anti-p504s (SP116) Rabbit Monoclonal Primary Antibody

REF 790-6011

08035130001

IVD Σ 50

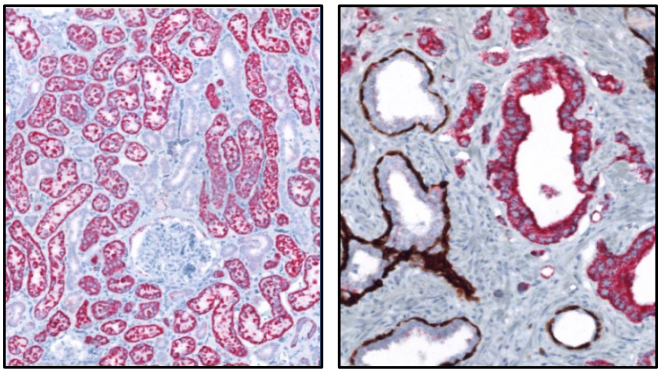

Figure 1. anti-p504s (SP116) antibody exhibiting cytoplasmic staining pattern in prostate tissue (left). Anti-p504s antibody and VENTANA Basal **Cell Cocktail (34βE12+p63) dual stain (right).**

INTENDED USE

Anti-p504s (SP116) Rabbit Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of α-methylacyl-CoA racemase (AMACR, also known as p504s) by light microscopy in sections of formalin-fixed, paraffin-embedded tissue stained on a BenchMark IHC/ISH instrument.

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

This antibody is intended for in vitro diagnostic (IVD) use.

SUMMARY AND EXPLANATION

The anti-p504s (SP116) Rabbit Monoclonal Primary Antibody (anti-p504s (SP116) antibody) is a monoclonal antibody produced against the p504s protein. p504s is a 42kDa protein located in mitochondria and peroxisomes. It plays a role in the b-oxidation of branched chain fatty acids.[1] While normal and benign prostate tissues express low levels of p504s, it is markedly overexpressed in most prostate carcinomas (PCa), including some atypical forms of PCa.[2]

First reported in 2000 to be overexpressed in PCa,[3] many subsequent studies have reported high sensitivity and specificity values for p504s in PCa and high-grade prostatic intraepithelial neoplasia (HGPIN).[4-7] Furthermore, several articles report that when p504s is used with antibodies for basal cell markers like high molecular weight cytokeratin (HMWCK) or p63, the differential staining patterns among benign glands, benign mimickers of PCa like HGPINs, and prostate carcinoma, improves diagnostic accuracy.[2,4,5,7-10] The differential staining pattern is particularly useful in the workup of clinically atypical prostate cases, as supported by numerous published reports.[10-15]

Note that p504s can be present in normal, atypical, and malignant prostate tissues to varying degrees, and the threshold for interpretation of p504s staining as positive is subjective. The recommended practice is to interpret p504s staining results in conjunction with basal cell markers and morphological features. Immunohistochemical detection of p504s with anti-p504s (SP116) antibody may be used as a complementary IHC stain with VENTANA Basal Cell Cocktail (34bE12 + p63) to assist in distinguishing morphologically difficult prostate tissue as benign, atypical or cancerous. (Note: Antibody clone 34bE12 detects HMWCK, a basal cell marker.)

The staining pattern for this antibody is cytoplasmic. It may be used as part of a panel of IHC studies.

PRINCIPLE OF THE PROCEDURE

Anti-p504s (SP116) antibody is a rabbit monoclonal antibody produced against recombinant human p504s protein. Anti-p504s (SP116) antibody binds to the p504s protein in formalin-fixed, paraffin-embedded (FFPE) tissue sections and exhibits a granular cytoplasmic staining pattern. This antibody can be visualized using *ultra*View Universal Alkaline Phosphatase Red Detection Kit (Cat. No. 760-501 / 05269814001) or as a dual stain in conjunction with VENTANA Basal Cell Cocktail (34βE12+p63) (Cat. No. 790-4536 / 06364497001) using *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001). Refer to the respective method sheet for further information.

MATERIAL PROVIDED

Anti-p504s (SP116) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of anti-p504s (SP116) antibody contains approximately 1.5 µg of a rabbit monoclonal antibody.

The antibody is diluted in Tris-HCl with a carrier protein and 0.10% ProClin 300, a preservative.

Specific antibody concentration is approximately 0.3 µg/mL. There is no known non-specific antibody reactivity observed in this product.

