


ultraView Universal DAB Detection Kit

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IVD  250

INTENDED USE

Ventana Medical Systems' (Ventana) *ultraView* Universal DAB Detection Kit is an indirect, biotin-free system for detecting mouse IgG, mouse IgM and rabbit primary antibodies. The kit is intended to identify targets by immunohistochemistry (IHC) in sections of formalin-fixed, paraffin-embedded and frozen tissue that are stained on the BenchMark, BenchMark XT, and BenchMark ULTRA instruments.

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

Immunohistochemistry (IHC) is a technique used in laboratories for diagnostic purposes. IHC uses specific primary antibodies to localize and bind to antigens in fixed or frozen tissue sections. The binding of the antibody to the antigen is visualized with an indirect detection method. The most common techniques for indirect methods use a secondary antibody directed against the species of primary antibody and an enzyme with a corresponding substrate-chromogen system. This combination results in a colored precipitate at the site of specific antibody binding. The *ultraView* Universal DAB Detection Kit uses an indirect method to visualize specific antibodies bound to antigens by depositing a brown colored precipitate.

PRINCIPLES OF THE PROCEDURE

The *ultraView* Universal DAB Detection Kit detects specific mouse and rabbit primary antibodies bound to an antigen in paraffin-embedded tissue sections. The specific antibody is located by a cocktail of enzyme labeled secondary antibodies. The complex is then visualized with hydrogen peroxide substrate and 3, 3'-diaminobenzidine tetrahydrochloride (DAB) chromogen, which produces a brown precipitate that is readily observed by light microscopy.

The staining protocol consists of numerous steps in which reagents are incubated for pre-determined times at specific temperatures. At the end of each incubation step, the BenchMark, BenchMark XT, and BenchMark ULTRA instrument washes the sections to remove unbound material and applies a liquid coverslip which minimizes the evaporation of the aqueous reagents from the slide.¹ 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.

For more detailed information on instrument operation, refer to the appropriate Operator's Manual. Figure 1 illustrates the indirect detection method.

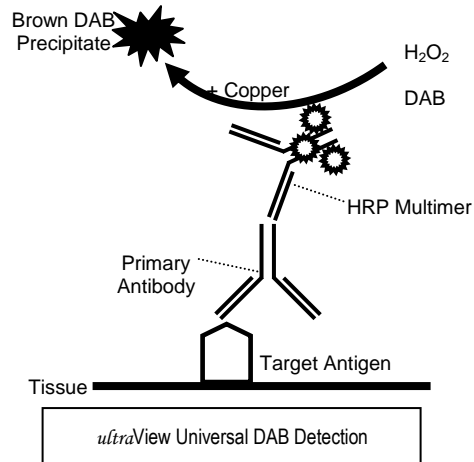


Figure 1. *ultraView* Universal DAB Detection Kit Reaction

MATERIAL AND METHODS

Reagents Provided

ultraView Universal DAB Detection Kit contains sufficient reagent for 250 tests.

One 25 mL dispenser	<i>ultraView</i> Universal DAB Inhibitor contains 3% hydrogen peroxide solution.
One 25 mL dispenser	<i>ultraView</i> Universal HRP Multimer contains a cocktail of HRP labeled antibodies (goat anti-mouse IgG, goat anti-mouse IgM, and goat anti-rabbit) (<50 µg/mL) in a buffer containing protein with ProClin 300, a preservative.
One 25 mL dispenser	<i>ultraView</i> Universal DAB Chromogen contains 0.2% 3, 3'-diaminobenzidine tetrahydrochloride in a proprietary stabilizer solution with a proprietary preservative.
One 25 mL dispenser	<i>ultraView</i> Universal DAB H ₂ O ₂ contains 0.04% hydrogen peroxide in a phosphate buffer solution.
One 25 mL dispenser	<i>ultraView</i> Universal Copper contains copper sulfate (5g/L) in an acetate buffer with proprietary preservative.

Reconstitution, Mixing, Dilution, Titration

The detection kit is optimized for use on BenchMark, BenchMark XT, and BenchMark ULTRA instruments. No reconstitution, mixing, dilution, or titration of kit reagents is required.

