VENTANA Basal Cell Cocktail (34βE12+p63)

REF	790-4536	Σ	50
	06364497001		
REF	790-1010	Σ	250
	06419445001		

IVD

Figure 1. VENTANA Basal Cell Cocktail (34βE12+p63) staining of normal basal cells in prostate tissue.

INTENDED USE

VENTANA Basal Cell Cocktail (34βE12+p63) is an antibody cocktail of anti-p63 (4A4) and anti-keratin (34βE12) mouse monoclonal antibodies. VENTANA Basal Cell Cocktail (34βE12+p63) is intended for laboratory use in the qualitative immunohistochemical detection of p63 and cytokeratin 5 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

VENTANA Basal Cell Cocktail (34βE12+p63) is an antibody cocktail of anti-p63 (4A4) and anti-keratin (34βE12) mouse monoclonal antibodies. The human tumor protein 63 (TP63, p63) is a 77 kDa protein localized to the cellular nucleus and member of the p53 family of transcription factors.[1] In the prostate, p63 is expressed in the basal cells of almost all normal and benign glands.[2,3] Cytokeratin 5 is a 62 kDa type II high molecular weight cytokeratin expressed in basal cells and not luminal cells of the prostate.[4,5] The 34βE12 antibody is commonly referred to as a basal cell high-molecular-weight-cytokeratin specific antibody. It recognizes cytokeratin 5, and by extension its in vivo partner cytokeratin 14, as well as cytokeratin 1 and its in vivo partner cytokeratin 10, which are not basal cell markers.[4,6]

In the prostate, p63 can be detected in the nucleus and cytokeratin 5 can be detected in the cytoplasm of basal cells from almost all normal and benign glands.[2-5] Invasive lesions that evolve from and involve the prostate can disrupt and eventually breach the basal membrane thus eliminating the presence of prostatic basal cells.[7] Thus, VENTANA Basal Cell Cocktail (34βE12+p63) may be used to detect basal cells to aid in the differentiation of benign and malignant prostate lesions.

This cocktail of antibodies has been reported to provide advantages in sensitivity over the use of anti-p63 (4A4) antibody or anti-keratin (34βE12) antibody alone in the detection of prostatic basal cells.[8,9,10] In addition, a small percentage of normal or benign glands may express only one of these antigens. Thus, the two components of this cocktail not only augment, but also complement each other in basal cell detection.[9]

PRINCIPLE OF THE PROCEDURE

VENTANA Basal Cell Cocktail (34βE12+p63) binds to p63 and keratin (34βE12) proteins in formalin fixed, paraffin-embedded (FFPE) tissue sections. This antibody can be visualized using *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001). Refer to the respective method sheet for further information.

MATERIAL PROVIDED

VENTANA Basal Cell Cocktail (34βE12+p63) (Cat. No. 790-4536) contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA Basal Cell Cocktail (34βE12+p63) contains approximately 8 µg of a mouse antibody cocktail.

VENTANA Basal Cell Cocktail (34βE12+p63) (Cat. No. 790-1010) contains sufficient reagent for 250 tests.

One 25 mL dispenser of VENTANA Basal Cell Cocktail (34βE12+p63) contains approximately 40 µg of a mouse antibody cocktail.

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

Specific antibody concentration is approximately 1.6 µg/mL. There is no known non-specific antibody reactivity observed in this product.

VENTANA Basal Cell Cocktail (34βE12+p63) is a cocktail of mouse monoclonal antibodies produced from cell culture supernatant [anti-p63 (4A4)] and ascites [anti-keratin (34βE12)] material.

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. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
4. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
5. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
6. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
7. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
8. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
9. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
10. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
11. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
12. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
13. Permanent mounting medium
14. Cover glass
15. Automated coverslipper
16. General purpose laboratory equipment
17. 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.[11] 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.

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 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.[12,13]
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 for recommended staining protocol.

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 790-4536 or P/N 790-1010.

Table 2. Recommended staining protocol for VENTANA Basal Cell Cocktail (34βE12+p63) with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	XT	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Standard	ULTRA CC1, 64 minutes, 95°C
Antibody (Primary)	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.

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."[14]

NEGATIVE REAGENT CONTROL

In addition to staining with VENTANA Basal Cell Cocktail (34βE12+p63), 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.

