

CONFIRM™ anti-Vimentin (V9) Primary Antibody

Catalog Number 790-2917

INDICATIONS AND USE

Intended Use

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

Ventana Medical Systems' (Ventana) CONFIRM anti-Vimentin (V9) Primary Antibody is a mouse monoclonal antibody (IgG1) directed against vimentin. This antibody is intended for use to qualitatively identify vimentin by light microscopy in sections of formalin fixed, paraffin embedded tissue on a Ventana automated slide stainer.

The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests. Prescription only.

Summary and Explanation

Vimentin antigen is expressed in endothelial cells, fibroblasts, smooth muscle cells and lymphoid cells. A number of tumors coexpress vimentin and cytokeratin, for example, thyroid carcinomas, pleomorphic adenomas of the salivary glands, ovarian carcinomas, and some renal carcinomas. Coexpression of desmin and vimentin has been reported in a number of soft tissue tumors, for example, rhabdomyosarcomas, leiomyosarcomas and alveolar soft tissue sarcomas.^{1,2,3,4,5}

Principles and Procedures

CONFIRM anti-Vimentin (V9) may be used as the primary antibody 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) that binds to the antigen, a secondary antibody (link antibody) that binds 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.

CONFIRM anti-Vimentin (V9) is optimally diluted for use with Ventana 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 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. Staining is completed after incubation with a substrate chromogen and optional counterstaining. For more detailed information on instrument operation, refer to the appropriate Ventana automated slide stainer Operator's Manual.

MATERIALS AND METHODS

Reagents Provided

CONFIRM anti-Vimentin (V9) contains sufficient reagent for 50 tests.

1 – 5 mL dispenser of CONFIRM anti-Vimentin (V9) contains approximately 12.5 µg (2.5 µg/mL) of a mouse monoclonal antibody directed against vimentin present in tissue. The antibody is diluted in a buffer containing carrier protein and preservative.

Reconstitution, Mixing, Dilution, Titration

This antibody is optimized for use on a Ventana automated slide stainer in combination with Ventana detection kits. 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. Ventana CONFIRM Negative Control Mouse Ig
2. Microscope slides, positively charged
3. Positive and negative tissue controls
4. Drying oven capable of maintaining a temperature of 70° C ± 5° C
5. Barcode labels (appropriate for negative control and primary antibody being tested)
6. 10% neutral buffered formalin
7. Staining jars or baths
8. Timer
9. Xylene
10. Ethanol or reagent alcohol
11. Deionized or distilled water
12. Biocare Medical's Decloaking Chamber* (ES® and NexES® IHC automated slide stainers)
13. Tissue-Tek® staining dishes*
14. ES, NexES IHC, BenchMark® Series automated slide stainers
15. Ventana VIEW™ DAB, AEC, Alkaline Phosphatase Red and Enhanced Alkaline Phosphatase Red detection kits*
16. Ventana Amplification Kit*
17. Ventana Endogenous Biotin Blocking Kit*
18. Detection kit specific software (ES automated slide stainer only)
19. Ventana APK Wash* (10X) (ES and NexES IHC automated slide stainers)
20. Ventana Liquid Coverslip™ (Low Temperature)* (ES and NexES IHC automated slide stainers)
21. Ventana EZ Prep™ (10X)* (BenchMark Series automated slide stainers)
22. Ventana Reaction Buffer (10X)* (BenchMark Series automated slide stainers)
23. Ventana Liquid Coverslip (High Temperature)* (BenchMark Series automated slide stainers)
24. Ventana Cell Conditioning 1 (Pre-dilute) (BenchMark Series automated slide stainers)
25. 10 mM sodium citrate (pH 6.0)
26. Ventana Hematoxylin counterstain
27. Ventana Bluing Reagent
28. Mounting medium
29. Cover glass
30. Light microscope (20-80X)

* As needed for specific applications.

Storage and Handling

Store at 2-8° C. Do not freeze. The user must validate any storage conditions other than those specified in the package insert.

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. Your local Ventana office should be contacted immediately if there is an indication of reagent instability.

Specimen Collection and Preparation for Analysis

Routinely processed, formalin fixed, paraffin embedded tissues are suitable for use with this primary antibody when used with Ventana detection kits and a Ventana automated slide stainer (see Materials and Reagents Needed, But Not Provided section). The recommended tissue fixative is 10% neutral buffered formalin.⁶ 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 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 70° C ± 5° C oven.

