510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K170005

B. Purpose for Submission:

Addition of the Dako Omnis staining platform and the EnVision FLEX visualization system to the Monoclonal Mouse Anti-Human Progesterone Receptor, Clone PgR 1294.

C. Measurand:

Human progesterone receptor protein

D. Type of Test:

Immunohistochemistry

E. Applicant:

Dako North America, Inc.

F. Proprietary and Established Names:

FLEX Monoclonal Mouse Anti-Human Progesterone Receptor, Clone PgR 1294, Ready-to-Use (Dako Omnis)

G. Regulatory Information:

1. Regulation section:

   21 CFR 864.1860

2. Classification:

   Class II

3. Product code:

   MXZ

4. Panel:

   Pathology (88)

H. Intended Use:

1. Intended use(s):

   For in vitro diagnostic use

   FLEX Monoclonal Mouse Anti-Human Progesterone Receptor, Clone PgR 1294, 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 progesterone receptor in formalin-fixed, paraffin-embedded tissue sections of human breast cancer. The antibody binds progesterone receptor-expressing cells and is useful in the assessment of progesterone 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:

FLEX Monoclonal Mouse Anti-Human Progesterone Receptor, Clone PgR 1294, Ready-to-Use (Dako Omnis) is utilized to perform a qualitative immunohistochemical (IHC) assay to identify progesterone receptor (PR) expression in formalin-fixed, paraffin embedded (FFPE) human breast cancer tissues. The antibody is provided in pre-diluted and provided in liquid form in a buffer containing stabilizing protein and 0.015 mol/L sodium azide.

The Dako Omnis is intended for fully automated slide-based staining of 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 an IHC slide staining process according to the assay protocol for the FLEX Monoclonal Mouse Anti-Human Progesterone Receptor, Clone PgR 1294, Ready-to-Use (Dako Omnis).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   FLEX Monoclonal Mouse Anti-Human Progesterone Receptor, Clone PgR 636, Ready-to-Use, (Link)

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

   K130861

3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Intended Use	Detection of progesterone receptor	Same
Antibody Type	Monoclonal, mouse origin	Same
Isotype	IgG	Same
Staining Pattern	Nuclear	Same
Positive Cell Type	Cells expressing progesterone	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**
Clone	Clone PgR 1294	Clone PgR 636
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)
Antibody Incubation Time*	10 minutes	20 minutes
Onboard Stability	80 hours	Not defined nor applicable
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, 25mL
Cap	Dako Omnis Closure including septum	White Nalgene Cap
Deparaffinization	Onboard 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 2x5 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:

FLEX Monoclonal Mouse Anti-Human Progesterone Receptor, Clone PgR 1294, Ready-to-Use (Dako Omnis) specifically binds to PR proteins located in the cell nucleus of PR-expressing cells. It is optimized for use on formalin-fixed, paraffin embedded (FFPE) breast cancer tissues. Automated immunohistochemical staining is performed on routinely

processed, FFPE specimens using a specific heat-induced epitope retrieval (HIER) method and incubation with the primary mouse monoclonal antibody PR, Clone PgR 1294. The procedure employs a series of incubation and wash steps, and 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. The tissue slide may then be counterstained with hematoxylin stain and coverslipped. The slide is 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 Mouse Anti-Human Progesterone Receptor, Clone PgR 1294, Ready-to-Use on the Dako Omnis, following the recommended staining procedure with the EnVision FLEX Visualization System. In each of these studies, 12 different paraffin embedded tissue blocks of human breast carcinoma were evaluated. The sample set represented the dynamic range of PR 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 with 3 sections. Each run also included 1 section from each block that was stained with a negative control reagent. Positive staining was defined as ≥ 1 % of tumor nuclei stained at any intensity. The results for both intra-run and inter-run were reported as overall agreement and the lower bound of the two-sided 95% confidence interval (95% CI). The acceptance criteria for the endpoint were at least 85% at the lower bound of a two-sided 95% confidence interval, using Wilson score confidence intervals.

The acceptance criteria were met, as staining calls for each tissue block stained with Anti-PR, Clone PgR 1294 did not differ within or between runs, and the slides stained with the negative control reagent were reported to have negative staining.

Inter-Laboratory Reproducibility:

Three sites with 1 investigator each evaluated tissue sections from a set of 20 different breast cancer specimens with approximately equal distribution of PR positive and negatives cases with 2 specimens around the cut-off. A fourth site was later added which tested 19 of the 20 original tissue sections; one was removed due to tissue degradation. Each site performed staining on 5 non-consecutive days over at least 20 days.

