed, paraffin-embedded (FFPE) tissue sections and operated by qualified professionals, in a pathology laboratory. The Dako Omnis can process up to 60 slides simultaneously in either parallel or batch processing with onboard deparaffinization.

The Dako Omnis Solution software is modular in its architecture. The modules are: stainer instrument software, workstation software, server software, including a database server, and connectivity to a Laboratory Information System (LIS). The end user interacts with the instrument via a touch screen interface or work station via screen and keyboard. The software parts identified to perform and control the slide staining process are distributed in the server software including the database access and the instrument software, including firmware modules. The software manages and controls the Dako Omnis Instrument to perform a immunohistochemical (IHC) slide staining process according to the assay protocol for the FLEX Monoclonal Rabbit Anti-Human Estrogen Receptor α, Clone EP1, Ready-to-Use (Dako Omnis)

J. Substantial Equivalence Information:

1. Predicate device name(s):

   FLEX Monoclonal Rabbit Anti-Human Estrogen Receptor α, Clone EP1, Ready-to-Use (RTU) antibody

2. Predicate 510(k) number(s):

   K120663

3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Intended Use	Detection of Estrogen Receptor α Protein	Same
Antibody Type	Monoclonal, rabbit origin	Same
Isotype	IgG	Same
Clone	EP1	Same
Staining Pattern	Nuclear	Same
Interpretation of results	Positive: ≥1% positive staining breast cancer cells	Same
Tissue Type	Formalin-fixed paraffin-embedded breast cancer	Same
Technology	Immunohistochemistry	Same
Storage	2-8 °C	Same

Differences		
Item	**Device**	**Predicate**
Configuration	Pre-diluted Ready-to-use with Dako Omnis	Pre-diluted Ready-to-use with Autostainer Link 48
Temperature Control/Incubation Temperature	Temperature controlled at 32 °C ± 2 °C	No temperature control (ambient temperature)

Differences		
Item	**Device**	**Predicate**
Antibody Concentration	14.8 µg/mL	3.7 µg/mL
Antibody Incubation Time	10 minutes	20 minutes
Staining	Dynamic gap staining: Reagent is applied and distributed across slide via active slide lids which enable capillary force spreading	Open slide staining: Reagent is applied and distributed across slide via gravitational spreading
Vial	Dako Omnis Large Vial, 30 mL	Automation Vial, 25 mL
Cap	Dako Omnis Closure including septum	White Nalgene Cap
Deparaffinization	On-board Dako Omnis with Clearify	EnVision FLEX High pH on external PT Module
Pre-Treatment (Antigen Retrieval)	EnVision FLEX High pH (30 minute heat-induced epitope retrieval)	EnVision FLEX High pH (20 minute heat-induced epitope retrieval)
Visualization	EnVision FLEX (Chromogen 5 minutes)	EnVision FLEX (Chromogen 2 x 5 minutes)

K. Standard/Guidance Document Referenced (if applicable):

ISO 14971: Medical Devices – Applications of risk management to medical devices, Version 5, August 1, 2012.

IEC 62304: Medical Device Software – Software Life Cycle Processes, Version 62304, Edition 1.1, April 4, 2016.

L. Test Principle:

Rabbit monoclonal anti-human antibody ER α, Clone EP1, specifically binds to the estrogen receptor-α antigen located in the nuclear region of cells of a variety of normal and neoplastic tissues. Automated immunohistochemical (IHC) staining on the Dako Omnis instrument is performed on formalin-fixed, paraffin-embedded (FFPE) human breast cancer specimens using a specific heat-induced epitope retrieval (HIER) method and incubation with the primary rabbit monoclonal antibody ER α, Clone EP1. The procedure employs a series of incubation and wash steps, a ready-to-use horseradish peroxidase (HRP)-linked visualization reagent. The enzymatic conversion of the added 3,3'-Diaminobenzidine (DAB+) chromogen results in the formation of a visible reaction product at the antigen site. After the IHC staining, specimens may be counterstained, cover slipped, and then visualized with a light microscope.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Intra-/Inter-Run Precision:

Studies to assess the intra-run and inter-run precision were conducted using the FLEX Monoclonal Rabbit Anti-Human Estrogen Receptor α, Clone EP1, Ready-to-Use (Dako Omnis), according to the instructions for use. In each of these studies, 12 different FFPE tissue blocks of human breast carcinoma were evaluated at one site. The sample set represented the dynamic range of ER protein expression including 3 cases around the cut-off. Five runs were performed on 5 non-consecutive days over a period of at least 20 days. Each run also included one section from each block that was stained with a negative control reagent (NCR). Positive staining was defined as ≥ 1 % of tumor nuclei stained at any intensity.

