

Cell Conditioning Solution (CC1)

Ventana Catalog Number 950-124

INDICATIONS AND USE

Intended Use

This reagent is intended for *in vitro* diagnostic use.

Ventana® Medical Systems' (Ventana) Cell Conditioning Solution (CC1) is a pre diluted solution used as a pretreatment step in the processing of tissue samples for immunohistochemistry (IHC) reactions carried out on Ventana BenchMark® and BenchMark XT automated slide stainers.

Summary and Explanation

CC1 is a tris based buffer which must not be diluted. CC1 is poured into the appropriate position (CC1 bottle) of the automated fluidics module on the automated slide stainer. The instrument applies CC1 automatically as required by the procedure being run on the automated slide staining system.

Principles and Procedures

Fixation of tissue by formalin results in the formation of covalent bonds between the aldehyde and amino groups present in the tissue. The formation of these bonds denatures protein and can result in the loss of antigenicity. In addition, the formaldehyde can form methylene bridges cross linking tissue proteins thus reducing the penetration of the tissue to large molecules such as antibodies.

CC1 is a tris based buffer with a slightly basic pH, which, at elevated temperatures is capable of hydrolyzing the covalent bonds formed by formalin in tissue. Removing these bonds allows renaturation of protein molecules and increases antibody accessibility. Often these changes result in significant gains in antibody binding and improved signal to noise ratios. The automated slide stainer automatically heats the slide to the appropriate temperature and time as selected by the user.

MATERIALS AND METHODS

Reagents Provided

1 - 2 L bottle of CC1; contains a tris based buffer and a preservative.

Reconstitution, Mixing, Dilution, Titration

This reagent is ready to use directly from the bottle and must not be diluted.

Materials and Reagents Needed But Not Provided

The following reagents and materials may be required but are not provided with this kit:

1. Ventana Negative Control Reagent or Rabbit Negative Control
2. Microscope slides, positively charged
3. Positive and negative tissue controls
4. Drying oven capable of maintaining a temperature of $70^{\circ}\text{C} \pm 5^{\circ}\text{C}$
5. Bar code labels (appropriate protocol)
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. BenchMark or BenchMark XT automated slide stainers
13. MIEW™ DAB, AEC, V Red (ALK PHOS) or Enhanced V Red detection kits*
14. Ventana Endogenous Biotin Blocking Kit*
15. Primary Antibody
16. Ventana EZ Prep™ solution*
17. Ventana LCS, Ventana's coverslip solution
18. Ventana Protease I, II or III*
19. Ventana Hematoxylin Counterstain*
20. Ventana Bluing Reagent*
21. Ventana Reaction Buffer
22. Mounting medium
23. Cover glass
24. Light microscope (20-80X)

* As needed for specific applications.

Storage and Handling

Store CC1 at room temperature (15 to 30°C). Keep out of direct sunlight. Do not freeze. The user must validate any storage conditions other than those specified in the package insert.

This reagent 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.

Specimen Collection and Preparation for Analysis

For tissue specimens, routinely processed, formalin fixed, paraffin embedded tissues are suitable for use with this reagent when used with Ventana detection kits 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 $70^{\circ}\text{C} \pm 5^{\circ}\text{C}$ oven.

WARNINGS AND PRECAUTIONS

1. Take reasonable precautions when handling reagents. Use disposable gloves when handling suspected carcinogens or toxic materials.
2. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
3. Do not smoke, eat or drink in areas where specimens or reagents are being handled.
4. Patient specimens and all materials contacting them should be handled as biohazardous materials and disposed of with proper precautions. Never pipette by mouth.
5. Avoid microbial contamination of reagents, as this could produce incorrect results.
6. This reagent may cause skin and eye irritation. It may also be irritating to mucous membranes and upper respiratory tract. An allergic respiratory or skin reaction may be possible in sensitized individuals.
7. CC1 is not flammable.
8. When used according to instructions, this product is not classified as a hazardous substance. The preservative in the reagent is ProClin 5000, containing the active ingredients 5-chloro-2-methyl-4-isothiazine-3-one and 2-methyl-4-isothiazolin-3-one. Symptoms of overexposure to ProClin 5000 include skin and eye irritation, and irritation of mucous membranes and upper respiratory tract. The concentration of ProClin 5000 in this product is 0.05% and does not meet the OSHA criteria for a hazardous substance. Systemic allergic reactions are possible in sensitive individuals.

INSTRUCTIONS FOR USE

CC1 is poured into the appropriate position (CC1 bottle) of the automated fluidics module on the Ventana automated slide stainer. CC1 is applied to tissue specimens following the removal of paraffin and prior to the application of other reagents used in the detection of the target analyte as required by the protocol being run. The instrument applies CC1 automatically. The user is given the option of selecting mild (30 minutes), standard (60 minutes), or extended (90 minutes) cell conditioning.

