CE

FLEX Monoclonal Rabbit Anti-Human Estrogen Receptor α

Clone EP1

Ready-to-Use

(Link)

Code IR084

Intended use

For in vitro diagnostic use.

FLEX Monoclonal Rabbit Anti-Human Estrogen Receptor α, Clone EP1, Ready-to-Use, (LINK), is intended for use in immunohistochemistry together with Autostainer Link instruments to semi-quantitatively detect human estrogen receptor in formalin-fixed, paraffin-embedded tissue sections of human breast cancer. The antibody labels estrogen receptor α-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.

Synonyms for antigen

ER α

Summary and explanation

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, 2).

Historical studies have shown that ER status is positively correlated with untreated outcome (ie. prognostic for well differentiated invasive breast cancer) and with response to anti-hormonal therapy e.g., tamoxifen (2). Estrogens have been found to be preferentially concentrated in the estrogen target organs of animals and in human breast cancers and it is well documented that the mitogenic effects of estrogen are mediated by ER. Investigations into the biological mechanisms for breast cancer growth have found that the growth rate is dependent on the presence of estrogen or progesterone or both in most breast cancers (2). Thus, estrogen receptor status in breast carcinomas is considered to be a validated prognostic and predictive factor for patient management for anti-hormonal therapy (2-4).

Refer to Dako's *General Instructions for Immunohistochemical Staining* 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.

Reagent provided

Ready-to-use monoclonal rabbit antibody provided in liquid form in a buffer containing stabilizing protein and 0.015 mol/L sodium azide.

Clone: EP1.

Immunogen

Recombinant protein of ER-alpha amino acids 1-300.

Specificity

In Western blotting of MCF7 cells lysates the antibody labels a major band of approximately 67 kDa, corresponding to the expected molecular weight of ER α. No cross-reactivity was seen with ER β.

Precautions

1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
4. As with any product derived from biological sources, proper handling procedures should be used.
5. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
6. Unused solution should be disposed of according to local, State and Federal regulations.

Storage

Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

Specimen preparation

The antibody can be used for labeling formalin-fixed, paraffin-embedded tissue sections. Tissue specimens should be cut into sections of approximately 4 µm.

including materials required but not supplied

Pre-treatment with heat-induced epitope retrieval (HIER) is required using Dako PT Link (Code PT100/PT101/PT200). For details, please refer to the PT Link User Guide. Optimal results are obtained by pretreating tissues using EnVision FLEX Target Retrieval Solution, High pH (50x) (Code K8000/K8004/K8023) for 20 minutes at 97 °C followed by 5 minutes in EnVision FLEX Wash Buffer (Code K8007).

Paraffin-embedded sections: Pre-treatment of formalin-fixed, paraffin-embedded tissue sections is recommended using the 3-in-1 specimen preparation procedure for Dako PT Link. After staining the sections must be dehydrated, cleared and mounted using permanent mounting medium.

The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, the use of Dako Silanized Slides (Code S3003) is recommended.

Staining procedure including materials required but not supplied

The recommended visualization system is EnVision FLEX, High pH, (Link) (Code K8000/K8023). The staining steps and incubation times are pre-programmed into the Autostainer Link software. The recommended reagent application volume is 1 x 200 µL or 2 x 150 µL per slide. Please refer to the proper Autostainer Link User Guide for detailed instructions on loading slides and reagents. If the protocols are not available on the used Autostainer platform, please contact Dako Technical Services. An installer to update DakoLink software with protocol and reagent information for Anti-ER α, clone EP1 for Autostainer Link software can be found at www.dako.com\Installer. All incubation steps should be performed at room temperature.

Optimal conditions may vary depending on specimen and preparation methods, and should be validated by each individual laboratory (5). A Dako Application Specialist/Technical Service Specialist can be contacted for information on re-programming of the protocol. Verify that the performance of the adjusted protocol is still valid by evaluating that the staining pattern is identical to the staining pattern described in “Performance characteristics”.

Counterstaining in hematoxylin is recommended using EnVision FLEX Hematoxylin (Link) (Code K8008). Non-aqueous, permanent mounting medium is recommended.

It is recommended that positive and negative controls should be run simultaneously using the same protocol as the patient specimens. Ideally the positive controls should include a low ER-expressing breast carcinoma tissue. As an alternative, benign cervix may also be used. The cells/structures should display reaction patterns as described for this tissue in “performance characteristics” in all positive specimens.