Further dilution may result in loss of antigen staining. The user must validate any such changes. Differences in tissue processing and pre-examination procedure in the laboratory may produce significant variability in results. For more information about controls, see the 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 detection kit:

1. Primary antibody
2. Negative control reagent
3. Positive and negative tissue controls (consult antibody package inserts for recommended types)
4. Amplification Kit (as needed for specific applications)
5. Protease 1 (as needed for specific applications)
6. Protease 2 (as needed for specific applications)
7. Protease 3 (as needed for specific applications)
8. Hematoxylin Counterstain (as needed for specific applications)

9. Hematoxylin II Counterstain (as needed for specific applications)
10. Bluing Reagent (as needed for specific applications)
11. Reaction Buffer Concentrate (10X)
12. Cell Conditioning Solution 1 (CC1) (as needed for specific applications)
13. Cell Conditioning Solution 2 (CC2) (as needed for specific applications)
14. Antibody Diluent (as needed for specific applications)
15. EZ Prep Concentrate (10X) Solution or EZ Prep Pre-Dilute (1X)
16. Liquid Coverslip (High Temperature)
17. BenchMark, BenchMark XT, or BenchMark ULTRA instrument
18. Microscope slides, positively charged
19. Drying oven capable of maintaining a temperature of $60^{\circ}\text{C} \pm 5^{\circ}\text{C}$
20. Bar code labels (appropriate bar code labels for negative reagent control and the primary antibody being tested)
21. Xylene (Histological grade)
22. Ethanol or reagent alcohol (Histological grade)
23. Staining jars or baths
24. Deionized or distilled water
25. Cover slip and cover slip method sufficient to cover tissue
26. Light microscope (20-80X)
27. Mild dishwashing detergent
28. Automated coverslipper such as Tissue-Tek SCA Automated Coverslipper, (as needed for specific applications)

Storage and Handling

Store at $2-8^{\circ}\text{C}$. Do not freeze. The user must validate any storage conditions other than those specified in the package insert. This detection kit can be used immediately after removal from the refrigerator.

To ensure proper reagent delivery and stability of each reagent, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every detection kit is expiration dated. When properly stored, the product is stable to the date indicated on the label. Do not use product 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 support representative should be contacted immediately if there is an indication of reagent instability.

Specimen Collection and Preparation for Analysis

Formalin-fixed, paraffin-embedded tissues are suitable for use with *ultraView* Universal DAB Detection Kit and a BenchMark, BenchMark XT, and BenchMark ULTRA instrument (see Materials and Reagents Needed but Not Provided section). The recommended tissue fixative is 10% neutral buffered formalin (NBF).² Variable results may occur as a result of tissue section thickness, fixation type, incomplete prolonged fixation or special processes such as decalcification of bone marrow preparations.

Each section should be cut to the appropriate thickness ($2-5\text{ }\mu\text{m}$) for the primary antibody being used and placed on a glass microscope slide. Slides containing the tissue section may be baked/heated for at least 2 hours (but not longer than 24 hours) in a $60^{\circ}\text{C} \pm 5^{\circ}\text{C}$ oven. Slide heating is used to dry the tissue post slide mounting and to enhance tissue adhesion to the glass. NOTE: Consult the primary antibody package insert to identify heating limitations. Extended heating of the tissue might result in decreased antigen availability.

Properly fixed and embedded tissues expressing the antigen will remain stable if stored in a cool location ($15^{\circ}-25^{\circ}\text{C}$). The Clinical Laboratory Improvement Act (CLIA) of 1988, 42CFR493.1259 (b) requires that "The laboratory must retain slides at least ten years from the date of examination and retain specimen blocks at least two years from date of examination." Each laboratory should validate the cut slide stability for their own procedures and environmental storage conditions.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic (IVD) use.
2. **Warning: Possible carcinogen.** The International Agency for Research on Cancer (IARC) and the US National Toxicology Program (NTP) have listed benzidine, a compound closely related to 3, 3'-diaminobenzidine tetrahydrochloride (DAB), as a known human carcinogen.
3. ProClin 300 is used as a preservative 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.
4. Materials of human or animal origin should be handled as potentially biohazardous and disposed of with proper precautions.
5. Take reasonable precautions when handling reagents. Avoid contact of reagents with eyes, skin, and mucous membranes. Use disposable gloves and wear suitable protective clothing when handling suspected carcinogens or toxic materials.
6. If reagents come in contact with sensitive areas, wash with copious amounts of water. Avoid inhalation of reagents.
7. Ensure that the waste container is empty prior to starting a run on the instrument. If this precaution is not taken, the waste container may overflow and the user risks a slip and fall.
8. Avoid microbial contamination of reagents as this may produce incorrect results.
9. Consult local and/or state authorities to determine the recommended method of disposal.
10. Refer to the product Safety Data Sheet for additional information.

