2006 01 T 2966 CP
IN THE SUPREME COURT OF NEWFOUNDLAND AND LABRADOR
TRIAL DIVISION

BETWEEN:

VERNA DOUCETTE **PLAINTIFF**

AND:

EASTERN REGIONAL INTEGRATED HEALTH AUTHORITY **DEFENDANT**

BROUGHT UNDER THE *CLASS ACTIONS ACT*
BEFORE THE HONOURABLE MR. JUSTICE THOMPSON,
CASE MANAGEMENT JUDGE

ANSWERS TO INTERROGATORIES

In answer to the Interrogatories of the Plaintiff dated March 30, 2007, I make oath, as a representative of Eastern Regional Integrated Health Authority, and say that to the best of my knowledge, information and belief, based on my review of medical literature and my experiences and interactions with oncologists, pathologists and other medical professionals on the topic of breast cancer and breast cancer testing, as follows:

1. *As to paragraph 20 of your Affidavit dated February 9, 2007, how many ER/PR tests were done on the Dako system from May 1997 to April 2004?*

Answer: There were 2214 ER/PR breast tissue tests conducted on the Dako system from 1997, when the Dako testing commenced, to April 1, 2004. Some, but very few, lymph node tissue samples as well as metastatic tissue samples may be included in this figure.

2. *How many ER/PR tests on the Ventana system from May 2004 to August 2005?*

Answer: There were 495 ER/PR breast tissue tests conducted on the Ventana system from April 1, 2004 to August 1, 2005.

3. *How many ER/PR tests were done on a year to year basis, stating total number of tests and total number of negatives for each year:*

Dako system	Total Tests	Negatives
May 1997 to December 31		
January 1, 1998 to December 31		
January 1, 1999 to December 31		
January 1, 2000 to December 31		
January 1, 2001 to December 31		
January 1, 2002 to December 31		
January 1, 2003 to December 31		
January 1, 2004 to December 31		

Ventana system

April 2004 to December 31
January 1, 2005 to July 31

Answer: The following is a summary of the total number of breast tissue ER/PR tests conducted on a year to year basis and the total number of clinically negative tests each year. In 1997, when testing commenced on the Dako system, a test was clinically positive if tumour tissue cells stained at 30% or more. This standard changed at the beginning of 2001 such that if tumour tissue cells stained at 10% or more, then the test was deemed positive.

Dako system	Total Tests	Total Negatives
May 1997 to December 31	137	57
January 1, 1998 to December 31	147	76
January 1, 1999 to December 31	360	126
January 1, 2000 to December 31	370	170
January 1, 2001 to December 31	374	143
January 1, 2002 to December 31	344	147
January 1, 2003 to December 31	373	89
January 1, 2004 to April 1, 2004	109	16

Ventana system

April 2004 to December 31	381	41
January 1, 2005 to July 31	114	19

4. *As to paragraph 21, of the 330 (763 less 433) false negatives found on retesting by Mount Sinai, how many occurred while the Dako system was in use?*

Answer: My Affidavit dated the 9th day of February 2007 reviewed test results from the perspective of treatment change rather than a change in test results. Of the 330 remaining patients calculated by question #4, a further 13 patients of the 330 patients calculated did not see a change in their test results but a change in treatment was recommended as the standard interpretation of what constituted an ER-positive result had changed between the time of the original testing and the Tumour Board's review. Of the remaining 317 patients, whose test results were different on retesting at Mount Sinai, a further 4 had a change in their diagnosis and another 4 saw their test results change from positive to negative. Therefore, there were 309 patients whose test results were different on retesting at Mount Sinai and 306 of those patients' original test results were obtained using the Dako system.

5. *As to paragraph 25, what criteria were used in the selection of the 101 patient samples for retesting?*

Answer: The 101 patients referred to in paragraph 25 of my Affidavit were not "selected" for retesting. A decision was made by an Ethics Committee during the retesting process that no further tissue samples for deceased patients would be sent for retesting unless a request was made by the deceased patient's family. At that time, 101 of the 176 deceased patients' tissue samples had been retested. A further 2 were retested upon request.

6. *As to paragraph 25(c), were the families or attending physicians of the 73 deceased patients notified that there would be no retesting?*

Answer: The families and attending physicians of the 73 deceased patients were advised through press releases and general advertising that the tissue samples of deceased breast cancer patients could be retested at the family's request.