Manual Deparaffinization Procedure

Required when using the ES or NexES IHC automated slide stainers or if deparaffinization is not selected on the BenchMark Series automated slide stainer:

- For instructions on when to label slides with barcode label, refer to the Instructions for Use section of the specific automated slide stainer Operator's Manual.
- Immerse the slides sequentially in 3 xylene baths for 5 ± 1 minutes each.
- Transfer the slides to 100% ethanol and immerse sequentially in 2 baths for 3 ± 1 minutes each.
- Transfer the slides to 95% ethanol and immerse them in a bath of this solution for 3 ± 1 minutes.
- Transfer the slides to 80% ethanol and immerse them in this solution for 3 ± 1 minutes.
- Transfer the slides to a bath of deionized or distilled water and dip a minimum of 10 times.
- Transfer slides to APK Wash (1X) or buffer solution as appropriate. For APK Wash, the slides should remain until you are ready to perform the staining run. For buffer solution, the slides should remain until you are ready to perform the antigen unmasking procedure. Do not allow the slides to dry.

Slides stained on the BenchMark Series automated slide stainers can be deparaffinized on the instrument. If this option is selected, apply barcode labels to slides and place slides on the instrument. If the option is not selected, follow the Manual Deparaffinization Procedure above.

WARNINGS AND PRECAUTIONS

- Take reasonable precautions when handling reagents. Use disposable gloves when handling suspected carcinogens or toxic materials (example: xylene or formaldehyde).
- Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
- Patient specimens and all materials contacting them should be handled as biohazardous materials and disposed of with proper precautions. Never pipette by mouth.
- Avoid microbial contamination of reagents, as this could produce incorrect results.
- Incubation times and temperatures other than those specified may give erroneous results. The user must validate any such change.
- The reagents have been optimally diluted, and further dilution may result in loss of antigen staining. The user must validate any such change.
- Consult local or state authorities with regard to recommended method of disposal.
- When used according to instructions, this product is not classified as a hazardous substance. The preservative in the reagent is either ProClin 300 or sodium azide (NaN₃). Symptoms of overexposure to ProClin 300 or NaN₃ include skin and eye irritation, and irritation of mucous membranes and upper respiratory tract. The concentration of ProClin 300 in this product is less than or equal to 0.05% and does not meet the OSHA criteria for a hazardous substance. Systemic allergic reactions are possible in sensitive individuals. The concentration of NaN₃ in this product is less than 0.1% and does not meet the OSHA criteria for a hazardous substance. Build up of NaN₃ may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide accumulation in plumbing.

INSTRUCTIONS FOR USE

Step by Step Procedure

Ventana primary antibodies have been developed for use on a Ventana automated slide stainer in combination with Ventana detection kits and accessories. Recommended staining protocols for the automated slide stainers are listed below in Table 1. The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the Operator's Manual. Other operating parameters for the automated slide stainers have been preset at the factory.

Table 1. Recommended Staining Protocols for CONFIRM anti-Vimentin (V9)

Procedure Type	Platform or Method	
	ES or NexES IHC	BenchMark Series
Deparaffinization	Off Line	Selected
Cell Conditioning (Antigen Unmasking)	10 mM sodium citrate (pH 6.0), 2 minutes, Decloaking Chamber, 120° C	Cell Conditioning 1, Standard
Enzyme (Protease)	None required	None required
Antibody (Primary)	Approximately 16 minutes, 37° C	Approximately 16 minutes, 37° C
A/B Block (Biotin Blocking)	Optional	Optional
Amplify (Amplification)	Optional	Optional
Counterstain (Hematoxylin)	Hematoxylin, 2 to 4 minutes	Hematoxylin, 2 to 4 minutes
Post Counterstain	Bluing, 2 to 4 minutes	Bluing, 2 to 4 minutes

The procedures for staining on the Ventana automated slide stainers are as follows. For more detailed instructions and additional protocol options, refer to your Operator's Manual.

ES and NexES IHC Automated Slide Stainers

Antigen Unmasking Required:

- Slides are to be deparaffinized through a series of xylene and gradient alcohols to water and then to appropriate buffer. Perform antigen unmasking procedure and transfer slides to APK Wash (1X).
- Load the primary antibody, appropriate detection kit dispensers and required accessory reagents onto the reagent tray and place the reagent tray on the automated slide stainer. Check bulk fluids and waste.
- Dry the painted end of the slide and then apply slide barcode label that corresponds to the antibody protocol to be performed.
- Load the deparaffinized, antigen unmasked, labeled slides from the APK Wash (1X). Avoid tissue drying.