The acceptance criteria for the percent agreements were at least 85% lower bounds of the two-sided 95% confidence intervals, using Wilson score. All specimens stained with the NCR were negative with no specific staining and < 1 background staining intensity. All specimens stained with ERα, Clone EP1 antibody had 100% overall agreement with regard to the ER status (positive and negative) for both intra- and inter-run precision.

Inter-Laboratory Reproducibility:

Reproducibility was evaluated at 4 sites with one pathologist each. At 3 sites, tissue sections from a set of 20 different breast cancer specimens (8 positive, 8 negative, and 4 near the cutoff) were evaluated. The fourth site assessed 17 of the 20 tissue specimens; 3 were excluded due to lack of remaining tissue and tissue degradation. Each site stained slides on 5 non-consecutive days over at least 20 days. Following staining, the study slides were blinded and randomized prior to evaluation by a pathologist.

The results for the inter-laboratory reproducibility study were reported as overall, positive, and negative percent agreement and the two-sided 95% confidence intervals (95% CI). The acceptance criteria of ≥ 85% at the lower bound of the two-sided 95% confidence intervals, using Wilson score confidence intervals, were met. Summary results of the inter-laboratory reproducibility study for the 17 specimens tested at all 4 laboratories is shown below.

Inter-Laboratory Reproducibility across All Sites:

	Positive	Negative	Total
Positive	173	7	180
Negative	2	158	160
Total	175	165	340

Positive Percent Agreement = 96.1% (95% CI: 92.2 – 98.1)
Negative Percent Agreement = 98.8% (95% CI: 95.6 – 99.7)
Overall Percent Agreement = 97.4% (95% CI: 95.0 – 98.6)

Summary results for each site are shown in the tables below. The comparisons between sites 1-3 included 20 specimens and comparisons with site 4 included 17 specimens.

Site 1 vs. Site 2:

	Positive	Negative	Total
Positive	46	2	48
Negative	14	38	52
Total	60	40	100

Positive Percent Agreement = 95.8% (95% CI: 86.0 – 98.8)
Negative Percent Agreement = 73.1% (95% CI: 59.7 – 83.2)
Overall Percent Agreement = 84.0% (95% CI: 75.6 – 89.9)

Site 1 vs. Site 3:

	Positive	Negative	Total
Positive	46	2	48
Negative	14	38	52
Total	60	40	100

Positive Percent Agreement = 95.8% (95% CI: 86.0 – 98.8)
Negative Percent Agreement = 73.1% (95% CI: 59.7 – 83.2)
Overall Percent Agreement = 84.0% (95% CI: 75.6 – 89.9)

Site 1 vs. Site 4:

	Positive	Negative	Total
Positive	38	2	40
Negative	7	38	45
Total	45	40	85

Positive Percent Agreement = 95.0% (95% CI: 83.5 – 98.6)
Negative Percent Agreement = 84.4% (95% CI: 71.2 – 92.3)
Overall Percent Agreement = 89.4% (95% CI: 81.1 – 94.3)

Site 2 vs. Site 3:

	Positive	Negative	Total
Positive	60	0	60
Negative	0	40	40
Total	60	40	100

Positive Percent Agreement = 100% (95% CI: 94.0 – 100)
Negative Percent Agreement = 100% (95% CI: 91.2 – 100)
Overall Percent Agreement = 100% (95% CI: 96.3 – 100)

Site 2 vs. Site 4:

	Positive	Negative	Total
Positive	45	0	45
Negative	0	40	40
Total	45	40	85

Positive Percent Agreement = 100% (95% CI: 92.2 – 100)
Negative Percent Agreement = 100% (95% CI: 95.6 – 100)
Overall Percent Agreement = 100% (95% CI: 95.0 – 100)

Site 3 vs. Site 4:

	Positive	Negative	Total
Positive	45	0	45
Negative	0	40	40
Total	45	40	100

Positive Percent Agreement = 100% (95% CI: 92.1 – 100)
Negative Percent Agreement = 100% (95% CI: 91.2 – 100)
Overall Percent Agreement = 100% (95% CI: 95.7 – 100)

Inter-Observer and Inter-Instrument Precision:

An inter-observer study was conducted in which the samples from the inter-laboratory reproducibility study were assessed independently by 3 pathologists. The results of the study are shown below.