7. *As to paragraph 25 (a) and (b), of the 103 deceased patients originally reported as negative and not retested by Mount Sinai, where was the retesting done and what were the results (ie. false negatives)?*

Answer: As of today's date there are 105 deceased patients whose results have been retested at Mount Sinai. Of those 105 patients, 68 saw no change in their results, 1 originally clinically positive result, on retesting, was determined to be clinically negative, and 36 patients' test results changed from clinically negative to clinically positive.

8. *Were the false negatives reported to the families or attending physicians?*

Answer: Through press releases and general advertising the families and attending physicians of deceased patients were advised that they could request the breast tissue test results.

9. *How many of the deceased patients were originally tested on the Dako system, and on retesting, were false negative?*

Answer: Of the 36 patients that saw a change in their test results, all were originally tested on the Dako system.

10. *As to paragraph 29, what controls were run in all instances?*

Answer: Technical controls were run in all instances. Technical controls are the inclusion of confirmed positive control patient tissue samples.

11. *What is the hospital policy on documentation of controls and on retention of the documentation?*

Answer: There is no written hospital or lab policy on the documentation of controls and the retention of such documentation. The lab policy, based on the manufacturer's protocol, was that a lab technologist would run a technical control with each batch of tests. In 1997 and 1998 all test results were interpreted in St. John's by one pathologist; therefore, only one control was required. However, in 1999 test results were sent to pathologists outside St. John's for interpretation. Therefore, several controls might be run for a single batch of tests such that each pathologist interpreting results would receive a control slide in addition to the patient's or several patients' test results. For tests interpreted in St. John's, the technical control slide would be filed with the test slides and maintained for 20 years.

As part of the lab's policy, the technologist would enter the date, time and number of tests run and number of controls run into the meditech computer system. By 2001, with computer upgrades, technologists included more particulars regarding the type of tests run. The pathologist would then sign out the slide(s) from the lab and produce a lab report. The report(s) should refer to both the technical and internal controls run.

12. *What were the controls used during the analytic phase (from paraffin section to the staining machine – antigen retrieval)?*

Answer: See responses to questions 10 and 11.

13. *Does the documentation show the controls were working in all documented cases?*

Answer: Not all pathologists referred to the technical and internal controls in their reports. I estimate that in 50% of all cases the pathologist referred to the technical controls in his or her report.

14. *What antibodies were used by Eastern Health while the Dako system was in use? (Estrogen and Progesterone).*

Answer: From 1997 to December 2000 the 1D5 ER clone was used and the 1A6 PR clone was used. From December 2000 to April 1, 2004 the 1D5 ER clone was used and the PgR 636 PR clone was used.

15. *What staining procedures were used at Mount Sinai in performing the retesting, Dako or Ventana?*

Answer: Mount Sinai used the Dako system.

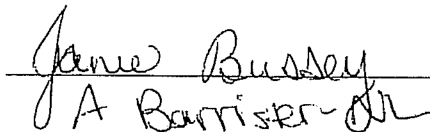
16. *What antibodies, both progesterone and estrogen, were used by Mount Sinai?*

Answer: On retesting at Mount Sinai the 6F11 ER clone was used and the PgR 1294 PR clone was used.

17. *Please provide a copy of the bench procedure for antigen retrieval during the use of the Dako system?*

Answer: Please see the bench procedures for 1D5 ER clone, 1A6 PR clone, and PgR 636 PR clone attached.

SWORN TO at St. John's, in the province of Newfoundland and Labrador, this 10th day of May, 2007, before me:



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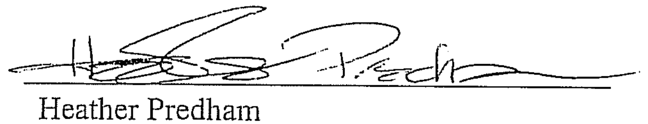

Heather Predham

Specification Sheet

Please note
Some information
is Lot dependent.

Monoclonal Mouse
Anti-Human Estrogen Receptor
Clone 1D5
Code No. M 7047
Lot 070. Edition 02.08.00

Presentation

Monoclonal mouse antibody supplied in liquid form as tissue culture supernatant (RPMI 1640 medium containing fetal calf serum) dialysed against 0.05 mol/L Tris/HCl, pH 7.2 containing 15 mmol/L NaN_3.

Mouse Ig concentration: 260 mg/L.

Isotype: IgG1, kappa.

Total protein concentration: 20.8 g/L.

Storage

2 - 8 °C.

Clone

1D5. (1).

Immunogen

Recombinant human estrogen receptor protein (1).

