Rabbit Monoclonal Negative Control Ig

REF 790-4795

06683380001

IVD Σ 250

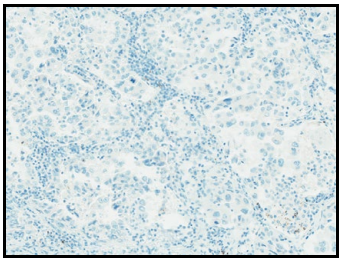

Figure 1. Rabbit Monoclonal Negative Control Ig on non-small cell lung carcinoma.

INTENDED USE

Rabbit Monoclonal Negative Control Ig is intended for laboratory use as a control for nonspecific binding of rabbit immunoglobulin in sections of formalin-fixed, paraffin-embedded tissue when stained on VENTANA BenchMark IHC/ISH platforms (BenchMark GX, BenchMark XT, and BenchMark ULTRA).

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

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

SUMMARY AND EXPLANATION

Rabbit Monoclonal Negative Control Ig may be used to aid in the identification of cells or tissue components that bind to antibodies in an antigen independent manner. The negative reagent control may demonstrate nonspecific binding to constituents located in the cell membrane, cytoplasm, nucleus, or extracellular regions of normal and abnormal tissues.

A negative reagent control must be run for every specimen to aid in the interpretation of results. A negative reagent control is used in place of the primary antibody to evaluate nonspecific staining. The incubation period for the negative reagent control should equal the primary antibody incubation period.

When panels of several antibodies are used on serial sections, a negative reagent control on one slide may serve as a negative or nonspecific binding background control for other antibodies.

PRINCIPLE OF THE PROCEDURE

Rabbit Monoclonal Negative Control Ig may be used in place of the primary antibody as a negative control for immunohistochemical staining of paraffin tissue sections. In general, immunohistochemical staining 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. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained and cover slipped. 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.

Rabbit Monoclonal Negative Control Ig is optimally diluted for use with VENTANA IHC detection kits and VENTANA BenchMark IHC/ISH platforms. 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 BenchMark IHC/ISH platforms 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. Staining is completed after incubation with a substrate chromogen and optional counterstaining. For more detailed information on instrument operation, refer to the appropriate VENTANA BenchMark IHC/ISH platform's Operator's Manual.

REAGENT PROVIDED

Rabbit Monoclonal Negative Control Ig contains sufficient reagent for staining 250 slides.

One 25 mL dispenser of Rabbit Monoclonal Negative Control Ig contains approximately 250 µg of a rabbit monoclonal antibody.

The antibody is diluted in 0.08 M PBS with 3% carrier protein and 0.05% ProClin 300, a preservative.

Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 10 µg/mL. There is no known non-specific antibody reactivity observed in this product.

Rabbit Monoclonal Negative Control Ig is a recombinant rabbit monoclonal antibody.

Refer to the appropriate VENTANA IHC detection kit package insert for detailed descriptions of: (1) Principles and Procedures, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General Limitations.

Reconstitution, Mixing, Dilution, Titration

This antibody is optimized for use on VENTANA BenchMark IHC/ISH platforms in combination with VENTANA OptiView DAB IHC Detection Kit, *ultra*View Universal DAB Detection Kit, and *ultra*View Universal Alkaline Phosphatase Red Detection Kit. No reconstitution, mixing, dilution, or titration is required.

MATERIALS REQUIRED BUT NOT PROVIDED

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

1. VENTANA rabbit monoclonal primary antibodies
2. Microscope slides, positively charged
3. Drying oven capable of maintaining a temperature of 60°C ± 5°C
4. Bar code labels (appropriate for negative reagent control and primary antibody being tested)
5. Xylene (Histological grade)
6. Ethanol or reagent alcohol (Histological grade)
   - 100% solution: Undiluted ethanol or reagent alcohol
   - 95% solution: Mix 95 parts of ethanol or reagent alcohol with 5 parts of deionized water
   - 80% solution: Mix 80 parts of ethanol or reagent alcohol with 20 parts of deionized water
7. Deionized or distilled water
8. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
9. OptiView Amplification Kit (Cat. No. 760-099 / 06396518001)
10. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
11. *ultra*View Universal Alkaline Phosphatase Red Detection Kit (Cat. No. 760-501 / 05269814001)
12. Amplification Kit (Cat. No. 760-080 / 05269814001)
13. EZ Prep Solution Concentrate (10X) (Cat. No. 950-102 / 05279771001)
14. Reaction Buffer Solution Concentrate (10X) (Cat. No. 950-300 / 05353955001)
15. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
16. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
17. ULTRA Cell Conditioning (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
18. Cell Conditioning 1 (CC1) (Cat. No. 950-124 / 05279801001)
19. Cell Conditioning 2 (CC2) (Cat. No. 950-123/ 05279798001)
20. Hematoxylin II Counterstain (Cat. No. 790-2208 / 05277965001)
21. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
22. Permanent mounting medium (Permount Fisher Cat. No. SP15-500 or equivalent)
23. Cover glass (sufficient to cover tissue, such as VWR Cat. No. 48393-060)
24. Automated coverslipper (such as the Tissue-Tek SCA Automated Coverslipper)
25. Light microscope
26. Absorbent wipes

STORAGE

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, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with VENTANA IHC detection kits and VENTANA BenchMark IHC/ISH platforms.

