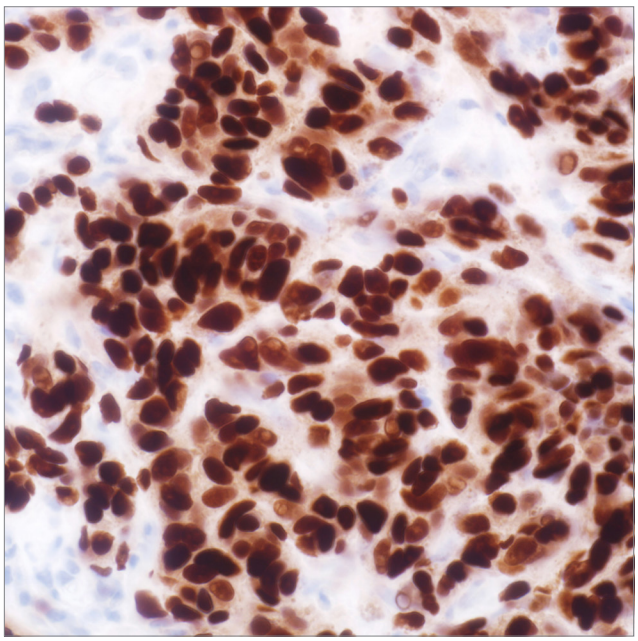
SOX-10 (SP267)
Rabbit Monoclonal Primary Antibody

For In Vitro Diagnostic Use (IVD)



Product Identification

Ventana REF	Roche #	Description
760-4968	07560389001	50 test dispenser

Symbol Definitions

KEY-CODE	keycode
A	ascites
E	serum
S	supernatant

Intended Use

SOX-10 (SP267) Rabbit Monoclonal Primary Antibody is intended for laboratory use in the detection of the SOX-10 protein in formalin-fixed, paraffin-embedded tissue stained on BenchMark immunohistochemical (IHC/ISH) automated slide stainers. 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

Sry-related HMG-BOX gene 10 (SOX-10) is a nuclear transcription factor that participates in neural crest development and in the specification and differentiation of cells of melanocytic lineage.[1] It has been recently shown to be a sensitive marker of melanoma, including conventional, spindled, and desmoplastic subtypes.[2] Anti-SOX-10 antibody was applied to a variety of neural crest-derived tumors, mesenchymal and epithelial neoplasms, and normal tissues. SOX-10 nuclear expression was found in 76 of 78 melanomas (97%) and 38 of 77 malignant peripheral nerve sheath tumors (49%), whereas S100 protein was expressed in 71 melanomas (91%) and 23 malignant peripheral nerve sheath tumors (30%).[2] SOX-10 was expressed by metastatic melanomas and nodal capsular nevus in sentinel lymph nodes, but not by other lymph node components such as dendritic cells which usually express S100 protein. In scar specimens, immature fibroblasts, epithelioid granulomas, and histiocytic proliferations can histopathologically mimic residual melanoma and even be positive for MiTF and S100.[3,4] However, SOX-10 is less likely to be expressed by fibroblasts or histiocytes. Anti-SOX-10 is also a useful marker in detecting both the in situ and invasive components of desmoplastic melanoma. It is known that the commonly used melanoma markers, anti-HMB-45 and anti-Melan-A, are poorly expressed in desmoplastic melanomas[5] while it has been reported that anti-SOX-10 was moderately to strongly expressed in all 28 desmoplastic melanomas tested[2]. These findings underscore the utility of anti-SOX-10 in the differential diagnosis of residual desmoplastic melanoma.[6] SOX-10 is diffusely expressed in schwannomas and neurofibromas. SOX-10 presence was not identified in any other mesenchymal or epithelial tumors except for myoepitheliomas and diffuse astrocytomas.[2] SOX-10 expression is seen in sustentacular cells of pheochromocytomas and paragangliomas, and occasionally carcinoid tumors from various organs, but is not seen in the tumor cells.[2] In normal tissues, SOX-10 is expressed in Schwann cells, melanocytes, and myoepithelial cells of salivary, bronchial, eccrine, and mammary glands. SOX-10 expression is also observed in mast cells in a variety of tissues and organs in both nuclear and cytoplasmic reaction.

