

Antibody Diluent

Catalog Number 251-018

INDICATIONS AND USE

Intended Use

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

Ventana® Medical Systems' (Ventana) Antibody Diluent is a buffered, proteinaceous solution used to dilute rabbit and mouse antibodies for use on Ventana automated slide stainers.

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. Caution: U.S. Federal law restricts this device to sale by or on the order of a physician.

Summary and Explanation

Concentrated antibodies are added to Antibody Diluent to obtain the appropriate antibody titer (determined by the individual user) necessary for optimal immunohistochemical staining. The optimally diluted antibody may be filled into a Ventana User-defined Antibody Dispenser (NexES® and ES®) or BenchMark® Prep Kit, or may be applied by hand in a titer mode.

Principles and Procedures

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.

MATERIALS AND METHODS

Reagents Provided

1 – 100 mL bottle of Antibody Diluent; contains 0.3% protein in 0.1M phosphate buffered saline (pH 7.3) and 0.05% ProClin 300, a preservative

Reconstitution, Mixing, Dilution, Titration

No reconstitution, mixing, dilution, or titration is required. This reagent is optimized for use on a Ventana automated slide stainer in combination with Ventana detection kits and ancillary reagents.

Materials and Reagents Needed But Not Provided

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

1. Ventana Negative Control Reagent*
2. Ventana Rabbit Negative Control*
3. Primary antibody
4. Microscope slides, positively charged
5. Positive and negative tissue controls
6. Drying oven capable of maintaining a temperature of 70° C ± 5° C
7. Bar code labels (appropriate for negative control or primary antibody being tested)
8. 10% neutral buffered formalin
9. Staining jars or baths
10. Timer
11. Xylene
12. Ethanol or reagent alcohol
13. Deionized or distilled water
14. Biocare Medical's Decloaking Chamber* (ES and NexES IHC automated slide stainers)
15. ES, NexES IHC, BenchMark and BenchMark XT automated slide stainers
16. iVIEW™ DAB, AEC, V Red (ALK PHOS) or Enhanced V Red detection kits
17. Ventana Endogenous Biotin Blocking Kit*
18. Detection kit specific software (ES automated slide stainer only)
19. Ventana APK Wash Solution* (ES and NexES IHC automated slide stainers)
20. Ventana Liquid Coverslip™ solution (ES and NexES IHC automated slide stainers)

21. Ventana EZ Prep™ solution* (BenchMark and BenchMark XT automated slide stainers)
22. Ventana Reaction Buffer* (BenchMark and BenchMark XT automated slide stainers)
23. Ventana LCS (BenchMark and BenchMark XT automated slide stainers)
24. Ventana Cell Conditioning 1 (CC1), Cell Conditioning 2 (CC2) (BenchMark and BenchMark XT automated slide stainers)*
25. Ventana Protease I, II, or III*
26. Ventana Hematoxylin*
27. Ventana Bluing Reagent*
28. Ventana User defined Antibody Dispenser* (ES and NexES IHC automated slide stainers) or Prep Kit* (BenchMark and BenchMark XT automated slide stainers)
29. Mounting medium
30. Cover glass
31. Light microscope (20-80X)
32. Micropipette and tips

* 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.

Antibody Diluent must be returned to storage conditions identified above immediately after use. Every reagent container 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.

The signs indicating contamination or instability of this product are turbidity of the solution, odor development, or precipitation of the solution. At the first sign of possible reagent instability, contact your local Ventana office.

Specimen Collection and Preparation for Analysis

Frozen Sections

The recommended procedure is to place the fresh tissue in OCT embedding medium and snap freeze in liquid nitrogen or dry ice cooled isopentane. The frozen tissue should be stored at -80° C until sectioning. Biopsies are sectioned at 4 to 6 µm in thickness, picked up on clean glass slides, and immediately placed into cold acetone (0-8° C) for 10 to 30 minutes. Upon removal from acetone, slides are allowed to air dry for 10 minutes to 1 hour.

Cytological Specimens

Cytospin preparations and blood smears should be prepared according to standard laboratory procedures:

1. Air dry slides for 30 minutes.
2. Fix in cold acetone (0 to 8° C) for 10 to 30 minutes.
3. Remove from acetone and air dry for 10 minutes to 1 hour.
4. Place in a desiccator with desiccant for a maximum of 10 days. If sections are to be stored longer than 10 days, wrap tightly in aluminum foil and store at -80° C.
5. Allow slides to come to room temperature after removal from -80° C, (for aluminum foil wrapped slides, allow to come to room temperature before removing aluminum foil to prevent condensation on the specimen).
6. Apply bar code labels to slides and load on the automated slide stainer.