The recommended negative control reagent is FLEX Universal Negative Control, Rabbit, Ready-to-Use (Link) (Code IR600).

Staining interpretation

The cellular staining pattern is nuclear. Cytoplasmic labeling, if observed, should be regarded as non-specific.

A positive result is defined as nuclear staining in ≥1% of tumor cells (6). This is consistent with ASCO/CAP’s recommended cut-off of ≥1% positive tumor cells for positive assessment (3).

Product specific limitation

1. False-negative results could be caused by degradation of the antigen in the tissues over time. Specimens should be stained within 2 months of mounting of tissues on slides when stored at room temperature(7).
2. For optimal and reproducible results, the ER protein requires target retrieval when tissues are routinely fixed (neutral buffered formalin) and paraffin embedded.
3. Use of Dako Monoclonal Rabbit Anti-Human ER α, Clone EP1 on tissues with fixatives other than formalin has not been validated.

Performance characteristics

Precision: 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 Precision: Following the standard EnVision FLEX, High pH protocol, three sections from each tissue block were stained with Monoclonal Rabbit Anti-Human ER α, Clone EP1. Concurrently one section from each block was stained with a negative control reagent.

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

Inter-instrument Precision: Staining three sections from each tissue block, the above procedure was performed on three different Autostainer instruments. Concurrently, one slide from each tissue block was stained with a negative control reagent.

Precision experiments with Monoclonal Rabbit Anti-Human ER α, Clone EP1 yielded consistent results with intra-run, inter-run and inter-instrument testing. Consistent test conditions were maintained throughout the study and reagents were stored at 2-8 ºC between test runs.

Normal tissues: Table 1 contains a summary of Monoclonal Rabbit Anti-Human ER α, Clone EP1 immunoreactivity on the recommended panel of normal of normal tissues. All tissues were formalin-fixed and paraffin-embedded and stained with Monoclonal Rabbit Anti-Human ER α, Clone EP1 according to the instructions in the package insert.

Table 1: Summary of Monoclonal Rabbit Anti-Human ER α, clone EP1 Normal Tissue Reactivity (8)

Tissue Type (# tested)	Positively Staining Tissue Elements
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
Kidney (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 (≤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

Method comparison: Monoclonal Rabbit Anti-Human ER α, Clone EP1 testing was performed with EnVision FLEX and scored according to ASCO/CAP guidelines (≥1% cut-off) (3). Anti-ER α cocktail (Clones 1D5 and ER-2-123) testing was performed using Dako ER/PR pharmDx Kit and scored using the Allred scoring guideline described in the package insert. The method comparison data are presented in Table 2. Using these respective scoring guidelines, Monoclonal Rabbit Anti-Human ER α, Clone EP1 was highly concordant with the ER α antibody component of Dako ER/PR pharmDx Kit, exhibiting values for overall, positive and negative agreement of 96.2%, 98.9% and 92.2%, respectively.

Table 2: Agreement between Anti-ER α, Clone EP1 and Anti-ER α Component of ER/PR pharmDx Kit

		Anti-ER α Component of ER/PR pharmDx Kit		
		Positive	**Negative**	Total
Monoclonal Rabbit Anti-Human ER α, Clone EP1	**Positive**	183	10	193
	Negative	2	119	121
	Total	185	129	314

Positive Percent Agreement = 183/185 = 98.9%
Negative Percent Agreement = 119/129 = 92.2%
Overall Percent Agreement = 302/314 = 96.2%
Kappa= 0.9203
95% Confidence Interval= 0.8761 – 0.9645

References

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4. Fitzgibbons FK, Page DL, Weaver D, Thor AD, Allred DC, Clark GM, et al. Prognostic factors in breast cancer: College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med 2000;124:966-78.
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8. M3634 IHC003-D03803 Report on file.

Monoclonal Rabbit Anti-Human ER α, Clone EP1 has been created by Epitomics Inc., using Epitomics' proprietary rabbit monoclonal antibody technology covered under Patent Nos. 5,675,063 and 7,402,409.

Explanation of symbols

REF	Catalogue number		Temperature limitation	IVD	In vitro diagnostic medical device
	Manufacturer	LOT	Batch code		Contains sufficient for <n> tests
	Use by		Consult instructions for use	EC REP	Authorized representative in the European Community

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