Principles and Procedures

The stated primary antibody may be used as the primary antibody for immunohistochemical staining of formalin-fixed, paraffin-embedded tissue sections. In general, immunohistochemical staining in conjunction with a streptavidin-biotin detection system allows the visualization of antigens via the sequential application of a specific antibody (primary antibody) to the antigen, a secondary antibody (link antibody) to the primary antibody, an enzyme complex and a chromogenic substrate with interposed washing steps. Alternatively, a biotin-free detection system may be used. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained and a coverslip applied. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

The stated primary antibody is optimally diluted to be compatible with Ventana Roche detection kits and automated slide stainers. Each step in the staining protocol includes incubation for a precise time at a specific temperature. At the end of each incubation step, the sections are rinsed by the Ventana Roche automated slide stainer to stop the reaction

and remove unbound material that would hinder the desired reaction in subsequent steps. To minimize evaporation of the aqueous reagents from the specimen-containing slide, a coverslip solution is applied in the slide stainer. For more detailed information on instrument operation, refer to the appropriate Ventana Roche automated slide stainer Operator's Manual.

Materials and Methods

Reagents Provided

One dispenser of the stated primary antibody contains sufficient prediluted reagent for 50 tests.

Product Composition	
Predilute: diluted in	Tris Buffer, pH 7.3-7.7, with 1% BSA and <0.1% Sodium Azide
Isotype	IgG

See product label for lot specific information for the following:

1. Antibody immunoglobulin concentration
2. Source details

Reconstitution, Mixing, Dilution, Titration

This antibody is optimized for use on a Ventana Roche automated slide stainer in combination with Ventana Roche detection systems. No reconstitution, mixing, dilution, or titration is required. Further dilution may result in loss of antigen staining. The user must validate any such changes. Differences in tissue processing and technical procedures in the laboratory may produce significant variability in results and require regular use of controls. (See Quality Control Procedures section)

Materials and Reagents Needed But Not Provided

The following reagents and materials may be required for staining but are not provided with the primary antibody:

1. Positive and negative control tissue
2. Microscope slides, positively charged
3. Drying oven capable of maintaining a temperature of 53-65°C
4. Bar code labels (appropriate bar code labels for negative control and the primary antibody being tested)
5. Staining jars or baths
6. Timer
7. Amplifier (when applicable)
8. Xylene or xylene substitute
9. Ethanol or reagent alcohol
10. Deionized or distilled water
11. ES®, NexES IHC®, BenchMark®, BenchMark® XT, and BenchMark® ULTRA automated slide stainers
12. iVIEW™ DAB (preferred), *ultra*View™, OptiView, AEC, V Red (ALK PHOS) and Enhanced V Red detection kits
13. Detection system specific software (ES® automated slide stainer only)
14. APK Wash Solution (ES® and NexES IHC® automated slide stainers)
15. Liquid Coverslip™ solution (ES® and NexES IHC® automated slide stainers)
16. EZ Prep™ solution (BenchMark®, BenchMark® XT, and BenchMark® ULTRA automated slide stainers)
17. Reaction Buffer (BenchMark®, BenchMark® XT, and Benchmark® ULTRA automated slide stainers)
18. LCS (BenchMark®, BenchMark® XT, and Benchmark® ULTRA automated slide stainers)
19. Hematoxylin or other counterstain
20. Negative Control Reagent
21. Mounting medium
22. Cover glass
23. Light microscope (40-400x)

Storage and Handling

Store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and stability of the antibody after every run, the cap must be replaced and the dispenser must be immediately placed 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 for the prescribed storage method.

There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be run simultaneously with unknown specimens. Contact Cell Marque customer service if there is a suspected indication of reagent instability.