Manual Antigen Unmasking Procedure

Manual antigen unmasking is required when using the ES or NexES automated slide stainer.

Antigen enhancement (cell conditioning) procedure (for tissue slides to be stained on ES, NexES, or manual methods):

- Prepare the Decloaking Chamber for use.
- Place the pan into the chamber. Note: Make sure that the outside of the pan is completely dry prior to placing it in the chamber. If the outside of the pan is wet, the Decloaking Chamber will make a cracking noise and any water in the chamber will cause a malfunction.
- Align the handles of the pot with the handles of the chamber.
- Fill the pan with 500 mL of deionized water and place the heat shield, (circular screen), in the center of the pot. Note: The heat shield keeps the plastic containers from warping.
- Place each Tissue-Tek staining dish, filled with 250 mL of 10 mM sodium citrate (pH 6.0) or EDTA buffer (pH 8.0) as indicated in the Recommended Staining Protocols (Table 1) in the package insert for each primary antibody, and the appropriate slides on the heat shield which is placed in the center of the pan. Cell Conditioning 1 (pH 8.5) may be substituted for EDTA Buffer and Cell Conditioning 2 (pH 6.0) may be substituted for 10 mM sodium citrate if desired. Up to 2 containers may be placed in the chamber, but make sure both are touching the heat shield.
- Put the lid on the Decloaking Chamber and secure (align the open arrow with the white dot on the pan handle. Grip the lid handle, and rotate clockwise to the closed position; when the lid is locked in the proper position, the Vent Lever will lower the weight on the vent nozzle).
- Turn the rheostat to 10 and lock into place (approximately 120° C).
- Turn on the Decloaking Chamber and monitor until the pressure reaches 17-25 psi and the temperature is 120° - 125° C. Once the Decloaking Chamber reaches the desired temperature, time for 2 minutes using a calibrated manual timer, as the Decloaking Chamber timer is not "real time" consistent. When the manual timer goes off, turn the Decloaker timer to the off position. The heat will turn off and the

light will turn from "heat on" to "keep warm". Note: Technician must monitor temperature and pressure conditions to confirm desired specifications are met.

9. Once the antigen enhancement procedure is completed, turn off the Decloaking Chamber.
10. The technician can monitor the declining pressure by periodically checking the pressure gauge. When pressure reaches 0 psi, the Decloaking chamber can be opened safely. Rotate the lid counterclockwise and remove it slowly, allowing steam to escape away from your hand. Note: Be very careful when opening lid, as surface and liquid temperatures remain high.
11. Remove the container of slides from the pan and place slide holders containing processed slides in a container of room temperature, deionized water.
12. Once rinsing is complete, place the slides in a Tissue-Tek slide rack filled with deionized water, for maintaining hydration while barcode labels are applied to slides. One by one, remove the slides from the slide rack and blot the frosted end dry, ensuring the tissue sections do not dry during the process. Label each slide with the appropriate barcode label, and return it to the slide container. Repeat this process for all slides.
13. Once all slides have been labeled, empty the deionized water from the slide container and refill it with APK Wash (1X). Slides should remain in this solution until ready to perform staining run.

BenchMark Series Automated Slide Stainers

1. Apply slide barcode label that corresponds to the antibody protocol to be performed.
2. Load the primary antibody, appropriate detection kit dispensers and required accessory reagents onto the reagent tray and place the reagent tray on the automated slide stainer. Check bulk fluids and waste.
3. Load the slides onto the automated slide stainer.

For All Instruments

1. Start the staining run.
2. At the completion of the run, remove the slides from the automated slide stainer.
3. For MIEW DAB and Alkaline Phosphatase Red detection kits, wash in a mild dishwashing detergent or alcohol to remove the coverslip solution; dehydrate, clear, and coverslip with permanent mounting media in the usual manner.
4. For AEC chromogen, do not dehydrate and clear. Mount AEC with aqueous mounting medium. The stained slides should be read within 2 to 3 days of staining, and are stable for at least 2 years if properly stored at room temperature (15° - 25° C).

