Reaction Buffer Concentrate (10X)

Catalog Number 950-300

INDICATIONS AND USE

Intended Use

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

Ventana® Medical Systems' (Ventana) Reaction Buffer Concentrate (10X) is a Tris based buffer solution (pH 7.6 ± 0.2) used to rinse slides between staining steps and provide a stable aqueous environment for the immunohistochemistry (IHC) or *in situ* hybridization (ISH) reactions carried out on BenchMark® and BenchMark XT automated slide staining systems.

Summary and Explanation

Reaction Buffer Concentrate (10X) is a Tris based buffer solution which must be diluted prior to use. Once diluted, Reaction Buffer (1X) solution is poured into the Reaction Buffer instrument bottle and placed in the appropriate position in the automated fluidics module of the automated slide stainer. The instrument applies Reaction Buffer (1X) solution automatically as required by the procedure being run. For additional information refer to the automated slide staining systems Operator's Manual.

Principles and Procedures

Ventana Reaction Buffer is used as a key component in maintaining a proper aqueous environment for many reactions to occur during IHC and ISH, such as general washing, antibody incubation and incubation of enzymes and other ancillaries when used on the automated slide stainer.

In general, ISH staining allows the visualization of target DNA or RNA sequences via the sequential binding of a labeled DNA or RNA probe to the target, a primary antibody against the labeled probe, and a secondary 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 target site.

The IHC reaction involves a specific antibody that is localized by a biotin conjugated secondary antibody formulation that recognizes rabbit and mouse immunoglobins. This step is followed by the addition of an avidin/streptavidin enzyme conjugate that binds to the biotin present on the secondary antibody. The specific antibody, secondary antibody streptavidin, enzyme complex is then visualized with a precipitating enzyme reaction product. Each step is incubated for a precise time and temperature. At the end of each incubation step, the Ventana automated slide stainer washes the sections with Reaction Buffer to stop the reaction and remove unbound material that would hinder the desired reaction in subsequent steps.

MATERIALS AND METHODS

Reagents Provided

1 - 2 L bottle of Reaction Buffer Concentrate (10X); contains a Tris based buffer and preservative.

Reconstitution, Mixing, Dilution, Titration

Reaction Buffer Concentrate (10X) must be diluted with nine parts of distilled or deionized water prior to use on the automated slide stainer. See Instructions for Use section.

Materials and Reagents Needed But Not Provided

1. Ventana Negative Control Reagent or Rabbit Negative Control*
2. Ventana Control Probes*
3. Microscope slides, positively charged
4. Positive and negative tissue controls
5. Drying oven capable of maintaining a temperature of 70° C ± 5° C
6. Bar code labels (appropriate for negative control and primary antibody or probe being tested)
7. 10% neutral buffered formalin
8. Staining jars or baths
9. Timer
10. Xylene
11. Ethanol or reagent alcohol
12. Deionized or distilled water
13. BenchMark or BenchMark XT automated slide stainers
14. Ventana ISH *i*VIEW $_{Blue}$ Detection Kit*
15. Ventana ISH Signal Clarifier*
16. Probe*
17. Ventana ISH Protease 1, 2 or 3*
18. Ventana ISH Red Counterstain*
19. Ventana *i*VIEW™ DAB (preferred), AEC, V Red (ALK PHOS) or Enhanced V Red detection kits*
20. Ventana Endogenous Biotin Blocking Kit*
21. Primary antibody*
22. Ventana EZ Prep solution
23. Ventana LCS, coverslip solution
24. Ventana Cell Conditioning 1 (CC1) or Cell Conditioning 2 (CC2)*
25. Ventana Protease I, II or III*
26. Ventana Hematoxylin counterstain*
27. Ventana Bluing Reagent*
28. Mounting medium
29. Cover glass
30. Light microscope (20-80X)
31. 20 L carboy

* As needed for specific applications.

Storage and Handling

Store concentrated reagent at room temperature (15° to 30° C) out of direct sunlight.

The diluted (1X) reagent may be stored at room temperature (15° to 30° C) out of direct sunlight until ready to use on the instrument. 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

Routinely processed, formalin fixed, paraffin embedded tissues are suitable for use with this reagent 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. 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.

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 has been optimally formulated for a 1:10 dilution. Further dilution may result in poor instrument performance and loss of staining. Any such change must be validated by the user.
7. When used according to instructions, this product is not classified as a hazardous substance. The preservative in the reagent is ProClin 300. Symptoms of overexposure include skin and eye irritation, and irritation of mucous membranes and upper respiratory tract. The concentration of ProClin 300 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.
8. Consult local or state authorities with regard to recommended method of disposal.

INSTRUCTIONS FOR USE

Refer to the automated slide stainer Operator's Manual for more detailed instructions.

To make the diluted solution from the 2 L bottle of Reaction Buffer Concentrate (10X):

1. Be sure the spigot of the 20 L graduated carboy (supplied with the automated slide stainer) is in the OFF position before filling.
2. Fill the empty 20 L carboy approximately 75% full with deionized or distilled water.
3. Pour contents of 2 L Reaction Buffer Concentrate (10X) bottle into the water in the carboy. Swirl to mix.

