

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

k103818

**B. Purpose for Submission:**

New device

**C. Measurand:**

Human progesterone receptor protein

**D. Type of Test:**

Qualitative

**E. Applicant:**

Ventana Medical Systems, Inc.

**F. Proprietary and Established Names:**

CONFIRM anti-Progesterone Receptor (PR) (1E2) Rabbit Monoclonal Primary Antibody

**G. Regulatory Information:**

1. Regulation section:

21 CFR § 864.1860, Immunohistochemistry reagents and kits

2. Classification:

Class II

3. Product code:

MXZ, Immunohistochemistry Assay, Antibody, Progesterone Receptor

4. Panel:

Pathology 88

**H. Intended Use:**

1. Intended use(s):

CONFIRM anti-Progesterone Receptor (PR) (1E2) Rabbit Monoclonal Primary Antibody is intended for laboratory use for the qualitative detection of progesterone receptor (PR) antigen in sections of formalin-fixed, paraffin-embedded tissue on a Ventana automated slide stainer with Ventana detection kits and ancillary reagents. CONFIRM anti-PR (1E2) is directed against an epitope present on human PR protein located in the nucleus of PR positive normal and neoplastic cells.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

2. Indication(s) for use:

CONFIRM anti-PR (1E2) is indicated as an aid in the management, prognosis, and prediction of hormone therapy for breast carcinoma.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Ventana BenchMark XT or BenchMark ULTRA automated slide stainer

**I. Device Description:**

The Ventana CONFIRM anti-Progesterone Receptor (PR) (1E2) Rabbit Monoclonal Primary Antibody is a qualitative immunohistochemical assay used to identify the

progesterone receptor (PR) antigen in normal and neoplastic tissues sections that have been formalin-fixed and paraffin-embedded. These antibodies are available as ready-to-use reagents and can only be used on the Ventana Benchmark immunostainer platforms.

The antibody is diluted in 0.05M Tris-HCl with 2% carrier protein, and 0.1% ProClin 300, a preservative. There is trace (~0.2%) fetal calf serum of U.S. origin from the stock solution. Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 1 µg/mL. It also contains a biotin conjugated secondary antibody formulation which recognizes rabbit or mouse immunoglobulins and a streptavidin-enzyme conjugate which binds to the biotin on the secondary antibody. CONFIRM anti-PR (1E2) is a rabbit monoclonal antibody produced as a cell culture supernatant. There is no known non-specific antibody reactivity observed in this product.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
Dako FLEX Monoclonal Mouse Anti-Human Progesterone Receptor Clone PGR636
2. Predicate 510(k) number(s):  
k020023
3. Comparison with predicate:

<b>Similarities</b>		
Item	Device	Predicate
Target	Human PR protein	Human PR protein
Assay method	IHC with secondary detection	IHC with secondary detection
Detection system	Indirect	Indirect
Sample	Formalin-fixed paraffin-embedded breast cancer specimens	Formalin-fixed paraffin-embedded breast cancer specimens

<b>Differences</b>		
Item	Device	Predicate
Antibody Type & clone	Monoclonal (Rabbit) 1E2	Monoclonal (Mouse) 636
Assay format	Ventana BenchMark XT and BenchMark Ultra autostainers	Dako Autostainer

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

Guidance for Industry - "Guidance for Submission of Immunohistochemistry Applications to the FDA"

**L. Test Principle:**

CONFIRM anti-PR (1E2) assay is intended to be used on breast carcinoma specimens. The CONFIRM anti-PR (1E2) primary antibody binds to human progesterone receptor located in the nuclear region of normal and neoplastic cells. Automated immunohistochemistry staining is performed on formalin-fixed, paraffin-

embedded tissue. The antigen in tissue sections is demonstrated through several steps. The specific antibody binds to the antigen present in tissue sections. The bound primary antibody is located by a biotin conjugated secondary antibody formulation which recognizes rabbit or mouse immunoglobulins. This step is followed by the addition of a streptavidin-enzyme conjugate which binds to the biotin on the secondary antibody. The primary antibody-secondary antibody-avidin enzyme complex is visualized by using a precipitating enzyme generated product. This is readily detected by light microscopy.

#### **M. Performance Characteristics:**

##### 1. Analytical performance:

###### a. *Precision/Reproducibility:*

Studies to assess the intra-run (within-run, intra-day) and inter-run (day-to-day) analytical precision of CONFIRM anti-PR (1E2) antibody was conducted using two Ventana IHC stainers: BenchMark XT and BenchMark Ultra instruments and the iView DAB Detection Kit with biotin blocker reagents (A/B Blocker). In each study, nine slides were stained with the CONFIRM anti-PR (1E2) antibody and one slide was stained with Negative Control Rabbit Ig antibody from six individual breast carcinoma tissue blocks. Of the six tissues, two were PR negative, two were PR low expression and two were PR high expression based on a cutoff of <1% tumor cells staining for negative, 1-10% for low and >10% for high expression. A total of 30 slides were stained on each of the two IHC stainers - BenchMark XT and BenchMark Ultra instruments. An acceptance criteria of at least 90% overall agreement rates for positive and negative staining status was set by the sponsor.

###### Intra-run Precision:

Intra-run precision of CONFIRM anti-PR (1E2) antibody on BenchMark XT was 100% concordant for all six cases. The background was reported as 0 on 100% of the tissues stained. Intra-run precision of CONFIRM anti-PR (1E2) antibody on BenchMark ULTRA was 100% concordant for all six cases. Background was below 0.25 on 100% of the tissues stained. The acceptance criteria set by the sponsor were met in these studies.

###### Inter-run Precision:

Inter-run precision was assessed in five separate nonconsecutive runs conducted over a 20 day period on the same BenchMark XT. There were a total of 30 slides per run. The same testing configuration was also performed on a BenchMark ULTRA instrument.

