

FLEX
Monoclonal Rabbit
Anti-Human AMACR
Clone 13H4
Ready-to-Use
(Dako Omnis)

English
Code GA060

Intended use	<p>For in vitro diagnostic use.</p> <p>FLEX Monoclonal Rabbit Anti-Human AMACR, Clone 13H4, Ready-to-Use (Dako Omnis), is intended for use in immunohistochemistry (IHC) together with the Dako Omnis instrument. Results aid in the classification of prostate adenocarcinomas (1-8). Differential classification is aided by the results from a panel of antibodies. 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 nonimmunologic histochemical stains.</p>
Synonyms for antigen	<p>Alpha methylacyl coenzyme A racemase, P504S (1, 9).</p>
Summary and explanation	<p>Monoclonal rabbit anti-AMACR (alpha methylacyl coenzyme A racemase) recognizes a 382 amino acid protein that was identified by cDNA library subtraction in conjunction with high throughput microarray screening of prostate adenocarcinoma (9). AMACR, also known as P504S, is an enzyme that is involved in bile acid biosynthesis and β-oxidation of branched-chain fatty acids (10). AMACR may be expressed in cells of premalignant high grade prostatic intraepithelial neoplasia (HGPIN) and prostate adenocarcinoma, and can be present at low or undetectable levels in glandular epithelial cells of normal prostate and benign prostatic hyperplasia (1-8). AMACR may be present in normal non-prostatic epithelium and in carcinomas from outside the prostate (1, 11-23).</p> <p>Refer to <i>Dako General Instructions for Immunohistochemical Staining</i> or the detection system instructions of IHC procedures for: Principle of Procedure, Materials Required, Not Supplied, Storage, Specimen Preparation, Staining Procedure, Quality Control, Troubleshooting, Interpretation of Staining, General Limitations.</p>
Reagent provided	<p>Ready-to-use monoclonal rabbit antibody provided in liquid form in a buffer containing stabilizing protein and 0.015 mol/L sodium azide.</p> <p><u>Clone:</u> 13H4 (2,3,9).</p>
Immunogen	<p>Full length recombinant AMACR (9).</p>
Specificity	<p>The specificity of monoclonal rabbit anti-AMACR was evaluated by immunocytochemistry and Western blot analysis. Anti-AMACR positively bound formalin-fixed HEK293 cells which overexpressed AMACR, but was unreactive with cells transfected with an empty plasmid. In Western blots of lysates from primary prostate carcinoma samples, the antibody labels a 54 kDa protein corresponding to the expected molecular weight of AMACR (2).</p>
Precautions	<ol style="list-style-type: none">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	<p>Store at 2-8 °C. During storage the cap should be closed. Do not use after expiration date stamped on vial. Onboard stability is 80 hours. Onboard time is tracked by the Dako Omnis software. 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.</p>

Staining protocol overview*

Step		Comments
Fixation/embedding	Formalin-fixed, paraffin-embedded	Onboard deparaffinization
Pre-treatment	EnVision FLEX, High pH (Code GV804)	30 min HIER
Antibody	Ready-to-use	10 min incubation
Negative Control	FLEX Negative Control, Rabbit (Code GA600)	10 min incubation
Visualization	EnVision FLEX (Code GV800) + EnVision FLEX+ Rabbit LINKER (Code GV809)	Block: 3 min; Link: 10 min; Polymer: 20 min; Chromogen: 5 min
Counterstain	Hematoxylin (Code GC808)	3 min incubation
Control Tissue	Prostate adenocarcinoma	Cytoplasmic staining
Slides	FLEX IHC Microscope Slides (Code K8020)	Recommended for greater adherence of tissue sections to glass slides
Mounting	Non-aqueous, permanent mounting required	After staining, the sections must be dehydrated, cleared and mounted using a permanent mounting method
Instrumentation	Dako Omnis	Reagents are provided in instrument-specific vials

*The user must always read the package insert for detailed instructions of the staining procedure and handling of the product.

Specimen preparation

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

Pre-treatment: Pre-treatment of formalin-fixed, paraffin-embedded tissue sections with heat-induced epitope retrieval (HIER) is required. Pretreating tissues with HIER using diluted EnVision FLEX Target Retrieval Solution, High pH (50x) (Dako Omnis), Code GV804, is recommended. Deparaffinization, rehydration and target retrieval are performed onboard Dako Omnis. Please refer to Dako Omnis Basic User Guide.

Dako Omnis ensures that the tissue sections do not dry out during the pre-treatment process or during the following immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, the use of FLEX IHC Microscope Slides, Code K8020 is recommended.

Staining procedure

Program: The staining steps and incubation times are pre-programmed into the Dako Omnis software. Please refer to the Dako Omnis Basic User Guide for detailed instructions on loading slides and reagents. If the protocols are not available in the Dako Omnis system, please contact Dako Technical Support. All incubation steps are performed at 32 °C onboard Dako Omnis.

Visualization: The recommended visualization system is EnVision FLEX, High pH (Dako Omnis), Code GV800 in combination with EnVision FLEX+ Rabbit LINKER (Dako Omnis), Code GV809. The visualization is performed onboard Dako Omnis.