INSTRUCTIONS FOR USE

Ventana *ultraView* Universal DAB Detection Kit has been developed for use on BenchMark, BenchMark XT, and BenchMark ULTRA instruments in combination with Ventana primary antibodies and accessories. The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument's Operator's Manual. Other operating parameters for the instrument have been preset at the factory.

For more detailed instructions and additional protocol options refer to your Operator's Manual. Whether a sample requires Cell Conditioning is antibody dependent. Please check the antibody package insert for directions.

BenchMark, BenchMark XT, and BenchMark ULTRA Instruments

1. Apply slide bar code label which corresponds to the protocol to be performed.
2. Load the primary antibody, appropriate detection kit dispensers, and required accessory reagent onto the reagent tray and place them on the automated slide stainer.
3. Check bulk fluids and empty waste.
4. Load the slides onto the automated slide stainer.
5. Start the staining run.
6. At the completion of the run, remove the slides from the automated slide stainer.
7. Proceed to Recommended Post-Instrument Processing Procedure.

Recommended Post-Instrument Processing Procedures

1. Wash slides in a mild dishwashing detergent to remove the coverslip solution.
2. Rinse slides thoroughly in distilled water to remove all detergent.
3. Dehydrate, clear, and coverslip with permanent mounting media.

Quality Control Procedures

Positive Tissue Control

Optimal laboratory practice is to include a positive control section on the same slide as the patient tissue. When this practice is employed, identification of a failure to apply primary antibody or other critical reagent to the patient test slide is easily detected. Additionally, positive controls should be selected on the basis of expression of the antigen. Low levels of antigen expression are suggested to prevent false negative results due to insufficient sensitivity. A tissue with weak positive staining is more suitable for optimal quality control and for detecting minor levels of reagent degradation.

A positive tissue control must be run with every staining procedure performed. Optimal laboratory practice is to include a positive control section on the same slide as the patient tissue. This practice helps to identify a failure to apply primary antibody or other critical

reagent to the patient test slide. A tissue with weak positive staining is more suitable for optimal quality control. The positive staining tissue components 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.

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 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 support representative 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 Mouse or Negative Control Rabbit, 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 a primary antibody or staining system in a diagnostic procedure, the specificity of the primary 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 Positive Tissue Control section in the primary antibody package insert and to the Quality Control recommendations of the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist,³ or the CLSI Approved Guideline⁴ 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 Performance Characteristics section of the primary antibody are suitable for assay verification.

Interpretation of Results

The *ultraView* Universal DAB Detection Kit causes a brown colored reaction product to precipitate at the antigen sites localized by the primary antibody. A qualified pathologist who is experienced in immunohistochemical procedures must evaluate controls and qualify the stained product before interpreting results. Staining of negative controls must be noted first, and these results compared to the stained material to verify that the signal generated is not the cause of nonspecific interactions.