Specificity/reactivity

The DAKO antibody reacts with the N-terminal domain (A/B region) of the receptor (3). In immunoblotting it reacts with the 67 kDa polypeptide chain obtained by transformation of *E. coli* and transfection of COS cells with plasmid vectors expressing estrogen receptor (1). The antibody further reacts with the cytosolic extracts of luteal endometrium and the human breast cancer cell line MCF-7 (1). The DAKO antibody reacts with estrogen receptor-α as revealed using a DNA-binding array (2).

Normal tissues: The antibody strongly labels the nuclei of cells known to contain abundant amounts of estrogen receptor, e.g. epithelial and myometrial cells of the uterus, and normal and hyperplastic epithelial cells in mammary glands. The staining is predominantly localized to the nuclei with no cytoplasmic staining. However, on cryostat sections a positive staining of estrogen receptor in the nucleus as well as in the cytoplasm can be seen. Tissues known to contain small or no detectable amounts of estrogen receptor, e.g. colonic epithelium, cardiac muscle cells, brain and connective tissue cells are consistently negative with the antibody.

Tumour cells: The antibody labels epithelial cells of breast carcinomas which express the estrogen receptor. A good correlation was found between the results of the biochemical quantification of estrogen receptor by the DCC technique and those obtained using this antibody (4-7). Except for occasional cases (i.e. sarcoma) no positive staining is observed in lymphoid tumours and non-lymphoid neoplasms such as melanomas (1).

Cross-reactivity: The antibody reacts with estrogen receptor from rat.

Staining procedures

Formalin-fixed and paraffin-embedded sections

Can be used on formalin-fixed, paraffin-embedded tissue sections. Antigen retrieval, such as heating in 10 mmol/L citrate buffer, pH 6.0, or in DAKO Target Retrieval Solution, code No. S 1700 is mandatory. Alternatively heat-induced epitope retrieval using Target Retrieval Solution, high pH, code No. S 3308, can be used in order to improve the immunostaining. The slides should not dry out during this treatment or during the following immunohistochemical staining procedure.

For tissue sections, a variety of sensitive staining techniques are suitable, including immunoperoxidase procedures, the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique and avidin-biotin methods such as LSAB methods.

The antibody gives an optimal staining at a dilution of 1:50 - 1:100 with the LSAB methods when tested on formalin-fixed, paraffin-embedded sections of human breast carcinoma.

Frozen sections and cell smears

Can be used for labelling acetone-fixed cryostat sections or fixed cell smears. For staining cell smears, the APAAP method is recommended.

The antibody may be used at a dilution at 1:50 -1:100 in the APAAP technique and avidin-biotin methods such as the LSAB methods, when tested on acetone-fixed cryostat sections of breast carcinoma.

These are guidelines only; optimal dilutions should be determined by the individual laboratory.

Automation

The antibody can be used on automated immunostaining systems.

Flow cytometry

The antibody is well-suited for flow cytometry (indirect technique) using Goat Anti-Mouse Immunoglobulins/FITC, code No. F 0479.

References

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(6) Mauri FA, Veronese S, Frigo B, Girlando S, Losi L, Gambacorta M, et al. ER1D5 and H222 (ER-ICA) antibodies to human estrogen receptor protein in breast carcinomas. Results of a multicentric comparative study. Appl Immunohistochem 1994;2:157-63.

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Specification Sheet

July 17/03 1/20

Monoclonal Mouse
Anti-Human Estrogen Receptor α
Clone 1D5
Code No. M 7047
Lot 032, Edition 06.03.02

Intended use

For in vitro diagnostic use.

DAKO Monoclonal Mouse Anti-Human Estrogen Receptor, Clone 1D5 (Anti-ER, 1D5), is intended for laboratory use to semi-quantitatively identify by light microscopy an epitope located on the N-terminal domain of the estrogen receptor in normal and pathological human cryostat and paraffin-embedded tissue processed in neutral buffered formalin. Positive results aid in the classification of normal and abnormal cells/tissues and serve as an adjunct to conventional histopathology. The clinical interpretation of any positive staining or its absence should be complemented by morphological and histological studies with proper controls. Evaluations should be made within the context of the patient's clinical history and other diagnostic tests by a qualified individual.

A variety of immunohistochemical staining methods are suitable for use with this antibody.

Introduction

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 on the membrane of cell nuclei 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 (1).

Measurement of the ER has been shown to be prognostically relevant for predicting overall survival and predicting relapse-free survival (2-4). 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 (2-5).

Refer to the General Instructions for Immunohistochemistry (IHC) or the Detection System Instructions of IHC procedures for:

(1) Principle of Procedure, (2) Materials Required, Not Supplied, (3) Staining Procedure, (4) Quality Control, (5) Troubleshooting, (6) Interpretation of Staining, (7) General Limitations.