Anti-p504s (SP116) antibody is a rabbit monoclonal antibody produced as purified cell culture supernatant.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
4. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
5. *ultra*View Universal Alkaline Phosphatase Red Detection Kit (Cat. No. 760-501 / 05269814001)
6. VENTANA Basal Cell Cocktail (34bE12+p63) (Cat. No. 790-4536 / 06364497001)
7. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
8. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
9. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
10. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
11. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
12. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
13. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
14. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
15. Permanent mounting medium
16. Cover glass
17. Automated coverslipper
18. General purpose laboratory equipment
19. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed FFPE tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark IHC/ISH instruments. The

recommended tissue fixative is 10% neutral buffered formalin.[16] Sections should be cut at approximately 4 µm in thickness and mounted on positively charged slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time. Ask your Roche representative for a copy of "Recommended Slide Storage and Handling" for more information.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. CAUTION: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. ProClin 300 solution is used as a preservative in this reagent. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
6. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining Ask your Roche representative for more information on how to use these types of slides.
7. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[17,18]
8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at dialog.roche.com.
11. Consult local and/or state authorities with regard to recommended method of disposal.
12. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Warning 	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
	P273	Avoid release to the environment.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362 + P364	Take off contaminated clothing and wash it before reuse.
	P501	Dispose of contents/ container to an approved waste disposal plant.

This product contains CAS # 55965-84-9, reaction mass of: 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories. Refer to Table 2 and Table 3 for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 790-6011.

Note: The Fast Red chromogen is soluble in alcohol and acetone. Do not use alcohol or acetone baths or extended xylene washes to dehydrate and clear slides. See *ultra*View Universal Alkaline Phosphatase Red Detection Kit method sheet for more details about the dual stain protocol and post-instrument processing procedures.

Table 2. Recommended staining protocol for anti-p504s (SP116) antibody with *ultra*View Universal Alkaline Phosphatase Red Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Standard	CC1, Standard	ULTRA CC1 64 minutes, 95°C
Antibody (Primary)	16 minutes, 37°C	16 minutes, 37°C	16 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

Table 3. Recommended dual staining protocol for anti-p504s (SP116) antibody with *ultra*View Universal Alkaline Phosphatase Red Detection Kit and VENTANA Basal Cell Cocktail (34βE12+p63) with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT [b]	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Standard	CC1, Standard	ULTRA CC1 64 minutes, 95°C
Antibody: VENTANA Basal Cell Cocktail **(34βE12+p63) DAB**	16 minutes, 37°C	16 minutes, 37°C	16 minutes, 36°C
DS Antibody: anti-p504s (SP116) RED	24 minutes, 37°C	16 minutes, 37°C	32 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

[b] BenchMark XT instrument requires "XT DS BCC uDAB-p504s uRed" procedure for optimal results.

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances."[19]

NEGATIVE REAGENT CONTROL

In addition to staining with anti-p504s (SP116) antibody, a second slide should be stained with the appropriate negative control reagent.

POSITIVE TISSUE CONTROL

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections.

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test specimen should be considered invalid.

The recommended positive control tissue is normal kidney. Proximal tubules should demonstrate moderate to high levels of staining while the distal tubules should exhibit low staining. Glomeruli should be negative for p504s expression.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for anti-p504s (SP116) antibody is cytoplasmic.

SPECIFIC LIMITATIONS

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Table 4. Sensitivity/Specificity of anti-p504s (SP116) antibody single stain and anti-p504s (SP116) antibody and VENTANA Basal Cell Cocktail (34βE12+p63) dual stain was determined by testing FFPE normal tissues.

Tissue	# positive / total cases for SINGLE stain	# positive / total cases for DUAL stain
Cerebrum [a]	0/3	0/3
Cerebellum	2/3	2/3
Adrenal gland	1/3	1/3
Ovary	1/3	1/3
Pancreas [b]	2/3	2/3
Lymph Node	0/3	0/3
Hypophysis	1/3	1/3
Testis [c]	2/3	2/3
Thyroid	0/3	0/3
Breast	0/3	0/3
Spleen	0/3	0/3
Tonsil	0/3	0/3
Thymus gland	0/3	0/3
Myeloid (Bone marrow)	0/3	0/3
Lung [d]	1/3	1/3
Heart	0/3	0/3
Esophagus	0/3	0/3
Stomach	3/3	3/3
Intestine	3/3	3/3
Colon	2/3	2/3
Liver	3/3	3/3
Tongue	3/3	3/3
Kidney	3/3	3/3
Prostate	43/64	43/64
Endometrium	2/3	2/3
Cervix	1/3	1/3
Skeletal muscle	0/3	0/3
Skin [e]	1/3	1/3
Peripheral nerve	0/3	0/3
Mesothelium	0/3	0/3
Urinary bladder	2/3	2/3
Parathyroid gland	2/3	2/3