4. Fill carboy to the 20 L fill line with deionized or distilled water. While adding the water, swirl the solution to ensure that the concentrate becomes fully mixed. If large quantities of bubbles occur during the filling step, allow the wash solution to settle. Once bubbles have settled, add the remaining volume of water necessary to make 20 L of wash solution.
5. Loosely screw the cap on the carboy. If cap is too tight, the solution will not feed properly from the spigot. The wash solution is now ready to use with the Ventana automated slide stainer.

The diluted Reaction Buffer (1X) Solution is poured into the appropriate position (Reaction Buffer bottle) of the automated fluidics module on the Ventana automated slide stainer. The Reaction Buffer (1X) Solution is applied automatically as required by the procedure being run on the Ventana automated slide stainer to rinse slides between staining steps and maintain a stable aqueous environment on the slide.

Prior to initial use of the Reaction Buffer (1X) solution in the user's laboratory, appropriate staining should be verified by staining a number of positive and negative controls. Ventana recommends positive controls be placed on the same slide as the patient tissue sample. Variable results may occur due to tissue fixation. The clinical interpretation of any staining or its absence 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.

Step by Step Procedure

Ventana reagents have been developed for use on a Ventana automated slide staining system 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 or probe 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 the Operator's Manual for the stainer.

BenchMark or BenchMark XT Automated Slide Stainers

1. Apply slide bar code label which corresponds to the antibody or probe protocol to be performed.
2. Load the probe or 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 iVIEW 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 medium 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).
8. For ISH *i*VIEW $_{Blue}$ Detection Kit, wash in a mild dishwashing detergent or alcohol to remove the coverslip solution.
9. Transfer the slides into a bath of distilled water for approximately 1 to 3 minutes. Shake off excess water.
10. View the level of this and all subsequent baths. Make sure that the solutions will completely cover the slides in the rack. Add new reagent to each container in sufficient quantity to cover the slides at all times. Be sure to remove excess fluid after each step.
11. Transfer the slides to a 90% ethanol for approximately 1 to 3 minutes.
12. Transfer the slides to a 100% ethanol for approximately 1 to 3 minutes.
13. Transfer the slides into a second bath of 100% ethanol for approximately 1 to 3 minutes.
14. Transfer the slides into the first xylene bath for approximately 1 to 3 minutes.
15. Transfer the slides into a second xylene bath for approximately 1 to 3 minutes. The slides may be left in this xylene bath until they are cover slipped.
16. Return all covers to dishes and turn off the fume hood

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 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

For immunohistochemistry, 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.

For *in situ* hybridization, a negative control must be run. Ventana recommends following the quality control recommendations of the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist.

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 or probe 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 immunohistochemistry counterstaining, the incubation length and potency of the hematoxylin used will range from a pale to dark blue coloration of cell nuclei.

For *in situ* hybridization counterstaining, the incubation length and potency of the ISH Red used will range from a pale to dark pink 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 control should be examined after the positive control to verify the specificity of the reaction. There should be no specific staining in the negative control. If staining occurs, it may indicate non specific cross reactivity to cells or cellular components. Intact cells should be used for interpretation of staining results since 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.

For immunohistochemical 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 (see Summary of Expected Results section).

For *in situ* hybridization tests, a negative result means that the RNA or DNA sequence targeted was either not detected or the copy number was below the sensitivity level of the kit, not that the sequence is absent in the cells assayed.

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. This product is for *in vitro* diagnostic use.
2. Immunohistochemistry and *in situ* hybridization testing are multiple step diagnostic processes that require specialized training in the selection of the appropriate reagents, samples, fixation, processing, preparation of the slide, and interpretation of the staining results.
3. For immunohistochemistry testing, false positive results may be seen because of non immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), endogenous alkaline phosphatase or endogenous biotin (example: liver, brain, breast, kidney), depending on the type of immunostain used.
4. 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.
5. Excessive or incomplete counterstaining may compromise proper interpretation of results.
6. 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.
7. Ventana provides antibodies, probes, 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.
8. 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. Reaction Buffer Concentrate (10X) Solution 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. This reagent has been optimally formulated for a 1:10 dilution. Further dilution may result in poor instrument performance and loss of staining. Any such change must be validated by the user.

SUMMARY OF EXPECTED RESULTS

1. Expected results are quantitative only when testing the sensitivity and specificity of each specific antigen or target nucleic acid sequence. As a stand alone reagent, this product cannot be tested for specificity or sensitivity.
2. Inter and intra run reproducibilities for 5 lots of Reaction Buffer Solution Concentrate (10X) were tested. 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, the probe 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, the probe or one of the common secondary reagents. Samples may have been improperly collected, fixed or deparaffinized.
3. For IHC, if excessive background staining occurs, high levels of endogenous biotin may be present. A biotin blocking step should be included. For probes, call your local Ventana office.
4. For IHC, 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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3. NCCLS. Quality Assurance for Immunocytochemistry: Approved Guideline. NCCLS document MM4-A- (ISBN 1-56238-396-5). NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 1999.
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