Reagent provided

Monoclonal mouse antibody provided in liquid form as cell culture supernatant dialysed against 0.05 mol/L Tris/HCl, pH 7.2, and containing 15 mmol/L NaN_3. Package size is 1 mL.

Clone: 1D5 (6). Isotype: IgG1, kappa.

Mouse IgG concentration: 290 mg/L. Total protein concentration: 22.5 g/L.

Immunogen

Soluble recombinant human estrogen-receptor protein (6).

Specificity

Anti-ER, 1D5 was produced in BALB/c mice using recombinant estrogen receptor (RER), a protein with molecular mass of 67 kDa (6). Anti-ER, 1D5 was shown to react with an epitope located in the N-terminal domain of ER. Anti-ER, 1D5 specifically binds to an antigen located primarily on the surface of cell nuclei of normal and some neoplastic mammary epithelial cells. Anti-ER, 1D5 does not recognize the ERβ (7).

Precautions

1. For In Vitro Diagnostic Use.

2. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, build-ups of NaN_3 may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing (8).

3. Minimize microbial contamination of reagents or increase in nonspecific staining may occur

Storage

Store at 2-8 °C or aliquot into convenient volumes and freeze at -20 °C. Avoid repeated freezing and thawing. Frozen antibodies may be stored in small aliquots until periodic assay verifications detect unacceptable changes in reactivity.

Fresh dilutions of the antibody should be made prior to use and are stable for up to eight hours at room temperature (20-25 °C). Unused portions of antibody preparations should be discarded after eight hours.

Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the user must verify the conditions (9).

There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens.

Specimen preparation

Formalin sections: Biopsy specimens may be preserved for IHC staining by formalin fixation followed by paraffin embedding.

DAKO Monoclonal Mouse Anti-Human Estrogen Receptor, code No. M 7047 can be used on tissues fixed in neutral buffered formalin, methacarn or Carnoy's fixative prior to paraffin embedding. The deparaffinized tissue sections must be treated with heat prior to the IHC staining procedure (10). Target retrieval involves immersion of tissue sections in a pre-heated buffer solution and maintaining heat, either in a water bath (95-99 °C), a steamer (95-99 °C), or an autoclave (121 °C). For greater adherence of tissue sections to glass slides, the use of silanized slides (DAKO code No. S 3003) is recommended. DAKO Target Retrieval Solution (code No. S 1700) or 10 x Concentrate (code No. S 1699) is recommended using a 20 minutes heating protocol.

Staining procedure

Dilution: DAKO Monoclonal Mouse Anti-Human Estrogen Receptor, code No. M 7047, may be used at a dilution of 1:35 when performing IHC using the DAKO EnVision, DAKO ENVision Doublestain or DAKO LSAB2 detection systems. These are guidelines only. Optimal antibody concentrations may vary depending on specimen preparation method, and should be determined by each individual laboratory. As negative control, DAKO Mouse IgG1, code No. X 0931, diluted to the same mouse IgG concentration as the primary antibody, is recommended.

Visualization: Follow the procedure for the detection system selected. The primary antibody dilution specified is suitable for a 10-minutes incubation when using an EnVision, EnVision Double stain or LSAB2 detection system.

Staining interpretation: The cellular staining pattern for anti-ER, 1D5 is nuclear. Cytoplasmic staining is considered to be background, non-specific staining, if seen.

There has been a variety of staining scales reported in the literature. Staining intensity has been reported using a staining intensity scale, and using the scale in conjunction with the percentage of cells staining (H score). The intensity score when stratified and compared to outcome, provided a greater statistical significance for DFS ($p = 0.003$ versus $p = 0.01$) compared to positive versus negative evaluation (2). Therefore, DAKO recommends using a semi-quantitative scoring system, e.g., 0-3 or negative, weak, moderate, and strong.

Product specific limitations

1. Wash buffers containing high levels of detergent can decrease the staining intensity with anti-ER, 1D5.
2. Occasional lymphoid tumours and non-lymphoid neoplasms such as melanomas are labelled.

Performance characteristics

Reproducibility: Eight serial sections from each of three different formalin-fixed, paraffin embedded blocks of breast carcinoma (prescreened for low antigen density) were collected for testing. Testing was performed as follows:

Intra-run reproducibility: Following the standard DAKO LSAB®2 Peroxidase Kit protocol (code No. K 0677), three slides from each tissue block were stained with Ready-to-Use DAKO® Mouse Anti-Human Estrogen Receptor, clone 1D5 (code No. N 1575). Concurrently, one slide from each block was stained with the supplied negative control reagent.