Examples of positive control tissues for this antibody are normal prostate and normal skin.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for VENTANA Basal Cell Cocktail (34βE12+p63) is cytoplasmic and nuclear.

SPECIFIC LIMITATIONS

Presence of nuclear or cytoplasmic staining may be observed which indicates positive staining but not necessarily the presence of basal cells in tissues other than prostate or tumors of the prostate. Interpretation by a qualified pathologist should be in conjunction with histological examination, relevant clinical information, and proper controls.

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 3. Sensitivity/Specificity of VENTANA Basal Cell Cocktail (34βE12+p63) was determined by testing FFPE normal tissues.

Pathology	# positive / total tissues	Pathology	# positive / total tissues
Cerebrum	0/3	Heart	0/3
Cerebellum	0/3	Esophagus	3/3
Adrenal gland	0/3	Stomach	0/3
Ovary	0/3	Small intestine	0/3
Pancreas	0/3	Colon	0/3
Parathyroid gland	1/3	Liver	0/3
Pituitary gland	0/3	Salivary gland	3/3
Testis	1/3	Kidney	1/3
Thyroid	0/3	Prostate	53/53
Breast	17/17	Endometrium	1/3
Spleen	0/3	Cervix	3/3
Tonsil	3/3	Skeletal muscle	0/3
Thymus	3/3	Skin	3/3
Bone marrow	0/3	Nerve	0/3
Lung	0/3	Mesothelium	2/3

Table 4. Sensitivity/Specificity of VENTANA Basal Cell Cocktail (34βE12+p63) was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total tissues
Glioblastoma (Cerebrum)	1/1
Meningioma (Cerebrum)	1/1
Ependymoma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Serous adenocarcinoma (Ovary)	1/1
Mucinous adenocarcinoma (Ovary)	1/1
Neuroendocrine neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	1/1
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	0/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	1/1
Ductal carcinoma in situ (Breast)	5/9
Lobular carcinoma in situ (Breast)	3/4
Invasive ductal carcinoma (Breast)	8/14
Invasive lobular carcinoma (Breast)	5/8
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	0/1
Adenocarcinoma (Lung)	1/1
Squamous cell carcinoma (Esophagus)	1/1
Adenocarcinoma (Esophagus)	1/1
Mucinous adenocarcinoma (Stomach)	0/1
Adenocarcinoma (Small intestine)	0/1
Gastrointestinal stromal tumor (GIST) (Small intestine)	0/1
Adenocarcinoma (Colon)	0/1
Gastrointestinal stromal tumor (GIST) (Colon)	0/1
Adenocarcinoma (Rectum)	0/1
Gastrointestinal stromal tumor (GIST) (Rectum)	0/1
Melanoma (Rectum)	0/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	0/1
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	4/148
Urothelial carcinoma (Prostatic urethra)	1/1
Leiomyoma (Uterus)	0/1
Adenocarcinoma (Uterus)	1/1
Clear cell carcinoma (Uterus)	1/1
Squamous cell carcinoma (Cervix)	1/2
Embryonal rhabdomyosarcoma (Striated muscle)	1/1
Basal cell carcinoma (Skin)	1/1
Squamous cell carcinoma (Skin)	1/1
Neurofibroma (Lumbar)	0/1
Neuroblastoma (Retroperitoneum)	0/1
Spindle cell rhabdomyosarcoma (Retroperitoneum)	0/1
Mesothelioma (Peritoneum)	1/1
Lymphoma; NOS	2/2
B-cell lymphoma; NOS	0/2
Hodgkin lymphoma	0/1
Urothelial carcinoma (Bladder)	1/1
Leiomyosarcoma	0/2
Osteosarcoma (Bone)	0/1

Precision

Precision studies for VENTANA Basal Cell Cocktail (34βE12+p63) were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark XT instrument.
- Between instrument precision on the BenchMark XT and BenchMark ULTRA instrument.
- Between platform precision between the 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 VENTANA Basal Cell Cocktail (34βE12+p63) were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

REFERENCES

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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
G	Updates to Specimen Preparation, Warnings and Precautions, Staining Procedure, Analytical Performance and Symbols sections. Added BenchMark ULTRA PLUS instrument.

INTELLECTUAL PROPERTY

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