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. 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 non-specific 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. 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. IHC 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. 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 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.⁵ Contact your local support representative 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.⁶
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. 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

- Each step of the detection kit procedure has been optimized on the BenchMark, BenchMark XT, and BenchMark ULTRA instruments and is preset. Because of variation in tissue fixation and processing, it may be necessary to increase or decrease the primary antibody incubation time on individual specimens. Primary antibody incubation time depends on the degree of tissue fixation, and may range from 8 to 32 minutes. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances"⁷ or "Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist".⁸
- The detection kit, in combination with Ventana primary antibodies and accessories, detects antigen that survives routine formalin fixation, tissue processing, and sectioning.
- This detection kit has been optimized for use with Reaction Buffer wash solution, primary antibodies, accessories, and BenchMark, BenchMark XT, and BenchMark ULTRA instruments. The use of Reaction Buffer wash solution is important to the proper function of the detection kit. Users who deviate from recommended test procedures are responsible for interpretation of patient results under these circumstances.
- This detection kit has been optimized for use with LCS (Predilute) or ULTRA LCS (Predilute), also known as LCS or Liquid Coverslip (High Temperature). LCS is a prediluted coverslip solution used both as a barrier between aqueous reagents and the air. The LCS barrier reduces evaporation and provides a stable aqueous environment for the IHC or *in situ* hybridization (ISH) reactions carried out on Ventana automated slide stainers.
- Incubation times and temperatures other than those specified may give erroneous results. The user must validate any such change.

PERFORMANCE CHARACTERISTICS

Reproducibility Studies

ultraView Universal DAB Detection Kit reproducibility testing was performed by staining serial sections from 3 neutral buffered formalin fixed, paraffin embedded tissues using 3 primary antibodies, a mouse IgG (anti-Ki67) stained on breast carcinoma, a mouse IgM (anti-CD15) stained on a Hodgkin's Lymphoma xenograft and a rabbit IgG (anti-S100) on a melanoma using BenchMark and BenchMark XT automated slide stainers. All primary antibodies were incubated for 16 minutes and slides counterstained using Hematoxylin II followed by Bluing Reagent. All slides stained with a primary antibody were compared against each other for staining appropriateness and intensity and scored by a qualified slide reader.

- Intra run reproducibility (same primary antibody stained on a platform compared) staining runs were performed one per day on 3 separate days using 2 different stainers: BenchMark and BenchMark XT instruments for a total of 6 staining runs. Intra run reproducibility for the BenchMark XT instrument was 100% (30 of 30 slides per primary antibody per run, a total of 90 slides stained per antibody across the 3 runs) and 100% (20 of 20 slides per primary antibody per run, a total of 60 slides per antibody across 3 runs for BenchMark instrument).
- Inter run reproducibility was calculated based on the number of slides stained in 3 runs per stainer type. Staining runs were performed one per day on 3 separate days using 2 different stainers: BenchMark and BenchMark XT instruments. Inter run reproducibility for the BenchMark XT instrument was 100% (90 of 90 slides, 30 slides for each primary antibody across 3 separate runs) and 100% (60 of 60 slides, 20 slides for each primary antibody across 3 separate runs for each platform) for the BenchMark instrument.
- Inter instrument reproducibility was calculated based on the number of slides stained in 6 runs across all stainer types. Staining runs were performed 1 per day on 3 separate days using 2 different stainers: BenchMark and BenchMark XT instruments. *ultraView* Universal DAB Detection Kit inter instrument reproducibility is 100% (150 of 150 stained slides, evaluated slides included all 3 primary antibodies).

The performance of the *ultraView* Universal DAB Detection Kit was also determined by staining 53 normal and disease state neutral buffer formalin fixed tissues using 20 primary antibodies. The selected antibodies represented species and isotypes commonly used to perform IHC. In 100% of the cases tested (53 of 53 cases stained) *ultraView* Universal DAB Detection Kit recognized the primary antibody and provided the appropriate clinical utility as determined by the qualified slide reader.

TROUBLESHOOTING

- 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.
- 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.
- If all of the paraffin has not been removed, there may be no staining. The deparaffinization procedure should be repeated.
- 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.
- If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged.
- For corrective action, refer to the Staining Procedure section, the instrument Operator's Manual or contact your local support representative.
- If a reagent dispenser does not dispense fluid, check the priming chamber or meniscus for foreign materials or particulates, such as fibers or precipitates. If the dispenser is blocked, do not use the dispenser and contact your local support representative. Otherwise, re-prime the dispenser by aiming the dispenser over a waste container, removing the nozzle cap, and pressing down on the top of the dispenser.

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