Inter-run reproducibility: Staining one slide from each tissue block, the above procedure was repeated on two additional days. Concurrently, one slide from each tissue block was stained with the supplied negative control reagent.

Reproducibility experiments with anti-ER, 1D5 yielded consistent results with intra- and inter-run testing. Consistent test conditions were maintained throughout the study and reagents were stored at 2-8°C between test runs.

Normal tissues: Distribution of ER throughout normal tissue has been reported in a variety of studies, summarized in a review article (11). The functionality of the ER is also explored in this review article. Immunoreactivity in a panel of normal tissues: Table 1 contains a summary of ER mmunoreactivity with the recommended panel of normal tissues. All tissues were formalin-fixed and paraffin embedded and stained with Anti-ER, 1D5 according to the instructions in the package insert.

TABLE 1

Summary of ER Normal Tissue Reactivity

TISSUE TYPE (# tested)	POSITIVE TISSUE ELEMENT STAINING AND STAINING PATTERN
Adrenal (4)	None
Bone Marrow (2)	None
Brain/Cerebellum (4)	None
Brain/Cerebrum (3)	None
Breast (3)	Mammary gland (1-2+ staining intensity, 2/3 tissues)
Cervix uteri (3)	squamous epithelium (1/3 tissues)
Colon (3)	None
Esophagus (3)	None
Heart (3)	None
Kidney (3)	None
Liver (3)	None
Lung (3)	None
Mesothelial Cells (3)	None
Ovary (3)	None
Pancreas (3)	None
Parathyroid (3)	None
Peripheral Nerve (3)	None
Pituitary (3)	None
Prostate (3)	fibromuscular stroma, 1+ staining intensity, cytoplasmic pattern, 1/3 tissues
Salivary Gland (3)	None
Skeletal Muscle (3)	None

Skin (3)	None
Small Intestine (3)	None
Spleen (4)	None
Stomach (3)	None
Testis (3)	None
Thymus (3)	None
Thyroid (3)	None
Tonsil (3)	None
Uterus (3)	Endometrium (1/3 tissues, 1+ staining intensity)

Reported staining in all tissues was nuclear, unless otherwise noted.

Abnormal Tissues: In pathological tissues, anti-ER, 1D5 was examined for specificity and sensitivity for breast cancer as well as for the evaluation of patients for endocrine therapy. Numerous studies of cases of breast cancer showed anti-ER, 1D5 to be safe and effective in this endeavor (2, 3, 10, 12-17). On frozen tissues, anti-ER, 1D5 immunoreacted with 63/93 (67.7%) cases of breast cancer and only 1/30 (3.3%) nonbreast cancers reacted positively (6). Direct comparison of anti-ER, 1D5 on formalin-fixed, paraffin-embedded (FFPE) specimens to the Abbott Estrogen receptor immunocytochemical assay using H222 on frozen sections of the same tumors has been completed by 7 different laboratories on more than 1000 specimens (2, 3, 10, 19-21). The Anti-ER, 1D5 specificity ranged from 51 to 88% while sensitivity ranged from 89% to 100%. When outcome to tamoxifen therapy or other measure of hormone reactivity is considered, one study reported that Anti-ER, 1D5 correlated with outcome to tamoxifen (7) while a second study reported the Anti-ER, 1D5 had a positive predictive value of 64% and negative predictive value of 92% (3).

Comparison of breast carcinoma ER concentration using anti-ER, 1D5 compared to dextran-coated charcoal (DCC) was performed by six different investigators. The relative scale for comparison is reported in Table 2. A total of 1,376 cases were assessed. Concordance was seen in 86.4% (1,188) of the cases, while 1D5+/DCC – was found.in 6.8% (94) of the cases and 1D5-/DCC+ was found in 6.8% (94) of the cases (3, 6, 13, 17, 23, 24).

TABLE 2. Correlation Between The IHC Score and DCC Mean Values

IHC *	ScoreDDC Mean* (fmol/mg)
-	8
+	22
++	33
+++	90
++++	365

* Cut-off positivity at ≤ 10 fmol/mg

Anti-ER, 1D5 was used to detect ER presence in a variety of tumors. No staining was found in carcinomas other than breast (0/14), malignant melanomas (0/2) or lymphoid tumors (0/6), while limited positivity was noted for sarcomas (1/8) (6).