Specimen Collection and Preparation for Analysis

Routinely processed, neutral-buffered formalin-fixed, paraffin-embedded, tissues are suitable for use with this primary antibody when used with Ventana Roche detection systems and a Ventana Roche automated slide stainer (see Materials, Reagents, and Equipment Needed But Not Provided section). The recommended tissue fixative is 10% neutral-buffered formalin. Variable results may occur as a result of prolonged fixation or special processes such as decalcification of bone marrow preparations.

Each section should be cut to the appropriate thickness (approximately 3 µm) and placed on a positively charged glass slide. Slides containing the tissue section may be baked for at least 2 hours (but not longer than 24 hours) in a 53-65°C oven.

Warnings and Precautions

1. Take reasonable precautions when handling reagents. Use disposable gloves and lab coats when handling suspected carcinogens or toxic materials (example: xylene).
2. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
3. Patient specimens and all materials contacting them should be handled as biohazardous materials and disposed of with proper precautions. Never pipette by mouth.
4. Avoid microbial contamination of reagents, as this could produce incorrect results.
5. Incubation times and temperatures other than those specified may give erroneous results.
6. The reagents have been optimally diluted, and further dilution may result in loss of antigen staining. The user must validate any such change.
7. When used according to instructions, this product is not classified as a hazardous substance. The preservative in the reagent is less than 0.1% sodium azide and does not meet the OSHA (USA) criteria for hazardous substance at the stated concentration. See SDS.

8. The user must validate any storage conditions other than those specified in the package insert.
9. Diluent may contain bovine serum albumin and supernatant may contain bovine serum. The products containing fetal bovine serum and products containing bovine serum albumin are purchased from commercial suppliers. Certificates of Origin for the animal source used in these products are on file at Cell Marque. The certificates support that the bovine sources are from countries with negligible BSE risk and state sources of bovine from USA and Canada.
10. As with any product derived from biological sources, proper handling procedures should be used.

Instructions For Use

Step by Step Procedure

Cell Marque's primary antibodies have been developed for use on Ventana Roche automated slide stainers in combination with Ventana Roche detection kits and accessories.

Recommended Staining Protocols:

Recommended Staining Protocol for anti-SOX-10 antibody with *ultra*View™ DAB IHC Detection BenchMark XT, BenchMark ULTRA and BenchMark GX instruments.

Recommended Staining Protocol with *ultra*View™	
Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard
Enzyme (Protease)	Not required
Antibody (Primary)	BenchMark ULTRA instrument: 32 minutes, 36˚C BenchMark XT instrument: 32 minutes, 37˚C BenchMark GX instrument: 32 minutes, 37˚C
Amplification	Not selected
Counterstain	Hematoxylin II, 8 minutes
Post Counterstain	Bluing, 4 minutes

Recommended Staining Protocol for anti-SOX-10 antibody with OptiView DAB IHC Detection Kit BenchMark XT, BenchMark ULTRA and BenchMark GX instruments.

Recommended Staining Protocol with OptiView	
Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, 32 minutes
Enzyme (Protease)	Not required
Pre-primary peroxidase inibition	Selected
Antibody (Primary)	BenchMark ULTRA instrument: 32 minutes, 36˚C BenchMark XT instrument: 32 minutes, 37˚C BenchMark GX instrument: 32 minutes, 37˚C
OptiView HQ Linker	8 minutes
OptiView HRP Multimer	8 minutes
Amplification	Not selected
Counterstain	Hematoxylin II, 8 minutes
Post Counterstain	Bluing, 4 minutes

Quality Control Procedures

Positive Tissue Control

A positive tissue control must be run with every staining procedure performed. This tissue may contain both positive and negative staining cells or tissue components and serve as both the positive and negative control tissue. Control tissues should be fresh autopsy, biopsy or surgical specimens prepared or fixed as soon as possible in a manner identical to the test sections. Use of a tissue section fixed or processed differently from the test specimen will serve to provide control for all reagents and method steps except fixation and tissue processing.