Inter-Observer Precision Results:

	Positive	Negative	Total
Positive	533	6	539
Negative	3	357	360
Total	536	363	899

Positive Percent Agreement = 98.9% (95% CI: 97.6 – 99.5)
Negative Percent Agreement = 99.1% (95% CI: 97.6 – 99.5)
Overall Percent Agreement = 99.0% (95% CI: 98.1 – 99.5)

Inter-instrument agreement was evaluated, where one observer evaluated all slides stained at three laboratories using three different instruments. A total of 20 specimens were stained in 5 runs across three different instruments, for a total of 300 comparisons. The results of the study are shown below.

Inter-Instrument Precision Results:

	Positive	**Negative**	**Total**
Positive	180	0	180
Negative	0	120	120
Total	180	120	300

Positive Percent Agreement = 100.0% (95% CI: 97.9 – 100)
Negative Percent Agreement = 100.0% (95% CI: 96.9 – 100)
Overall Percent Agreement = 100.0% (95% CI: 98.7 – 100)

Lot-to-Lot Reproducibility:

A single site, blinded, randomized study using 3 lots of antibody and FFPE breast cancer tissues expressing differing levels of ER was conducted to assess lot-to-lot reproducibility. Three separate automated runs using a total of 30 different FFPE breast cancer tissue specimens tested in triplicate for a total of 270 data points, plus positive and negative control slides, were stained on Dako Omnis instruments. Slides were scored and staining characteristics recorded by a single pathologist. One data point was excluded due to folded tissue for a total of 269 evaulable data points. Lot-to-lot reproducibility across the 3 lots was evaluated by calculating the negative percent agreement, positive percent agreement, and overall percent agreement. The results are shown below.

Inter-Lot Reproducibilty Results:

	Positive	**Negative**	**Total**
Positive	125	0	125
Negative	1	143	144
Total	126	143	269

Positive Percent Agreement = 100.0% (95% CI: 97.0 – 100)
Negative Percent Agreement = 99.3% (95% CI: 96.2 – 99.9)
Overall Percent Agreement = 99.6% (95% CI: 97.9 – 99.9)

b. *Linearity/assay reportable range:*

Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Stability:

Reagent shelf-life stability was assessed at specified time intervals (0, 8 10, 12, and 14 months) at 2-8 °C using 3 manufactured lots of primary antibody. Additionally, on-board stability was assessed by placing the same 3 manufactured lots of primary antibody at 18 °C (in-use conditions) at various time intervals during the course of the study. One of the 3 antibody lots was also subjected to a transport simulation test, i.e., 3 freeze/thawing cycles (freeze at -18°C and thaw at 2-8°C) followed by incubation at 35-39 °C for 16-24 hours and then incubation at 28-32 °C for 4 days.

Results demonstrated a shelf-life stability of 12 months when stored at 2-8 °C, and on-board stability of 80 hours by the end of the study. The transportation stability testing met the acceptance criteria of no more than 0.5 grade specific staining intensity with the aged antibody when compared to baseline and the freshly diluted EP1 clone antibody.

Controls:

Positive (breast carcinoma or benign cervix) and negative controls (normal colon or endothelium of breast carcinoma or benign cervix) 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:

Three independent samples from 30 normal tissue types were tested with the ERα, Clone EP1 and Dako EnVision FLEX visualization system. Results demonstrated negative immunoreactivity with most tissues expected to be negative, and positive nuclear staining in a subset of normal cells from seven tissue types including epithelial cells and/or stromal cells from breast, cervix, esophagus, ovary, prostate, tonsil and uterus. The distribution of ERα, Clone EP1 normal tissue reactivity is summarized in the table below.