[a] Purkinje cells, [b] Islets of Langerhans, [c] Rare Sertoli cells,[d] Columnar epithelium of large bronchus, [e] Sebaceous glands

Table 5. Sensitivity/Specificity of anti-p504s (SP116) antibody single stain and anti-p504s (SP116) antibody and VENTANA Basal Cell Cocktail (34βE12+p63) dual stain was determined by testing FFPE neoplastic tissues.

Pathology	# positive / total cases for SINGLE stain	# positive / total cases for DUAL stain
Glioblastoma (Cerebrum)	0/1	0/1
Meningioma (Cerebrum)	0/1	0/1
Anaplastic ependymoma (Cerebrum)	0/1	0/1
Oligodendroglioma (Cerebrum)	0/1	0/1
Serous carcinoma (Ovary)	2/2	2/2
Neuroendocrine neoplasm (Pancreas)	1/1	1/1
Adenocarcinoma (Pancreas)	0/1	0/1
Seminoma (Testis)	0/1	0/1
Embryonal carcinoma (Testis)	0/1	0/1
Medullary carcinoma (Thyroid)	1/1	1/1
Papillary carcinoma (Thyroid)	1/1	1/1
Ductal carcinoma in situ (Breast)	1/1	1/1
Invasive ductal carcinoma (Breast)	2/2	2/2
B-Cell Lymphoma; NOS	0/3	0/3
Small cell carcinoma (Lung)	0/1	0/1

Pathology	# positive / total cases for SINGLE stain	# positive / total cases for DUAL stain
Squamous cell carcinoma (Lung)	1/1	1/1
Adenocarcinoma (Lung)	0/1	0/1
Squamous cell carcinoma (Esophagus)	1/1	1/1
Adenocarcinoma (Esophagus)	1/1	1/1
Mucinous adenocarcinoma (Stomach)	1/1	1/1
Gastrointestinal stromal tumor (GIST)	0/2	0/2
Adenocarcinoma (Gastrointestinal)	2/3	2/3
Hepatocellular carcinoma (Liver)	1/1	1/1
Hepatoblastoma (Liver)	0/1	0/1
Clear cell carcinoma (Kidney)	1/1	1/1
Adenocarcinoma (Prostate)	110/111	110/111
Prostatic adenocarcinoma (Metastatic)	1/1	1/1
Leiomyoma (Uterus)	0/1	0/1
Adenocarcinoma (Uterus)	0/1	0/1
Clear cell carcinoma (Uterus)	0/1	0/1
Squamous cell carcinoma (Cervix)	1/2	1/2
Embryonal rhabdomyosarcoma	0/1	0/1
Melanoma (Rectum)	0/1	0/1
Basal cell carcinoma (Skin)	0/1	0/1
Squamous cell carcinoma (Skin)	1/1	1/1
Neurofibroma (Lumbar)	0/1	0/1
Neuroblastoma	0/1	0/1
Mesothelioma	1/1	1/1
Hodgkin lymphoma (Lymph Node)	0/1	0/1
Urothelial carcinoma (Bladder)	1/1	1/1
Leiomyosarcoma	0/1	0/1
Spindle cell rhabdomyosarcoma	0/1	0/1

Precision

Precision studies for anti-p504s (SP116) antibody were completed to demonstrate:

- Within run and between day precision on a BenchMark ULTRA instrument.
- Between instrument precision on the BenchMark GX, BenchMark XT and BenchMark ULTRA instrument.
- Between platform precision between BenchMark GX, BenchMark XT, and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

Precision on the BenchMark ULTRA PLUS instrument was demonstrated using representative assays. Studies included Within-run Repeatability, Between-day and Between-run Intermediate Precision. All studies met their acceptance criteria.

CLINICAL PERFORMANCE

Clinical performance data relevant to the Intended purpose of anti-p504s (SP116) antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
D	Updates to Principle of the Procedure, Material Provided, Specimen Preparation, Warnings and Precautions, Staining Procedure, Negative Reagent Control, Analytical Performance, Symbols, and Contact Information sections. Added BenchMark ULTRA PLUS instrument.

INTELLECTUAL PROPERTY

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