References

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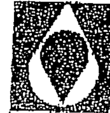
M 7047/HEW/08.03.02



DAKO

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Ordering Information: 800/235-5763
Technical Information: 800/424-0021
Fax: 805/566-6688

Sept 5/03
1-25

Specifications

DAKO Monoclonal Mouse Anti-Human Progesterone Receptor, clone PgR 636 for Immunoenzymatic Staining

IMMUNOGEN: Formalin-fixed recombinant full length A-form of human progesterone receptor[1]
CLONE: PgR 636[1]

CODE NO.: M3569
Primary Antibody
Lot No. 110
Total Protein Concentration: 14.4 mg/mL (Refractometry)
Mouse IgG Concentration: 238 µg/mL (Single Radial Immunodiffusion)
Subclass: IgG_1, kappa

INTENDED USE:
For In Vitro Diagnostic Use
DAKO Monoclonal Mouse Anti-Human Progesterone Receptor, Clone PgR 636 (Anti-PR, PgR 636) is intended for laboratory use for the semi-quantitative detection of progesterone receptor by light microscopy in normal and pathological human paraffin-embedded tissue processed in neutral buffered formalin. This antibody is indicated for use as an aid in the management, prognosis and prediction of outcome of breast cancer. Positive results aid in the classification of normal and abnormal cells/tissues and serve as an adjunct to conventional histopathology. The clinical interpretation of any positive staining or its absence should be complemented by morphological and histological studies with proper controls. Evaluations should be made within the context of the patient's clinical history and other diagnostic tests by a qualified individual.

Refer to the General Instructions for Immunohistochemistry (IHC) or the Detection System Instructions of IHC procedures for: (1) Principle of Procedure, (2) Materials Required, Not Supplied, (3) Storage, (4) Specimen preparation, (5) Staining Procedure, (6) Quality Control, (7) Troubleshooting, (8) Interpretation of Staining, (9) General Limitations

SUMMARY AND EXPLANATION:
INTRODUCTION
The role of steroid hormone receptors in breast cancer is well-known.[2,3] The absence of ER and PR predicts early recurrence and poor survival of breast cancer patients.[4-7] Also, the presence of ER and PR in tumors predicts the potential for benefit from tamoxifen and other endocrine-related therapies. Measurement of ER and PR can be determined semi-quantitatively using IHC or quantitatively using DCC or EIA. Correlation between the semi-quantitative and quantitative evaluations of PR have ranged from 73 to 91% depending on the laboratory and antibody used.[8-10]

SPECIFICITY
Anti-PR, PgR 636 has been demonstrated to react with the PR-A and PR-B forms by Western blot of whole cell extracts and reacts with both free and hormone-bound PR.[1] The epitope has been mapped to the amino terminal domain shared by PR-A and PR-B.

REAGENTS PROVIDED:
M3569
Anti-PR, PgR 636 is available in a 0.2 mL or 1 mL volume as a mouse anti-human monoclonal antibody tissue culture supernatant in 50 mM Tris/HCl, pH 7.2, containing 15 mM NaN_3 and stabilizing protein.

M3569 may be used at a dilution of 1:50 when performing IHC using the DAKO LSAB®2 detection system. These are guidelines only. Optimal antibody concentrations may vary depending on specimen and preparation method, and should be determined by each individual laboratory.

MATERIALS REQUIRED, NOT SUPPLIED:
Refer to the General Instructions for IHC and/or the Detection System Instructions. In addition, use the following negative reagent control.

DAKO Mouse IgG_1, Code No. X0931

PRECAUTIONS:
1. For In Vitro Diagnostic Use.
2. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, build-ups of NaN_3 may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing.[11]
3. Minimize microbial contamination of reagents or increase in nonspecific staining may occur.

STORAGE:
Store at 2-8 °C.

SPECIMEN PREPARATION:
Biopsy specimens may be preserved for IHC staining by formalin fixation followed by paraffin embedding.

Anti-PR, PgR 636 can be used on tissues fixed in neutral buffered formalin, methacarn, or Carnoy's fixative prior to paraffin embedding. The deparaffinized tissue sections must be treated with heat prior to the IHC staining procedure.[10] Target retrieval involves immersion of tissue sections in a pre-heated buffer solution and maintaining heat, either in a water bath (95-99 °C), a steamer (95-99 °C), or an autoclave 121 °C). For greater adherence of tissue sections to glass slides, the use of silanized slides (DAKO Code No. S3003) is recommended. DAKO Target Retrieval Solution (Code No. S1700) or 10x Concentrate (Code No. S1699) is recommended using a 20-40 minute heating protocol.