Tissue Type	Positive Nuclear Staining Tissue Elements	Tissue Type	Positive Nuclear Staining Tissue Elements
Adrenal	0/3	Ovary	3/3 Stromal cells (10-25%) 1/3 Epithelial cells (50%)
Bone marrow	0/3	Pancreas	1/3 Stromal cells (<5%)
Breast	3/3 Epithelial cells (<5-80%)	Placenta	0/3
Cerebellum	0/3	Pituitary	3/3 Pituitary cells (<5-<10%)
Cerebrum	0/3	Prostate	3/3 Epithelial cells (<5-10%)
Cervix	3/3 Epithelial cells (15->80%) 3/3 Stromal cells (50-80%)	Salivary gland	0/3
Colon	0/3	Skin	1/3 Epithelial cells (5%)
Endometrium	3/3 Epithelial cells (>80->90%) 2/3 Stromal cells (>90%) 2/3 Muscle cells (>90%)	Small intestine	0/3
Esophagus	0/3	Spinal cord	0/3
Fallopian tube	3/3 Epithelial cells (80-90%) 3/3 Stromal cells (60-90%)	Spleen	0/3
Heart	0/3	Stomach	0/3
Kidney	0/3	Testis	0/3
Liver	0/3	Thyroid	0/3
Lung	0/3	Tonsil	1/3 Epithelial cells (1%)
Lymph node	0/3	Ureter	0/3
Muscle, skeletal	0/3	Urinary bladder	1/3 Epithelial cells (5%) 1/3 Stromal cells (5%)
Nerve, peripheral	0/3	Uterus	3/3 Epithelial cells (25-100%) 3/3 Stromal cells (25->90%) 3/3 Muscle cells (15->90%)

f. Assay cut-off:

A positive staining result is defined as ≥1% of tumor cell nuclei staining of any intensity and a negative result is <1% tumor cell nuclei staining of any intensity.

2. Comparison studies:

a. Method comparison with predicate device:

A blinded, randomized concordance study was conducted at 2 sites using 250 FFPE samples, split evenly (n=125) between each site, and tested with both the subject and predicate devices according to the instructions for use. Breast cancer tissue samples were pre-screened with the predicate to ensure that there was an approximately equal distribution of ERα-positive (n=107) and ERα-negative specimens (n=123) and that there was a number of samples expressing ERα staining levels near the cut-off (n=20). Of the 250 samples, 12 samples were excluded because they did not meet pre-established inclusion criteria (10 samples were the incorrect specimen type and 2 failed QC).

The method comparison study demonstrated levels of agreement, as noted in the table below.

Subject Device		Predicate Device		
		Positive	**Negative**	**Total**
	Positive	114	10	124
	Negative	0	114	114
	Total	114	124	238

Positive Percent Agreement = 100% (95% CI: 96.7 – 100)
Negative Percent Agreement = 91.9% (95% CI: 85.8 – 95.6)
Overall Percent Agreement = 95.8% (95% CI: 92.4 – 97.7)

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):

Not applicable

4. Clinical cut-off:

A positive result is defined as nuclear staining of ≥1% of tumor cells at any stain intensity.

5. Expected values/Reference range:

Not applicable

N. Instrument Name:

Dako Omnis

O. System Descriptions:

1. Modes of Operation:
   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?
   Yes ____X____ or No _____
   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?
   Yes ____X____ or No _____

2. Software:
   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
   Yes ___X_____ or No _____

3. Specimen Identification:

   Glass specimen slides are identified by the Dako Omnis through a built-in barcode reader in the Dako Omnis loading station. Labels are generated at the Dako Omnis Workstation through the Laboratory Information System (LIS).

4. Specimen Sampling and Handling:

   Specimen sampling, which includes FFPE tissues, is performed by clinicians. The specimens are processed by trained healthcare professionslas, and the FFPE tissue specimen slides are manually placed into slide racks and loaded into the Dako Omnis loading station, which has a 60 slide capacity.

5. Calibration:

   The Dako Omnis automatically prompts for any required maintenance when needed, and displays an overview of upcoming maintenance actions and guides the user through the procedures.

6. Quality Control:

   The Dako Omnis Solution Software on the Dako Omnis autostainer continuously monitors the workflow during the staining run. If the Dako Omnis Solution Software detects a inapropriate external or internal anomoly related to the instrument, errors will be presented to the user through the built-in touch screen interface with appropriate instructions for resolving the error.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not Applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

R. Conclusion:

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