Quality Control Procedures

Positive Tissue Control

A positive tissue control must be run with every staining procedure performed. An example of a positive control for CONFIRM anti-Vimentin (V9) is tonsil. The positive staining tissue components (cytoplasmic staining of endothelial cells and lymphoid cells) are used to confirm that the antibody was applied and the instrument functioned properly. This tissue may contain both positive and negative staining cells or tissue components and may 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. Such tissues may monitor all steps of the procedure, from tissue preparation through staining. Use of a tissue section fixed or processed differently from the test specimen will 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.

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

Unexplained Discrepancies

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

Negative Reagent Control

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 slide should be stained with CONFIRM Negative Control Mouse Ig or CONFIRM Negative Control Rabbit Ig, as appropriate. If an alternative negative reagent control is used, dilute to the same concentration as the primary antibody antiserum with Ventana Antibody Diluent. The diluent alone may be used as an alternative to the previously described negative reagent controls. 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.

Assay Verification

Prior to initial use of an antibody or staining system in a diagnostic procedure, the specificity of the antibody 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 Checklist⁷, or the NCCLS Approved Guideline⁸ or both documents). These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters. Tissues listed in the Summary of Expected Results section of this insert are suitable for assay verification.

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.

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 kit used for expected color reactions. Depending on the incubation time 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 positive staining, any results with the test specimens should be 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 should be considered invalid.

Nonspecific staining, if present, will have a diffuse appearance. Sporadic light staining of connective tissue may also be observed in tissue sections that are excessively formalin fixed. Intact cells should be used for interpretation of staining results, as necrotic or degenerated cells often stain nonspecifically.

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. If necessary, use a panel of antibodies to 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

General Limitations

1. Immunohistochemistry is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents and tissues, 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 be a consequence of variations in fixation and embedding methods, or 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 and proper controls as well as other diagnostic tests. This antibody is intended to be used in a panel of antibodies. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents 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.
5. Ventana 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.⁹ Contact your local Ventana office with documented unexpected reactions.
8. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.¹⁰
9. When used in blocking steps, normal sera from the same animal source as the secondary antisera may cause false negative or false positive results due to autoantibodies or natural antibodies.
10. False positive results may be seen because of nonimmunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), alkaline phosphatase, or endogenous biotin (example: liver, brain, breast, kidney) depending on the type of immunostain used.¹¹
11. 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

1. The antibody has been optimized for a 16 minute incubation time in combination with Ventana detection kits and the Ventana 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. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".¹²
2. The antibody, in combination with Ventana detection kits and accessories, detects antigen that survives 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

1. Specificity of CONFIRM anti-Vimentin (V9) was determined by a study that showed appropriate staining of normal and neoplastic tissues. The following results were found in normal human tissue: for adrenal, brain (cerebellum and medulla), small intestine, kidney, liver, lung, skeletal muscle, myocardium, ovary, pancreas, placenta, prostate, skin, stomach, testis, tonsil, and uterus, 1 out of 1 case tested positive. The results of a study for expression in neoplasms are as follows: for melanoma, 5 out of 5 cases tested positive; for thyroid medullary carcinoma, Schwannoma, and lymphoma, 3 out of 3 cases tested positive; for leiomyosarcoma,

and chondrosarcoma, 1 out of 1 case tested positive; and for colon adenocarcinoma, 0 out of 2 cases tested positive.

2. Sensitivity is dependent upon the preservation of the antigen. Any improper tissue handling during fixation, sectioning, embedding or storage which alters antigenicity weakens vimentin detection by CONFIRM anti-Vimentin (V9) and may generate false negative results.
3. Intra run reproducibility of staining was determined by staining 5 slides containing the same tissue. Five of 5 slides stained positively. All slides stained with the same staining intensity. Users should verify within run reproducibility results by staining several sets of serial sections with low, medium and high antigen density in a single run.
4. Inter run reproducibility of staining was determined by staining slides containing the same tissue on 5 different instrument runs. Five of 5 slides stained positively. All slides stained with similar staining intensity. Users should verify between run reproducibility results by staining several sets of serial sections with low, medium and high antigen density on different days.

TROUBLESHOOTING

1. If the positive control exhibits weaker staining than expected, other positive controls run concurrently should be checked to determine if it is due to 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 barcode label. If the slide is labeled properly, other positive controls run concurrently should be checked to determine if it is due to 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 may be included.
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 the primary antibody 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.
7. For corrective action, refer to the Step By Step Procedure section of the automated slide stainer Operator's Manual or contact your local Ventana office.

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