A tissue with weak positive staining is more suitable for optimal quality control and for detecting minor levels of reagent degradation. Positive tissue control for the stated primary antibody may include the following:

Positive Tissue Control	
Tissue	Visualization
Melanoma	Nuclear
Skin Melanocytes	Nuclear

Known positive tissue controls should be utilized only for monitoring the correct performance of processed tissues and test reagents, not as an aid in determining a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate appropriate positive staining, results with the test specimens must be considered invalid.

Negative Tissue Control

The same tissue used for the positive tissue control may be used as the negative tissue control. The variety of cell types present in most tissue sections offers internal negative control sites, but this should be verified by the user. The components that do not stain should demonstrate

the absence of specific staining, and provide an indication of non-specific background staining. If specific staining occurs in the negative tissue control sites, results with the patient specimens must be considered invalid.

Unexplained Discrepancies

Unexplained discrepancies in controls should be referred to your local Ventana Roche office immediately. If quality control results do not meet specifications, patient results are invalid. See the Troubleshooting section of this insert. Identify and correct the problem, then repeat the entire procedure with the patient samples.

Negative Control Reagent

A negative control reagent must be run for every specimen to aid in the interpretation of results. A negative control reagent is used in place of the primary antibody to evaluate nonspecific staining. The slide should be treated with negative control reagent, matching the host species of the primary antibody, and ideally having the same IgG concentration. The incubation period for the negative control reagent should equal the primary antibody incubation period.

Interpretation of Results

The immunostaining procedure run on Ventana Roche automated slide stainers causes a colored reaction product to precipitate at the antigen sites localized by the primary antibody. Refer to the appropriate detection system package insert for expected color reactions. A qualified pathologist experienced in immunohistochemistry procedures must evaluate positive and negative tissue controls before interpreting results.

Positive Tissue Control

The stained positive tissue control should be examined first to ascertain that all reagents are functioning properly. The presence of an appropriately colored reaction product within the target cells is indicative of positive reactivity. Refer to the package insert of the detection system used for expected color reactions. Depending on the incubation length and potency of the hematoxylin used, counterstaining will result in a pale to dark blue coloration of cell nuclei. Excessive or incomplete counterstaining may compromise proper interpretation of results. If the positive tissue control fails to demonstrate appropriate positive staining, any results with the test specimens are considered invalid.

Negative Tissue Control

The negative tissue control should be examined after the positive tissue control to verify the specific labeling of the target antigen by the primary antibody. The absence of specific staining in the negative tissue control confirms the lack of antibody cross reactivity to cells or cellular components. If specific staining occurs in the negative tissue control, results with the patient specimen are considered invalid. Nonspecific staining, if present, will have a diffuse appearance. Sporadic light staining of connective tissue may also be observed in sections from tissues that are not optimally fixed. Intact cells should be used for interpretation of staining results. Necrotic or degenerated cells show non-specific staining.

Patient Tissue

Patient specimens should be examined last. Positive staining intensity should be assessed within the context of any background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen in question was not detected, not that the antigen is absent in the cells or tissue assayed. A panel of antibodies may aid in the identification of false negative reactions (see Summary of Expected Results section). The morphology of each tissue sample should also be examined utilizing a hematoxylin and eosin stained section when interpreting any immunohistochemical result. The patient's morphologic findings and pertinent clinical data must be interpreted by a qualified pathologist.

Limitations

1. Color does not affect performance.
2. This reagent is "for professional use only" as immunohistochemistry is a multiple step process that requires specialized training in the selection of the appropriate reagents, tissues, fixation, processing; preparation of the immunohistochemistry slide; and interpretation of the staining results.
3. For laboratory use only.
4. For *in vitro* diagnostic use.
5. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may result from variations in fixation and embedding methods, as well as from inherent irregularities within the tissue.
6. Excessive or incomplete counterstaining may compromise proper interpretation of results.
7. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology, other histopathological criteria as well as other diagnostic tests. This antibody is intended to be used in a panel of antibodies if applicable. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents, diagnostic panels, and methods used to produce the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
8. Cell Marque provides antibodies at optimal dilution for use as instructed. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users in any circumstance must accept responsibility for interpretation of patient results.
9. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated because of biological variability of antigen expression in neoplasms, or other pathological tissues. Contact Cell Marque customer service with documented unexpected reactions.
10. This product is not intended for use in flow cytometry; performance characteristics have not been determined.
11. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.
12. When used in blocking steps, normal sera from the same animal source as the secondary antisera may cause false negative or false positive results because of the effect of autoantibodies or natural antibodies.
13. False positive results may be seen because of non immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (example: liver, brain, breast, kidney) subject to the type of immunostaining technique used.