Counterstaining: The recommended counterstain is Hematoxylin (Dako Omnis), Code GC808. The counterstaining is performed onboard Dako Omnis.

Mounting: After staining onboard Dako Omnis the sections must be dehydrated, cleared and mounted using a permanent mounting method.

Quality control: Positive and negative control tissues as well as negative control reagent should be run simultaneously using the same protocol as the patient specimens. The positive control tissue should include prostate carcinoma and the cells/structures should display reaction patterns as described for this tissue in the "Performance characteristics" section. The recommended negative control reagent is FLEX Universal Negative Control, Rabbit (Dako Omnis), Code GA600.

Staining interpretation Performance characteristics

The cellular staining pattern is cytoplasmic.

Normal tissues:

Tissue Type (# Tested) (1-4, 11-14)	Labeled Tissue Elements
Adrenal (12) ¹	0/12
Bladder (7) ¹	0/7
Brain (5) ¹	0/5
Breast (12) ¹	0/12
Colon (24) ^{11,12}	20/24 Colonic surface epithelium, focal and luminal
Colon, hyperplastic polyp (28) ¹²	1/28
Endometrium (17) ^{1,11}	0/17
Gallbladder (10) ¹	10/10 Epithelial cells
Heart (5) ¹	0/5
Kidney (12) ¹	12/12 Tubular epithelial cells
Liver (31) ^{1,13}	18/31 Hepatocytes
Lung (18) ^{1,11}	12/18 Bronchial epithelial cells
Lymph Node (7) ¹	0/7

Ovary (14) ^{1,11}	0/14
Pancreas (15) ¹	0/15
Prostate, normal (34) ^{1,11}	0/34
Prostate, benign (156) ^{2,3,4}	11/156
Salivary gland (7) ¹	0/7
Skin (10) ^{1,11}	0/10
Small Intestine (18) ¹	0/18
Spleen (12) ¹	0/12
Stomach (13) ¹	0/13
Stomach, non-neoplastic epithelium (44) ¹⁴	2/44
Testes (5) ¹	0/5
Thyroid (5) ¹	0/5










Abnormal tissues: Anti-AMACR is immunoreactive with the majority of prostate carcinomas tested (1-7,11). The epithelial cells of hyperplastic prostate glands should be negative or only show a weak focal cytoplasmic staining reaction. The majority of neoplastic cells of prostate adenocarcinoma should show a moderate to strong cytoplasmic staining reaction.

Tissue Type (# Tested) (1, 11-23)	Labeled Tumors
Adrenal cortical tumor (20) ¹	1/20
Basal cell carcinoma of skin (20) ¹	0/20
Bile duct cholangiocarcinoma (14) ¹	2/14
Breast adenocarcinoma (315) ^{1,11,15}	54/315
Carcinoid tumors; lung and GI (10) ¹	0/10
Colon carcinomas (233) ^{11,12,16,17}	
Colon (35) ^{11,16}	16/35
Well differentiated (58) ¹²	45/58
Moderately differentiated (88) ¹²	66/88
Poorly differentiated (30) ¹²	11/30
Metastatic (22) ¹⁷	7/22
Endometrioid carcinoma (10) ¹	0/10
Germ cell tumors (14) ¹	0/14
Hepatocellular carcinoma (72) ^{1,13}	59/72
Lung carcinoma (300) ^{1,11,18}	
Carcinoma (28) ¹¹	4/28
Adenocarcinoma (151) ^{1,18}	75/151
Squamous cell carcinoma (121) ¹⁸	27/121
Melanoma (41) ^{1,11}	2/41
Neuroendocrine carcinoma; GI, lung and liver (167) ^{1,18}	95/167
Ovary adenocarcinoma (76) ^{1,11,17}	2/76
Pancreas adenocarcinoma (13) ¹	1/13
Pleural mesothelioma (16) ¹	0/16
Prostate adenocarcinoma (640) ^{1-7,11}	587/640
Renal tumors (105) ^{19,20}	
Clear cell carcinoma (77) ^{19,20}	17/77
Chromophobe (24) ^{19,20}	1/24
Oncocytoma (29) ^{19,20}	4/29
Bellini/urothelial (5) ¹⁹	0/5
Mucinous and spindle cell carcinoma (5) ¹⁹	5/5
Papillary (96) ^{19,20}	96/96
Sarcomatoid (15) ²⁰	0/15
Salivary gland tumors (28) ¹	1/28
Small cell carcinoma; lung and skin (15) ¹	0/15
Soft tissue epithelioid sarcoma (12) ¹	1/12
Soft tissue synovial sarcoma (6) ¹	0/6
Squamous cell carcinoma; skin and mucosa (25) ¹	0/25
Stomach adenocarcinoma (413) ^{1,14,21,22}	262/413
Undifferentiated carcinoma (27) ¹	0/27
Thymoma (8) ¹	0/8
Thyroid tumors (54) ¹	0/54
Urothelial carcinoma (50) ^{1,20,23}	
Urothelial carcinoma (17) ²⁰	2/17
Transitional cell carcinoma, bladder (29) ¹	9/29
Clear cell adenocarcinoma (4) ²³	4/4

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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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