(over)

STAINING PROCEDURE:
Follow the procedure for the detection system selected. When performing IHC with the LSAB2 detection system, use a 1:50 dilution in a 10-30 minute incubation with the diluted Anti-PR, PgR 636.

PERFORMANCE CHARACTERISTICS:
The cellular staining pattern for anti-PR, PgR 636 is nuclear. A positive staining result is defined as more than 10% of tumor cells with stained nuclei of any intensity.

Normal tissues: Distribution of PgR throughout normal tissue has been reported in a variety of studies. The nuclei of uterine gland cells were found to be strongly immunoreactive. Weaker immunostaining was observed in the nuclei of endometrial and prostatic stromal cells.

Immunoreactivity in a panel of normal tissues: Table 1 contains a list of positive tissues with PgR immunoreactivity. All tissues were formalin-fixed and paraffin embedded and stained with Anti-PR, PgR 636 according to the instructions in the package insert using the LSAB2 detection system (DAKO Code No. K0675). Negative tissues included adrenal (4), bone marrow (2,) brain/cerebellum (4), brain/cerebrum (3), colon (3), esophagus (3), heart (3), kidney (3), liver (3), lung (3), mesothelial cells (3), ovary (3), pancreas (3), parathyroid (3), peripheral nerve (3), salivary gland (3), skeletal muscle (3), skin (3), small intestine (3), spleen (4), stomach (3), testis (3), thymus (3), thyroid (3), and tonsil (3).

TABLE 1: Summary of PgR Normal Tissue Reactivity

TISSUE TYPE (# tested)	POSITIVE TISSUE ELEMENT STAINING AND STAINING PATTERN	
Breast (3)	Ductal epithelial cells	3+ staining intensity, 3/3 tissues
Cervix uteri (3)	Glandular epithelial cells	2+ staining intensity, 1/3 tissues
	Stromal fibroblasts	2+ staining intensity, 2/3 tissues
Pituitary (3)	Pituicytes	2+ staining intensity, 1/3 tissues
Prostate (3)	Stromal fibroblasts	2+ staining intensity, 1/3 tissues
Uterus (3)	Endometrial stroma	2+ staining intensity 3/3 tissues
	Myometrium	2+ staining intensity, 3/3 tissues
	Endometrial glands	2+ staining intensity, 2/3 tissues

A second survey of normal tissues demonstrated positivity in endometrium and weak positivity in prostate after heat-induced epitope retrieval using the LSAB+ detection system. Negative tissues included esophagus, testis, breast, liver, kidney, skeletal muscle, placenta, adrenal, tonsil, lung, colon, skin, pancreas, spleen, thyroid, stomach and cardiac muscle.[1]

Abnormal tissues: Ninety seven breast cancer tissues were tested using the DAKO anti-PR, PgR 636 with the LSAB2 detection system, which had been previously assessed for PR expression using the PR-EIA. Correlation between the 2 assays was 90.7% while specificity was 94% and sensitivity was 87.2%. In another study, 31 breast carcinomas previously tested with the DCC assay were stained using the LSAB+ detection system. Positive staining was reported for 21/23 positive tumors, while 6/8 remained negative (91% sensitivity and 75% specificity).[1]

Anti-PR, PR 636 with peroxidase/antiperoxidase detection system was used to immunostain a variety of 60 different tumor types. Breast cancer (5/11), uterine (2/2), ovarian (2/6), and endometrial (2) carcinomas stained strongly. Medullary carcinoma of the thyroid (1/2) and testicular yolk sac tumor were positive. Other tumors including melanoma, lymphoma and neuroendocrine and neural tumors were negative for PR expression.[1]

Reproducibility
Eight serial sections from each of three different formalin-fixed, paraffin embedded blocks of breast carcinoma were collected for testing. Testing was performed as follows:

Intra-run reproducibility: Following the standard DAKO EnVision®+ Peroxidase Kit protocol (Code No. K4007), three slides from each tissue block randomly distributed through the staining order, were stained with Ready-to-Use DAKO® Mouse Anti-Human Progesterone Receptor, clone PgR 636 (Code No. N1608). Concurrently, one slide from each block was stained with the negative control reagent (Code No. N1015).

Inter-run reproducibility: Staining one slide from each tissue block, the above procedure was repeated on two additional days with another technician staining on the third staining procedure. Concurrently, one slide from each block was stained with the negative control reagent.

Reproducibility experiments with PgR 636 yielded consistent results with intra- and inter-run testing. Consistent test conditions were maintained throughout the study and reagents were stored at 2-8 °C between test runs.