The recommended tissue fixative is 10% neutral buffered formalin.[1] The amount used is 15 to 20 times the volume of tissue. No fixative will penetrate more than 2 to 3 mm of solid tissue or 5 mm of porous tissue in a 24-hour period. A 3 mm or smaller section of tissue should be fixed no less than 4 hours and no more than 8 hours. Fixation can be performed at room temperature (15-25°C).[2]

Approximately 4 µm thick sections should be cut and picked up on glass slides. The slides should be positively charged. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic (IVD) use.
2. ProClin 300 preservative is used in this solution. 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.
3. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
4. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
5. Avoid microbial contamination of reagents as it may cause incorrect results.
6. Consult local and/or state authorities with regard to recommended method of disposal.
7. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Risk Phrase Guide located at www.ventana.com.

STAINING PROCEDURE

Rabbit Monoclonal Negative Control Ig primary antibody has been developed for use on VENTANA BenchMark IHC/ISH platforms in combination with OptiView DAB IHC Detection Kit, *ultra*View Universal DAB Detection Kit, and *ultra*View Universal Alkaline Phosphatase Red Detection Kit and accessories. Refer to the appropriate primary antibody package insert for the staining protocol.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument's Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding immunohistochemistry staining procedures.

Unexplained Discrepancies

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 patient samples.

Assay Verification

Prior to initial use of an antibody or staining system in a diagnostic procedure, the specificity of the assay should be verified by testing it on a series of tissues with known immunohistochemistry performance characteristics representing known positive and negative tissues (refer to the Quality Control Procedures previously outlined in this section of the product insert and to the Quality Control recommendations of the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist,[3] or the CLSI Approved Guideline[4] or both documents). These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters.

Interpretation of Results

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

Slides are to be processed with the test specimens following the instructions found in the appropriate VENTANA primary antibody package insert.

LIMITATIONS

General Limitations

1. Immunohistochemistry is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the immunohistochemistry slide, and interpretation of the staining results.
2. 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, or from inherent irregularities within the tissue.
3. Excessive or incomplete counterstaining may compromise proper interpretation of results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies as well as other diagnostic tests. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents and methods used to interpret 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.
5. Ventana Medical Systems, Inc. provides antibodies and reagents at optimal dilution for use when the provided instructions are followed. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.
6. This product is not intended for use in flow cytometry, performance characteristics have not been determined.
7. 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.[5]
8. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[6]
9. 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) depending on the type of immunostain used.[7]
10. 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.

SPECIFIC LIMITATIONS

Rabbit Monoclonal Negative Control Ig has been optimized on VENTANA BenchMark IHC/ISH platforms in combination with VENTANA detection kits and ancillary reagents. Some staining of collagen as well as weak muscle staining was observed when the antibody was used with a protease digestion step selected in the protocol. Cross-reactivity (specific staining) was observed on pheochromocytomas.

TROUBLESHOOTING

If inappropriate staining is observed on either the positive or negative tissue control, ensure that instrument maintenance has been followed for the VENTANA BenchMark IHC/ISH platform.

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, and reproducibility were conducted and the results are listed in Table 1 and Table 2 and in the Reproducibility section.

Specificity

Table 1. Specificity of Rabbit Monoclonal Negative Control Ig was determined by testing the same Tour of Body array, representing formalin-fixed, paraffin-embedded normal tissues, on the BenchMark GX, BenchMark XT and BenchMark ULTRA platforms across several instrument protocol parameters (n=25).