Inter-run precision of CONFIRM anti-PR (1E2) antibody on BenchMark XT was 100% concordant for all six cases. The background was reported as below 0.5 on 100% of the tissues stained. Inter-run precision of CONFIRM anti-PR (1E2) antibody on BenchMark ULTRA was 100% concordant for all six cases. Background was below 0.25 on 100% of the tissues stained. The acceptance criteria set by the sponsor were met in these studies.

###### Intra-platform Reproducibility

Four slides from six cases were stained with CONFIRM anti-PR (1E2)

antibody and one slide from each case was stained with Negative Control Rabbit Ig antibody on three separate BenchMark XT instruments. The same testing configuration was used for the BenchMark ULTRA instrument. The acceptance criteria set by the sponsor was as follows: The percentage of cells staining shall not vary in binning categories (as defined in protocol) on a minimum of 80% (positive) tissues tested. Background shall be  $\leq 0.5$  on a minimum of 90% of samples stained. This study met the acceptance criteria set by the sponsor and showed acceptable reproducibility with no variation in staining quality and patterns.

- b. *Linearity/assay reportable range:*  
Not applicable
  - c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*  
Controls: Positive and negative control slides should be stained with each staining run. The pathologist is responsible for assuring the proper performance of this test.  
Stability: Real-time stability tests and ship-stress tests were conducted to determine the shelf life of the reagent. Three lots of the reagent were tested. The acceptance criteria set by the sponsor were as follows: Positive cases – nuclear staining of 51% - 100% of the tumor cells and no more than 0.5 difference in staining intensity when compared to the Day 0 slide stain and less than or equal to 0.5+ non-specific staining. Background staining (0-4+) must not be greater than 0.5+. The acceptance criteria were met in these studies. Based on these studies, the expiration date of this reagent is set at 18 months.
  - d. *Detection limit:*  
Not applicable
  - e. *Analytical specificity:*  
A total of 90 formalin-fixed and paraffin-embedded tissues covering a wide range of normal human tissues types were tested with the CONFIRM anti-PR (1E2) antibody. The antibody demonstrated negative immunoreactivity with most tissues. Positive immunoreactivity was noted in breast, uterus, and cervix (uterine) tissues as expected.
  - f. *Assay cut-off:*  
A negative staining result is defined as  $< 1\%$  tumor cells staining and a positive staining result is defined as  $\geq 1\%$  tumor cells staining of any intensity.
2. Comparison studies:
- a. *Method comparison with predicate device:*  
The Ventana CONFIRM anti-PR (1E2) antibody was compared to the predicate device Dako FLEX Monoclonal Mouse Anti-Human Progesterone Receptor Clone PGR636.  
  
A total of 336 breast carcinoma slides were tested in 3 different sites on the two Ventana platforms and the Dako Autostainer Plus. The acceptance criteria set by the sponsor were as follows: greater than 85% positive and negative agreement rates across all sites for CONFIRM anti-PR (1E2) staining

on both the BenchMark ULTRA and XT compared to the Dako Autostainer Plus, with the lower bound 95% CI of at least 77% for all comparisons.

CONFIRM anti-PR (1E2) on BenchMark ULTRA and FLEX anti-PR Clone PgR 636 on Dako Autostainer Plus

	FLEX anti-PR Clone PgR 636		
CONFIRM anti-PR (1E2)	Positive	Negative	Total
Positive	200	7	207
Negative	9	104	113
Total	209	111	320
Positive Percent Agreement	200/209	95.7% (92.0-97.7)	(95% CI)
Negative Percent Agreement	104/111	93.7% (87.6-96.9)	(95% CI)

CONFIRM anti-PR (1E2) on BenchMark XT and FLEX anti-PR Clone PgR 636 on Dako Autostainer Plus

	FLEX anti-PR Clone PgR 636		
CONFIRM anti-PR (1E2)	Positive	Negative	Total
Positive	186	9	195
Negative	18	100	118
Total	204	109	313
Positive Percent Agreement	186/204	91.2% (86.5-94.3)	(95% CI)
Negative Percent Agreement	100/109	91.7% (85.0-95.6)	(95% CI)

An additional assessment of the staining of the ductal carcinoma in-situ (DCIS) component was also performed. The primary objective of this study was to determine if the performance of the staining in areas of human breast carcinoma specimens using the CONFIRM anti-PR (1E2) with BenchMark ULTRA was equivalent to that using the FLEX anti-PR (636) with the Dako Autostainer Plus. All slides from the method comparison study that contained invasive tumor as well as DCIS component were assessed. There were no pre-set acceptance criteria. Of the PR positive cases, >99% of cases had positive staining in the DCIS component as well as the invasive component.

CONFIRM anti-PR (1E2) on BenchMark ULTRA and FLEX anti-PR Clone PgR 636 on Dako Autostainer Plus

FLEX anti-PR Clone PgR 636 (Dako)			
CONFIRM anti-PR (1E2)	Positive	Negative	Total
Positive	97	3	100
Negative	2	17	19
Total	99	20	119
Positive Percent Agreement	97/99	98.0%	
Negative Percent Agreement	17/20	85.0%	

CONFIRM anti-PR (1E2) on BenchMark XT and FLEX anti-PR  
Clone PgR 636 on Dako Autostainer Plus

FLEX anti-PR Clone PgR 636 (Dako)			
CONFIRM anti-PR (1E2)	Positive	Negative	Total
Positive	94	1	95
Negative	5	19	24
Total	99	20	119
Positive Percent Agreement	94/99	94.9%	
Negative Percent Agreement	19/20	95.0%	

- 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):
  - 4. Clinical cut-off:  
Not applicable
  - 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.