Formalin Fixed, Paraffin Embedded

Routinely processed, formalin fixed, paraffin embedded tissues are suitable for use with this reagent when used with Ventana primary antibodies, detection kits, ancillary reagents, and automated slide stainer (see Materials and Reagents 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 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 or the BenchMark XT automated slide stainer:

1. For instructions on when to label slides with bar code label, refer to the Instructions for Use section of the specific automated slide stainer.
2. Immerse the slides sequentially in 3 xylene baths for 5 ± 1 minutes each.
3. 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) solution or buffer solution as appropriate. For APK Wash solution, 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 or BenchMark XT automated slide stainers can be deparaffinized on the instrument. If this option is selected, barcode slides and place them 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.
- Do not smoke, eat or drink in areas where specimens or reagents are being handled.
- 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.
- Symptoms of overexposure may include skin and eye irritation and irritation to mucous membranes and upper respiratory tract. ACGIH (TLV): none; OSHA (PEL): N/A; IARC: not listed; NTP: not listed.
- Consult local or state authorities with regard to recommended method of disposal.

INSTRUCTIONS FOR USE

Step by Step Procedure

Antibody Diluent provides consistent staining with primary rabbit and mouse antibodies on Ventana automated slide stainers. Titration of concentrated antibodies is usually accomplished by serial two fold dilutions. One hundred µl of each dilution is necessary for titration on Ventana automated slide stainers. The events carried out by the automated slide stainer are dependent upon the options selected for each procedure being run. For exact sequence of events consult your Operator's Manual. 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.

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 deparaffinized through a series of xylene and gradient alcohols to water and appropriate buffer. Perform antigen unmasking procedure and transfer slides to APK Wash (1X).
- Load the primary antibody and appropriate detection kit dispensers and required accessory reagents onto the reagent tray and place them on the automated slide stainer. Check bulk fluids and waste.
- Dry the painted end of the slide and then apply slide bar code 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.

Antigen Unmasking Not Required:

- Apply barcode labels to the slide. Slides are then deparaffinized through a series of xylene and gradient alcohols to water and then to APK Wash (1X).
- Load the primary antibody and appropriate detection kit dispensers and required accessory reagents onto the reagent tray and place them on the automated slide stainer.
- Load the deparaffinized labeled slides from the APK Wash (1X). Avoid tissue drying.

BenchMark or BenchMark XT Automated Slide Stainers

- Apply slide bar code label which corresponds to the antibody protocol to be performed.
- Load the primary antibody and appropriate detection kit dispensers and required accessory reagents onto the reagent tray and place them on the automated slide stainer. Check bulk fluids and waste.
- Load the slides onto the automated slide stainer.

For All Instruments

- Start the staining run.
- At the completion of the run, remove the slides from the automated slide stainer.
- For MIEW DAB and Ventana Red kit, 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.
- For AEC chromogen, do not dehydrate and clear. Mount AEC with aqueous mounting medium. The stained slides should be read within two to three days of staining, and are stable for at least two years if properly stored at room temperature (20 ° C to 28° C).

Quality Control Procedures

Positive Tissue Control

A positive tissue control must be run with every staining procedure performed. For examples of positive control material, refer to the Ventana primary antibody package insert of interest.

Positive controls 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 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, 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 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 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. See 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 Negative Control Reagent (mouse) or Rabbit Negative Control, 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 section outlined in the Ventana primary antibody of choice package insert, to the Quality Control recommendations of the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist, and the NCCLS Approved Guideline). 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.

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 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 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 sections from excessively formalin fixed tissues. Intact cells should be used for interpretation of staining results. 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. 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. 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 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. 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 your local Ventana office with documented unexpected reactions.
7. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.
8. 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 autoantibodies or natural antibodies.
9. 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 activity, or endogenous biotin (example: liver, brain, breast, kidney) depending on the type of immunostain used.
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.

SUMMARY OF EXPECTED RESULTS

1. Expected results are quantitative only when testing the sensitivity and specificity of each specific antigen. As a stand alone reagent, this product cannot be tested for specificity or sensitivity. Refer to the appropriate Ventana primary antibody package insert for expected patient sample results. Appropriate sample control results verify the reagents and system are working properly.
2. Sensitivity is dependent upon the preservation of the antigen. Any improper tissue handling during fixation, sectioning, embedding or storage which alters antigenicity weakens primary antibody detection and may generate false negative results.
3. Intra lot reproducibility was tested for 5 lots of Antibody Diluent. All samples were determined to be concordant within the specification range for all reagent properties tested.
4. Inter lot reproducibility was tested for 5 lots of Antibody Diluent. All samples were determined to be concordant within the specification range for all reagent properties tested.

TROUBLESHOOTING

1. If the positive control exhibits weaker staining than expected, other positive controls run during the same instrument 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 run 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.
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.
7. For corrective action, refer to the Step By Step Procedure section, the automated slide stainer Operator's Manual or contact your local Ventana office.

REFERENCES

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