

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

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

k120663

**B. Purpose for Submission:**

New device

**C. Measurand:**

Human Estrogen Receptor  $\alpha$

**D. Type of Test:**

Semi-quantitative, immunohistochemistry

**E. Applicant:**

Dako North America, Inc

**F. Proprietary and Established Names:**

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

**G. Regulatory Information:**

1. Regulation section:

21 CFR 864.1860, Immunohistochemistry reagents and kits

2. Classification:

Class II

3. Product code:

MYA, Estrogen receptor immunohistochemistry antibody assay

4. Panel:

Pathology (88)

## H. Intended Use:

1. Intended use(s):

For in vitro diagnostic use.

FLEX Monoclonal Rabbit Anti-Human Estrogen Receptor  $\alpha$ , Clone EP1, Ready to-Use, (LINK), is intended for use in immunohistochemistry with EnVision™ FLEX, High pH visualization kit together with Autostainer Link 48 to semi-quantitatively detect human estrogen receptor in formalin-fixed, paraffin-embedded tissue sections of human breast cancer. The antibody labels estrogen receptor  $\alpha$ -positive 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.

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 Autostainer Link 48

EnVision™ FLEX, High pH visualization kit

## I. Device Description:

The Dako Monoclonal Rabbit Anti-Human Estrogen Receptor (ER)  $\alpha$ , Clone EP1 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 Dako automated stainer (Autostainer Link 48) with the EnVision™ FLEX, High pH visualization kit. A vial of RTU ER  $\alpha$ , Clone EP1 contains 12mL of reagent available in liquid form in a 0.01 mol/L phosphate buffered saline, containing stabilizing protein and 0.015 mol/L sodium azide. The ER  $\alpha$  EP1 antibody target concentration is 3.7 $\mu$ g/mL with acceptable concentration range of 3.5 $\mu$ g/mL to 3.8 $\mu$ g/mL.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

ER  $\alpha$  component of the Dako ER/PR pharmDx™ Kit.

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

k042884

3. Comparison with predicate:

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Intended Use	Semi-quantitative detection of Estrogen Receptor $\alpha$	Semi-quantitative detection of Estrogen Receptor and Progesterone Receptor
Staining Pattern	Nuclear	Same
Positive Cell Type	Cells expressing estrogen receptor protein	Same
Tissue Type	Formalin-fixed paraffin-embedded breast cancer	Same
Technology	Immunochemistry	Same
Storage	2-8 °C	Same

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Antibody Type	Monoclonal, rabbit	Monoclonal, mouse
Isotype	IgG	IgG1, kappa; IgG2a, kappa
ER clone(s)	EP1	1D5 and ER-2-123
Interpretation of results	Positive: $\geq 1\%$ positive staining tumor cells	Allred Scoring Method: 3 to 8= positive
Configurations	Pre-diluted Ready-to-Use with Dako Autostainer Link 48 instrument	Pre-diluted Ready-to-Use with Dako instruments

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

- In Vitro Diagnostic Devices: Guidance for the Preparation of 510(k) Submissions.

- Guidance for Industry: Guidance for Submission of Immunohistochemistry Applications to the FDA.

#### **L. Test Principle:**

Rabbit monoclonal anti-human antibody ER  $\alpha$ , Clone EP1, specifically binds to the estrogen receptor- $\alpha$  antigen located in the nuclear region of a variety of normal and neoplastic tissues. Automated immunohistochemical staining is performed on routinely processed, formalin-fixed, paraffin-embedded (FFPE) specimens using a specific heat-induced epitope retrieval (HIER) method and incubation with the primary rabbit monoclonal antibody ER  $\alpha$ , Clone EP1. The procedure employs a ready-to-use horseradish peroxidase (HRP)-linked visualization reagent. And the enzymatic conversion of the added DAB+ chromogen results in the formation of a visible reaction product at the antigen site. Specimen may then be counterstained and coverslipped and visualized with a light microscope.