The results for the inter-laboratory reproducibility study were reported as positive and negative percent agreements (PPA and NPA) and the corresponding two-sided 95%

confidence intervals (95% CI). The acceptance criteria for the endpoints were at least 85% at the lower limit of a two-sided 95% confidence interval, using Wilson score confidence intervals. Summary results of the inter-laboratory reproducibility study for the 19 specimens tested at all 4 laboratories is shown below.

Inter-Laboratory Reproducibility Results across All Sites:

	Positive	Negative	Total
Positive	185	15	200
Negative	1	179	180
Total	186	194	380

Positive Percent Agreement = 92.5% (95% CI: 88.0 – 95.4)
Negative Percent Agreement = 99.4% (95% CI: 96.9 – 99.9)
Overall Percent Agreement = 95.8% (95% CI: 93.3 – 97.4)

Summary results for each site are shown in the tables below. Please note that comparisons with between sites 1-3 contained 20 specimens and comparisons between site 4 contained 19 specimens due to a missing specimen at time of testing.

Site 1 vs. Site 2:

	Positive	Negative	Total
Positive	37	0	37
Negative	18	45	63
Total	55	45	100

Positive Percent Agreement = 100% (95% CI: 90.6 – 100)
Negative Percent Agreement = 71.4% (95% CI: 59.3 – 81.1)
Overall Percent Agreement = 82.0% (95% CI: 73.3 – 88.3)

Site 1 vs. Site 3:

	Positive	Negative	Total
Positive	37	0	37
Negative	19	44	63
Total	56	44	100

Positive Percent Agreement = 100% (95% CI: 90.6 – 100)
Negative Percent Agreement = 69.8% (95% CI: 57.6 – 79.8)
Overall Percent Agreement = 81.0% (95% CI: 72.2 – 87.5)

Site 1 vs. Site 4:

	Positive	Negative	Total
Positive	35	0	35
Negative	15	45	60
Total	50	45	95

Positive Percent Agreement = 100% (95% CI: 90.1 – 100)
Negative Percent Agreement = 75.0% (95% CI: 62.8 – 84.2)
Overall Percent Agreement = 84.2% (95% CI: 75.6 – 90.2)

Site 2 vs. Site 3:

	Positive	Negative	Total
Positive	55	0	55
Negative	1	44	45
Total	56	44	100

Positive Percent Agreement = 100% (95% CI: 93.5 – 100)
Negative Percent Agreement = 97.8% (95% CI: 88.4 – 99.6)
Overall Percent Agreement = 99.0% (95% CI: 94.6 – 99.8)

Site 2 vs. Site 4:

	Positive	Negative	Total
Positive	50	0	50
Negative	0	45	45
Total	50	45	95

Positive Percent Agreement = 100% (95% CI: 92.9 – 100)
Negative Percent Agreement = 100% (95% CI: 92.1 – 100)
Overall Percent Agreement = 100% (95% CI: 96.1 – 100)

Site 3 vs. Site 4:

	Positive	Negative	Total
Positive	50	1	51
Negative	0	44	55
Total	50	45	95

Positive Percent Agreement = 98.0% (95% CI: 89.7 – 99.7)
Negative Percent Agreement = 100% (95% CI: 92.0 – 100)
Overall Percent Agreement = 98.9% (95% CI: 94.3 – 99.8)

Inter-Observer/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	504	6	510
Negative	8	382	390
Total	512	388	900

Positive Percent Agreement = 98.8% (95% CI: 97.5 – 99.5)
Negative Percent Agreement = 97.9% (95% CI: 96.0 – 99.0)
Overall Percent Agreement = 98.4% (95% CI: 97.4 – 99.1)

Inter-instrument agreement was evaluated, where one observer evaluated all slides stained at 3 laboratories using 3 different instruments. A total of 20 specimens were stained in 5 runs across 3 different instruments, for a total of 300 comparisons. The results of the study are shown below.

Inter-Instrument Precision Results:

	Positive	**Negative**	**Total**
Positive	165	0	165
Negative	7	128	135
Total	172	128	300

Positive Percent Agreement = 100.0% (95% CI: 97.7 – 100)
Negative Percent Agreement = 94.8% (95% CI: 89.7 – 97.5)
Overall Percent Agreement = 97.7% (95% CI: 95.3 – 98.9)

Lot-to-Lot Reproducibility:

The lot-to-lot reproducibility of the Anti-PR, Clone PgR 1294 primary antibody was evaluated across 3 manufactured lots of antibody, using 30 human breast carcinoma tissue samples with differing levels of PR expression, including 6 specimens around the cut-off. Samples were tested in triplicate for each lot for a total of 9 serial sections per specimen. The results were reported as positive percent agreement (PPA) and negative percent agreement (NPA) with the two-sided 95% confidence intervals (95% CI). The acceptance criteria for the endpoints were at least 85% at the lower bound of a two-sided 95% confidence interval, using Wilson score confidence intervals.