Prior to initial use of the CC1 in the user's laboratory, appropriate staining should be verified by staining a number of positive and negative tissues with known performance characteristics for antibodies which require antigen enhancement. Assay verification on a daily basis may be accomplished through the proper use of positive and negative controls. Ventana recommends positive controls be placed on the same slide as the patient tissue sample. Users can adjust assay parameters to optimize staining results with the mild, standard, and extended options as well as adjusting the incubation time of the selected primary antibody by adjusting the digestion conditions.

Step by Step Procedure

Ventana reagents have been developed for use on a Ventana automated slide stainers in combination with Ventana detection kits and accessories. Recommended staining protocols for the automated slide stainers are described in the package insert of the primary antibody of interest. 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. For more detailed instructions and additional protocol options refer to your Operator's Manual.

BenchMark or BenchMark XT Automated Slide Stainers

1. Apply slide bar code label, which corresponds to the protocol to be performed.

2. Load the primary antibody, appropriate detection kit and required accessory reagents onto the reagent tray and place them on the automated slide stainer. Check bulk fluids and waste.
3. Load the slides onto the automated slide stainer.
4. Start the staining run.
5. At the completion of the run, remove the slides from the automated slide stainer.
6. 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.
7. 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 to 28° C).

Quality Control Procedures

Positive Tissue Control

A positive tissue control must be run with every staining procedure performed.

The positive staining tissue components are used to confirm that the reagents were 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 should be used 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

For IHC, 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 a staining system in a diagnostic procedure, the specificity of the system should be verified by testing it on a series of samples with known staining performance characteristics representing known positive and negative samples (refer to the Quality Control Procedures previously outlined in this section of the product 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 primary antibody lot, or whenever there is a change in assay parameters.

Interpretation of Results

The Ventana automated staining procedures cause colored reaction products. Refer to the appropriate detection kit package insert for expected color reactions. A qualified pathologist 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. Intensity of the counterstain will be depended on the incubation time selected.

For IHC counterstaining, the incubation length and potency of the hematoxylin used will range from 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.

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.

For IHC tests, 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 sample should also be examined utilizing a hematoxylin and eosin stained section when interpreting any results. The patient's morphologic findings and pertinent clinical data must be interpreted by a qualified pathologist.

LIMITATIONS

General Limitations

1. IHC testing is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, samples, fixation, processing, preparation of the slide, and interpretation of the staining results.
2. For IHC testing, 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), endogenous alkaline phosphatase activity, or endogenous biotin (example: liver, brain, breast, kidney) depending on the type of immunostain used.
3. 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 or false negative results. Inconsistent results may result from variations in fixation and embedding methods, or from inherent irregularities within the tissue.
4. Excessive or incomplete counterstaining may compromise proper interpretation of results.
5. 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 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.
6. Ventana provides 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.
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. Contact your local Ventana office with documented unexpected reactions.

Specific Limitations

1. CC1 must be examined for microbial contamination prior to use. The signs indicating contamination or instability of this product are: turbidity of the solution, odor development or precipitation. At the first sign of possible reagent contamination or instability, call your local Ventana Office.

2. CC1 has been optimally formulated for use on Ventana automated slide stainers. Dilution of this product will result in poor instrument performance and possible loss of staining.

SUMMARY OF EXPECTED RESULTS

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.

1. Cell conditioning solution CC1 is a tris based buffer used as a pretreatment step in the processing of tissue samples for immunohistochemistry or in situ hybridization reactions. This reagent is a stand alone reagent that can not be tested for sensitivity or specificity; it is a qualitative chemical enhancement to the target antigen and may be used as a tool to enhance staining. CC1 was tested with a variety of antibodies representing Breast, Lymphoma, and Leukemia panels at various incubation times with specific tissue types. The antibodies include PR, ER, ki67, p53, CD5, CD3, CD4, CD10, CD79a, Bcl-2, CD15, CD30, Cyclin D1, Synaptophysin, Keratin, CD8, CD4 and Vimentin. The tissue types include Breast Carcinoma, Tonsil, Mantle Cell Lymphoma, Spleen, Adrenal Tumor, Schwannoma, Nerve Sheath Tumor, Colon Carcinoma, Thyroid Carcinoma, Thymus, Lymphoma, and Hodgkins Lymphoma.
2. Inter and intra run reproducibility of staining was determined by taking the difference in staining intensity from each of three slides per case between three runs. The intensity of staining from slide to slide within and between runs when read by three qualified pathologists did not vary by more than one reading grade for all cases.

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. Samples may have been improperly collected, fixed or deparaffinized.
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 intervals of 4 minutes 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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CONTACT INFORMATION

Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, Arizona 85755
USA

+1 520 887 2155

+1 800 227 2155 (USA)



www.ventanamed.com



Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
Germany