#### **M. Performance Characteristics (if/when applicable):**

##### 1. Analytical performance:

###### *a. Precision:*

Precision study was conducted with serial sections from 12 different formalin-fixed paraffin-embedded human breast carcinoma tissue blocks, representing a dynamic range of ER expression which included samples around the cutoff. Testing was performed by three operators at a single site, utilizing a single instrument (Autostainer Link 48) per operator. Tissue sections were stained with ER  $\alpha$ , clone EP1 antibody using Dako EnVision™ FLEX visualization system protocol.

Study was focused on proportion scores based on a 0 to 5 scale and the goal was to demonstrate that estrogen receptor status (positive or negative) will remain the same within a specimen tested across all variables. A specimen is considered estrogen receptor-positive if  $\geq 1\%$  tumor cells display nuclear staining of any intensity.

Intra-run: Triplicate slides from each of 12 specimens were stained with ER  $\alpha$ , clone EP1 antibody on one Autostainer Link 48 instrument by one operator in a single run. Concurrently one section from each block was also stained with a negative control reagent.

Inter-run: A single slide from each specimen was stained with ER  $\alpha$ , clone EP1 antibody on the same one Link 48 instrument by the same one operator and repeated for five non-consecutive days. Concurrently, one section from each tissue block was also stained with a negative control reagent.

Inter-instrument/Inter-operator: A single slide from each of 12 specimens were stained with ER  $\alpha$ , clone EP1 antibody on three unique Autostainer Link 48 instruments by three different operators on the same day. Concurrently,

one slide from each tissue block was also stained with a negative control reagent

All 12 specimens stained with ER  $\alpha$ , EP1 clone antibody for intra-run, inter-run and inter-operator had a 100% concordance agreement with regard to the Estrogen Receptor Status (Positive or Negative). And all Specimens stained with the NCR were negative with 0 specific staining and <1 background staining.

*Reproducibility:*

Study was conducted at 2 U.S sites and 1 foreign site. A set of 18 randomized (9 positive and 9 negative) breast cancer tissue blocks expressing different levels of ER  $\alpha$  which also included samples around the cutoff, were selected by screening cut sections of FFPE tissues. Tissue sections were stained on Dako Autostainer Link 48 instruments by performing 5 separate automated runs at each study site for 5 non-consecutive days. The first automated run for 18 specimens included a control slide stained with ER  $\alpha$ , clone EP1 antibody, and a negative control reagent (NCR) slide. A total of 270 tissue sections were employed for the staining runs performed with the ER  $\alpha$ , clone EP1 primary antibody reagent. Comparative results for the 3 study sites were within the established value of  $\geq 85\%$  set for negative percent agreement, positive percent agreement, and total percent agreement. Summary results of site-to-site reproducibility study using rabbit monoclonal ER  $\alpha$  antibody EP1 clone are shown in the tables below:

**Site 1 vs. Site 2 reproducibility study**

		Site 1		
		Positive	Negative	Total
Site 2		44	2	46
	Positive	44	2	46
	Negative	0	44	44
	Total	44	46	90

Positive Percent Agreement = 97.8% (95% CI: 92.25-99.38)  
 Negative Percent Agreement = 95.7% (95% CI: 92.25-99.38)  
 Overall Percent Agreement = 97.8% (95% CI: 92.25-99.38)

**Site 1 vs. Site 3 reproducibility study**

		Site 1		
		Positive	Negative	Total
Site 3		44	1	45
	Positive	44	1	45
	Negative	0	45	45
	Total	44	46	90

Positive Percent Agreement = 98.9% (95% CI: 93.91-99.80)

Negative Percent Agreement = 98.9% (95% CI: 94.04-99.80)  
 Overall Percent Agreement = 98.9% (95% CI: 93.91-99.80)

**Site 2 vs. Site 3 reproducibility study**

		Site 2		
Site 3		Positive	Negative	Total
	Positive	45	0	45
	Negative	1	44	45
	Total	46	44	90