REFERENCES:

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2. Henderson C. Breast cancer. In: Harrison's Principles of Internal Medicine, 12th edition, Wilson JD, Braunwald E, Isselbacher KJ, Petersdorf RG, Martin JB, Fauci AS, Root RK (eds) McGraw-Hill, Inc. New York, 1991
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11. Department of Health, Education and Welfare, National Institute for Occupational Safety and Health, Rockville, MD. "Procedures for the decontamination of plumbing systems containing copper and/or lead azides." DHHS (NIOSH) Publ. No. 78-127, Current 13. August 16, 1976

DAKO

Specification Sheet

Please note
Some information
is Lot dependent.

MONOCLONAL MOUSE
ANTI-HUMAN PROGESTERONE RECEPTOR, 1A6
CODE NO.: M3529
LOT NO.: 070

Immunogen
Synthetic peptide

Clone
1A6

Presentation
Anti-progesterone receptor is a mouse monoclonal antibody supplied in liquid form as tissue culture supernatant (containing fetal calf serum) dialyzed against 0.05M Tris-HCl, pH 7.2, 15mM sodium azide. Contains carrier protein.
Protein Concentration: 1.6 mg/mL (Refractometry, excluding carrier protein)
Mouse IgG Concentration: 24 μg/mL (Single Radial Immunodiffusion)
Subclass: IgG_1, kappa

Specificity
Monoclonal anti-progesterone receptor binds to the A/B region of human progesterone receptor.

Reactivity
Normal cells:
Anti-progesterone receptor, clone 1A6 has been found to immunohistochemically label the nuclei of normal breast and uterine epithelial cells on routinely processed tissue.[1] The nuclei of myometrial cells and stromal cells of the uterus also stain positively. Anti-progesterone receptor, clone 1A6 has also been observed to stain islet cells in pancreas. Cytoplasmic staining of certain epithelial components has been observed. The significance of cytoplasmic staining in unknown.

Tumor cells:
In immunohistochemistry, anti-progesterone receptor has been demonstrated to stain the nuclei of cells from various histologic subtypes of breast carcinoma including infiltrating ductal, lobular, mucinous and carcinoid.[1]

The cytosol-based assay of assessing progesterone receptor status has been compared to an immunohistologic assay using monoclonal antibody 1A6 on routinely processed breast carcinoma specimens. The receptor status as determined by the two assays was found to agree in 83% of the cases tested.[1]

Staining Procedure
Paraffin Sections:
Anti-progesterone receptor can be used on formalin-fixed, paraffin-embedded tissue sections.

The deparaffinized tissue sections must be treated with heat prior to the immunohistochemical (IHC) staining procedure. For greater adherence of tissue sections to glass slides, the use of silanized slides (DAKO® Code No. S3003) is recommended.

When using the water bath method, preheat a Coplin jar containing 10mM citrate buffer, pH 6.0 as well as a water bath to 95-99°C. When the temperature has stabilized, place tissue sections into the Coplin jar containing the preheated buffer. Heat the tissue sections for 40 minutes. For improved staining results and a shorter incubation time, DAKO® Target Retrieval Solution (Code No. S1700) can be used in place of the 10mM citrate buffer. Under these conditions the incubation time in the water bath may be reduced to 20 minutes. After thermal treatment, allow the jar with buffer and slides to cool for 20 minutes at room temperature. Rinse well with distilled water and place slides into buffer (Tris, PBS, etc.)

(OVER)

A number of staining techniques are suitable as listed below:

- avidin-biotin procedure
- three-stage immunoperoxidase procedure
- peroxidase anti-peroxidase (PAP) procedure
- alkaline phosphatase anti-alkaline phosphatase procedure (APAAP)
- labelled strept-avidin biotin (LSAB) procedure
- enhanced polymer (DAKO Envision™ System) procedure

Anti-progesterone receptor may be used at a dilution of 1:10 in the LSAB method determined on formalin-fixed, paraffin-embedded tissue. These are guidelines only; optimal dilutions should be determined by the individual laboratory.

Cryostat Sections and Cell Smears:
Anti-progesterone receptor can also be used to label cryostat sections or cell smears.

Storage

Store at 2-8°C or -20°C. Avoid repeated freeze-thaw cycles.

References

1. Kell DL, et al. Immunohistochemical analysis of breast carcinoma estrogen and progesterone receptors in paraffin-embedded tissue: Correlation of clones ER1D5 and 1A6 with a cytosol-based hormone receptor assay. Appl Immunohistochem, 1993; 1(4): 275

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.