Tissue	# positive / total replicate tissue	Tissue	# positive / replicate tissue
Cerebrum	0/75	Thymus	0/75
Cerebellum	0/75	Myeloid (bone marrow)	0/75
Adrenal gland	0/75	Lung	0/100
Ovary	0/50	Heart	0/75
Uterus	0/25	Esophagus	0/75
Pancreas	0/75	Stomach	0/75
Parathyroid gland	0/75	Small intestine	0/75
Hypophysis	0/75	Colon	0/75
Testis	0/75	Liver	0/75
Thyroid	0/100	Salivary gland	0/75
Breast	0/75	Kidney	0/75
Spleen	0/75	Prostate	0/75
Tonsil	0/75	Cervix	0/75
Endometrium	0/75	Skin	0/75
Skeletal muscle	0/50	Heart Mesothelium	0/50
Nerve	0/75	Pheochromocytoma	25/25

Sensitivity/Specificity

Table 2. Specificity of Rabbit Monoclonal Negative Control Ig was determined by testing the same Tour of Tumor array, which represented a variety of formalin-fixed, paraffin-embedded neoplastic tissues, on the BenchMark GX, BenchMark XT and BenchMark ULTRA platforms across several instrument protocol parameters (n=25).

Pathology	# positive / replicate tissue
Glioblastoma	0/25
Atypical meningioma	0/25
Malignant ependymoma	0/25
Malignant oligodendroglioma	0/25
Ovarian Serous adenocarcinoma	0/25
Ovarian adenocarcinoma	0/25
Islet cell carcinoma	0/25
Pancreatic adenocarcinoma	0/25
Seminoma	0/25
Testicular Embryonal carcinoma	0/25
Medullary carcinoma	0/25
Thyroid papillary carcinoma	0/25
Breast intraductal carcinoma	0/25
Breast invasive ductal carcinoma	0/50
Spleen diffuse B-cell lymphoma	0/25
Lung small cell undifferentiated carcinoma	0/25
Lung squamous cell carcinoma	0/25
Lung adenocarcinoma	0/25
Esophageal squamous cell carcinoma	0/25
Esophageal adenocarcinoma	0/25
Gastric mucinous adenocarcinoma	0/25
Intestine adenocarcinoma	0/25
Intestine malignant interstitialoma	0/25
Hepatocellular carcinoma	0/25
Hepatoblastoma	0/25
Renal clear cell carcinoma	0/25
Prostatic adenocarcinoma	0/50
Uterine Leiomyoma	0/25
Endometrial adenocarcinoma	0/25
Endometrial clear cell carcinoma	0/25
Uterine squamous cell carcinoma	0/50
Embryonal rhabdomyosarcoma	0/25
Anal malignant melanoma	0/25
Basal cell carcinoma	0/25
Skin squamous cell carcinoma	0/25
Soft tissue neurofibroma	0/25
Retroperitoneal neuroblastoma	0/25
Abdominal cavity malignant mesothelioma	0/25
Mediastinum diffuse malignant lymphoma	0/25
Hodgkin lymphoma	0/25
Diffuse malignant lymphoma	0/25
Bladder transitional cell carcinoma	0/25
Low grade leiomyosarcoma	0/25
Osteosarcoma	0/25
Spindle cell rhabdomyosarcoma	0/25

Pathology	# positive / replicate tissue
Moderate malignant leiomyosarcoma	0/25
Colon adenocarcinoma	0/25
Abdominal cavity interstitialoma	0/25
Rectal adenocarcinoma	0/25
Rectal moderate malignant interstitialoma	0/25
Pheochromocytoma (tissue marker)	25/25

Appropriate negative staining results and acceptable background were observed when the antibody was tested using several cell conditioning protocols such as CC1 cell conditioning selected from 32 to 92 minutes and CC2 at 32 minutes. The Rabbit Monoclonal Negative Control Ig also showed appropriate negative staining results and acceptable background when an antibody incubation time from 8 to 60 minutes was selected.

Reproducibility

Reproducibility studies for Rabbit Monoclonal Negative Control Ig were completed to demonstrate:

- Inter-lot reproducibility of the antibody.
- Intra-run and Inter-run reproducibility on a BenchMark XT instrument.
- Intra-platform reproducibility on the BenchMark XT instrument and the BenchMark ULTRA instrument.
- Inter-platform reproducibility between the BenchMark XT instrument, BenchMark GX instrument, and BenchMark ULTRA instrument.

The Rabbit Monoclonal Negative Control Ig demonstrated ≥90% reproducibility between antibody lots, across multiple instrument runs, across multiple slides, and between instrument staining platforms.

Detection Kit and Ancillary Reagent Compatibility

Detection kit and ancillary reagent compatibility studies for Rabbit Monoclonal Negative Control Ig were completed and demonstrated that compatibility of the antibody with the OptiView DAB IHC Detection Kit, *ultra*View Universal DAB Detection Kit, and *ultra*View Universal Alkaline Phosphatase Red Detection Kit. The antibody also demonstrated appropriate negative staining results and acceptable background when used with the OptiView DAB Amplification Kit or the Amplification Kit.

All studies met their acceptance criteria.

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