Positive Percent Agreement = 98.9% (95% CI: 94.04-99.81)  
 Negative Percent Agreement = 98.9% (95% CI: 93.91-99.80)  
 Overall Percent Agreement = 98.9% (95% CI: 93.97-99.80)

**Combined reproducibility study sites Percent Agreement:**

	Positive	Negative	Total
Positive	133	3	136
Negative	1	133	134
Total	134	136	270

Positive Percent Agreement = 98.15% (CI: 95.75 – 99.20)  
 Negative Percent Agreement = 98.15% (CI: 96.77 – 99.62)  
 Total Percent Agreement = 98.52% (CI: 96.25 – 99.42)

Lot-to-Lot Reproducibility study:

A single site, blinded, randomized, comparative study using 3 lots of antibody and full tissue sections of FFPE breast cancer tissue expressing differing levels of ER was conducted to assess lot-to-lot reproducibility. Three separate automated runs (11, 11 and 8 specimens) using a total of 30 different FFPE breast cancer tissue specimens, plus a positive and negative control slides, on Dako Autostainer Link48 instruments were stained with 3 different lots of the primary antibody and the appropriate negative control reagent (NCR). In addition, slides were scored and staining characteristics recorded by a single licensed pathologist. Lot-to-lot reproducibility within the 3 lots was evaluated by calculating the negative percent agreement, positive percent agreement, and total percent agreement of each paired lot. The range of negative percent agreement was above 92.9% to 100% across all lots, positive percent agreement was 100% across all lots and the total percent agreement was 96.7 to 100%.

*b. Linearity/assay reportable range:*

Not applicable

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

Stability:

Stability was tested for 3 manufactured lots of RTU rabbit monoclonal anti-human ER  $\alpha$ , clone EP1 at specified time intervals in real time and in-use/on-board stability at 2-8<sup>0</sup> C, 25<sup>0</sup>C, 37<sup>0</sup>C and 45<sup>0</sup>C. Prior to testing one lot of the antibody was also subjected to a rigorous transport simulation test i.e., 3 freeze/thawing cycles and incubation at 35<sup>0</sup>C – 39<sup>0</sup>C for 16-24 hours and at 28<sup>0</sup>C – 32<sup>0</sup>C for 4 days. Results demonstrated an interim shelf life of 12 months when store at 2 -8<sup>0</sup>C, based on the acceptance criteria of a positive specific staining in the nucleus of ER  $\alpha$ -positive cells and that results of specimens stained with the aged Rabbit Monoclonal anti-Human ER  $\alpha$ , should vary by no more than 0.5 grade specific staining intensity when compared to baseline and the freshly diluted EP1 clone antibody.

Control:

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:*

Sections from 90 normal tissues were tested with the ER  $\alpha$ , clone EP1 and Dako EnVision™ FLEX visualization system. Results demonstrated negative immunoreactivity with most tissues expected to be negative and positive nuclear staining was observed 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 ER  $\alpha$ , Clone EP1 normal tissue reactivity is summarized in the table below:

<b>Tissue type (# tested)</b>	<b>Positively Staining Tissue Elements</b>
Adrenal (3)	0/3
Bone marrow (3)	0/3
Breast (2)	2/2 Glandular epithelial cells (20%), nuclear
Cerebellum (3)	0/3
Cerebrum (3)	0/3
Cervix (3)	3/3 Epithelial cells (30%), nuclear
	3/3 Stromal cells (30%), nuclear
Colon (3)	0/3
Esophagus (3)	1/3 Epithelial cells (<1%), nuclear