For all the studies, staining calls for each tissue block stained with Anti-PR, Clone PgR 1294 did not differ within lots or between lots, and the slides stained with the negative control reagent were reported to have negative staining. The results are summarized below.

Inter-Lot Reproducibilty Results:

	Positive	**Negative**	**Total**
Positive	126	0	126
Negative	0	144	144
Total	126	144	270

Positive Percent Agreement = 100.0% (95% CI: 97.0 – 100)
Negative Percent Agreement = 100% (95% CI: 97.4 – 100)
Overall Percent Agreement = 100% (95% CI: 98.6 – 100)

b. *Linearity/assay reportable range:*

Not applicable

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

Stability:

Real-time stability tests using 3 lots of the device and simulated ship-stress tests using 1 lot of the device were conducted to determine the shelf life of the reagent. Additionally, all 3 lots were subjected to in-use/onboard testing with each lot being subjected to a minimum of 80 hours of time on-board the instrument. Based on the testing, the current product shelf-life is set at 12 months with 80 hours of on-board stability.

Controls:

Positive and negative tissue controls should be run simultaneously using the same protocol as the patient specimens in each staining run. Ideally, the positive controls should include a low to moderate PR-expressing breast carcinoma tissue. As an alternative, benign cervix may also be used. Studies on formalin-fixed, paraffin-embedded cervix have shown variations in progesterone receptor expression based on the menstrual cycle. For negative tissue controls, colon epithelium or endothelium present in cervix or breast carcinoma may be used. In addition, each case should have a negative reagent control slide included in the staining run.

d. *Detection limit:*

Not applicable

e. *Analytical specificity:*

Three samples from 30 tissue types for a total of 90 samples were stained with the FLEX Monoclonal Mouse Anti-Human Progesterone Receptor, Clone PgR 1294, Ready-to-Use on the Dako Omnis, using the recommended staining procedure with EnVision FLEX Visualization System. The cell types that most commonly demonstrated nuclear positivity were epithelial cells, stromal cells, and interstitial cells. Nuclear PR expression was also observed in breast glandular cells, cervix stromal cells, endometrium (epithelial, muscle, and stromal cells), the fallopian tube (epithelial and stromal cells), ovary (epithelia and stromal cells), pancreas insular cells, prostate (epithelial and stromal) , and uterus (smooth, stromal, and glandular cells). Low nuclear PR expression (< 10% of cells) was observed in colon smooth muscle cells, the pituitary, salivary gland, testis stromal cells, and urinary bladder stromal cells. Weak cytoplasmic immunoreactivity was also observed in adrenal

glomerular cells.

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:

This study was a three-site, blinded, randomized, comparative study using 300 FFPE breast cancer specimens containing differing levels of PR expression. Samples were run on both the current assay, FLEX Monoclonal Mouse Anti-Human Progesterone Receptor, Clone PgR 1294, Ready-to-Use (Dako Omnis), and on the predicate, FLEX Monoclonal Mouse Anti-Human Progesterone Receptor, Clone PgR 636, Ready-to-Use, (Dako Link) according to their respective instructions for use. In total, there were 600 stained sections (300 per device). Twenty-two slides were excluded from analysis due to insufficient or absent invasive tumor cells or tissue shredding, resulting in a total of 289 specimens tested per device. Appropriate positive and negative control slides were included. Stained slides were interpreted by pathologists and the intensity and the proportion of cells staining were recorded. PR expression in DCIS also was evaluated. Positive staining was defined as ≥ 1 % of tumor nuclei stained at any intensity.

The acceptance criteria for the PPA and NPA endpoints were at least 85% at the lower bounds of the two-sided 95% confidence intervals, using Wilson score confidence intervals. The results were a PPA of 96.7% with a lower bound of the two-sided 95% confidence interval being 93.2% and an NPA of 100% with a lower bound of the two-sided 95% confidence interval being 95.4%. A table of the results is shown below.

Subject Device		**Predicate Device**		
		Positive	**Negative**	**Total**
	Positive	202	7	209
	Negative	0	80	80
	Total	202	87	289

Positive Percent Agreement = 96.7% (95% CI: 93.2 – 98.4)
Negative Percent Agreement = 100% (95% CI: 95.4 – 100)
Overall Percent Agreement = 97.6% (95% CI: 95.1 – 98.8)

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.