14. As with any immunohistochemistry test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells or tissue assayed.
15. The antibody is optimized for the incubation time specified in the Instructions for Use section in combination with Ventana Roche detection kits and the Ventana Roche automated slide stainers. Because of variation in tissue fixation and processing, it may be necessary to increase or decrease the primary antibody incubation time on individual specimens.
16. Cell Marque antibodies, when used in combination with Ventana Roche detection systems and accessories, detects antigen(s) that survive routine formalin fixation, tissue processing, and sectioning. Users who deviate from recommended test procedures are responsible for interpretation and validation of patient results.

Summary of Expected Results

See the following tables of reactivity:

Normal Study			
Tissue	# Stained (+)	Total #	Notes
Brain	6	6	
Adrenal Cortex	0	3	
Ovary	0	3	
Pancreas	0	3	
Parathyroid	0	3	
Pituitary	0	3	
Testis	2	3	Spermatocytes +
Thyroid	0	3	
Breast	3	3	Myoepithelium +
Spleen	0	3	
Tonsil	0	11	
Thymus	0	3	
Bone Marrow	0	3	
Lung	0	3	
Heart	0	3	
Esophagus	0	3	
Stomach	0	3	
Small Intestine	0	3	
Colon	0	3	
Liver	0	3	
Salivary Gland	3	3	
Gall Bladder	0	3	
Kidney	0	3	
Bladder	0	3	
Prostate	0	3	
Uterus	0	4	
Fallopian Tube	0	2	
Ureter	0	3	
Cervix	0	2	
Skeletal Muscle	0	3	
Smooth Muscle	0	3	
Skin	6	6	Melanocytes + , sweat gland +
Peripheral Nerve	3	3	
Mesothelium	0	3	
Fat	0	3	
Placenta	0	3	

This antibody shows nuclear reactivity in neurons, nerves, myoepithelial cells and melanocytes. It is not immunoreactive with basal cells of the prostate.

Disease Tissue Study			
Tissue	# Stained (+)	Total #	Notes
Melanoma	19	21	
Neurofibroma	18	18	
Desmoplastic melanoma	6	6	
Malignant peripheral nerve sheath tumor	3	3	
Schwannoma	2	2	
Lung adenocarcinoma	0	7	

This antibody stains tumors as indicated in literature.

Troubleshooting

1. If the positive control exhibits weaker staining than expected, other positive controls run during the same staining run should be checked to determine if it is because of the primary antibody or one of the common secondary reagents.
2. If the positive control is negative, it should be checked to ensure that the slide has the proper bar code label. If the slide is labeled properly, other positive controls used on the same instrument run should be checked to determine if it is because of the primary antibody or one of the common secondary reagents. Tissues may have been improperly collected, fixed or deparaffinized. The proper procedure should be followed for collection, storage and fixation.
3. If excessive background staining occurs, high levels of endogenous biotin may be present. A biotin blocking step should be included unless a biotin-free detection system is being used in which case any biotin present would not be a contributing factor to background staining.
4. If all of the paraffin has not been removed, the deparaffinization procedure should be repeated.
5. If specific antibody staining is too intense, the run should be repeated with incubation time shortened by 4 minute intervals until the desired stain intensity is achieved.
6. If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged. Other possibilities that could have adverse affect on tissue adhesion include insufficient drying of the tissue section on the slide prior to staining or fixation in formalin that was not properly neutral-buffered. Tissue thickness may also be a contributing factor.

For corrective action, refer to the Instructions for Use section or contact Cell Marque customer service.

References

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Disclaimers

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CM Template #2.0
Implemention date 16 Nov 2015