<b>Tissue type (# tested)</b>	<b>Positively Staining Tissue Elements</b>
Kidney (3) 0/3	0/3
Liver (3)	0/3
Lung (3)	0/3
Mesothelial cells (3)	0/3
Muscle, cardiac (3)	0/3
Muscle, skeletal (3)	0/3
Nerve, peripheral (3)	0/3
Ovary (3)	3/3 Follicular epithelium (20-40%), nuclear
	2/3 Stromal cells (10-30%), nuclear
Pancreas (3)	0/3
Parathyroid (3)	0/3
Pituitary (3)	0/3
Prostate (3)	3/3 Stromal cells (<5-30%), nuclear
Salivary gland (3)	0/3
Skin (3)	0/3
Small intestine (3)	0/3
Spleen (3)	0/3
Stomach (3)	0/3
Testis (3)	0/3
Thymus (3)	0/3
Thyroid (3)	0/3
Tonsil (3)	2/3 Epithelial cells ( $\leq 1\%$ ), nuclear
	1/3 Germinal center lymphocytes (<1%), nuclear
Uterus (3)	3/3 Myometrium (<1-40%) nuclear
	3/3 Glandular epithelium (50-80%), nuclear
	3/3 Stromal cells (30-80%), nuclear

*f. Assay cut-off:*

A positive staining result is defined as  $\geq 1\%$  of tumor cells with stained nuclei and negative result is  $< 1\%$  nuclei tumor cells stained.

2. Comparison studies:

*a. Method comparison with predicate device:*

A blinded, randomized concordance study was conducted at 3-sites using FFPE full tissue sections containing invasive breast cancer and ductal carcinoma *in situ* (DCIS) that exhibited differing levels of ER  $\alpha$  expression. A set of 173 different FFPE invasive breast cancer tissue samples were pre-screened with the ER  $\alpha$  component of the ER/PR pharmDx kit to ensure that there was an approximately equal distribution of ER  $\alpha$  -positive and ER  $\alpha$  -negative specimens and that a number of samples expressing ER  $\alpha$  staining levels near the cut-off value of  $> 1\%$  to  $10\%$  stained tumor cells were

adequately represented. All tissue sections were stained using FLEX monoclonal rabbit anti-human estrogen receptor  $\alpha$ , clone EP1, Ready to-Use (RTU) with EnVision™ FLEX. All slides were scored according to ASCO/CAP guidelines ( $\geq 1\%$  cut-off), and also with ER  $\alpha$  component of the Dako ER/PR pharmDx™ Kit, scored according to the Allred scoring guideline. All slides were scored according to this summarized accepted guideline below:

**Scoring Guidelines**

<b>Proportion Score (PS) (proportion of positive tumor cells)</b>	<b>Intensity Score (IS) (average intensity of positive tumor cells)</b>
0: No cells stained	0: Negative
1: $>0$ to $<1/100$	1: Weak
2: $\geq 1/100$ to $1/10$	2: Intermediate
3: $>1/10$ to $1/3$	3: Strong
4: $>1/3$ to $2/3$	
5: $>2/3$ to 1	

**Criteria for Assignment of ER Status**

<b>ER Status</b>	<b>ASCO/CAP Guidelines</b>	<b>Allred Scoring(PS + IS)</b>
Negative (N)	$<1\%$ tumor cells	Allred Score = 0 or 2
Positive (P)	$\geq 1\%$ tumor cells	Allred Score $\geq 3$

Using these respective scoring guidelines, concordance study demonstrated accepted agreement in the proportion of cells stained and intensity between the test device and predicate. Results are summarized in the table below:

<b>RTU EP1 Antibody (ASCO/CAP Score)</b>		<b>ER <math>\alpha</math> Component of ER/PR pharmDx Kit (Allred Score, Predicate Device)</b>		
		Positive	Negative	Total
Positive		84	0	84
Negative		4	85	89
Total		88	85	173

Positive Percent Agreement =  $84/88 = 95.5\%$  (CI: 94.17 – 99.09)

Negative Percent Agreement =  $85/85 = 100.0\%$  (CI: 94.23 – 99.10)

Total Percent Agreement (Concordance) =  $169/173 = 97.7\%$  (CI: 94.20 - 99.09)

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  $\geq 1\%$  of tumor cells. Please see table (criteria for assignment of ER Status) in section M 2(a).

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.