

## 10X SSC

Catalog Number 950-110

### INDICATIONS AND USE

#### Intended Use

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

Ventana Medical Systems' (Ventana®) 10X SSC, Sodium Chloride Sodium Citrate buffer solution is used for stringency washes and to rinse slides between staining steps and provide a stable aqueous environment for the *in situ* hybridization reactions carried out on a Ventana automated slide stainer.

#### Summary and Explanation

10X SSC must be diluted prior to use.

Once diluted, 2X SSC buffer solution is poured into the appropriate position (SSC) of the automated fluidics module on the Ventana automated slide stainer. The instrument applies 2X SSC automatically as required by the procedure being run. For additional information refer to the automated slide stainer Operator's Manual.

#### Principles and Procedures

The reliability of nucleic acid binding is controlled by stringency of the hybridization environment. One of the major controlling factors of stringency is the salt concentration. Salt provides ions that partially mask the negative charge on the phosphate backbone of DNA and RNA. The higher the salt concentration the lower the stringency which translates to less energy required to hold two strands of nucleic acid together. 10X SSC is a sodium citrate, sodium chloride buffer that needs to be used at a final 2X working concentration for hybridization reactions. The Ventana automated slide stainer automatically further dilutes the 2X SSC solution as needed for stringency washes.

Technical Note: 10X SSC comes as a 5X concentrate (10X salt concentrate) which when diluted five fold provides a 2X SSC buffer for hybridization reactions.

### MATERIALS AND METHODS

#### Reagents Provided

One 2 liter bottle of 10X SSC contains sodium chloride, sodium citrate and a preservative.

#### Reconstitution, Mixing, Dilution, Titration

10X SSC must be diluted with four parts of distilled or deionized water prior to use.

1. Pour the entire contents of the 2 liter 10X SSC solution into the 20 liter carboy.
2. Using a 1 or 2 liter graduated cylinder, add 8 liters of deionized or distilled water to the 20 liter carboy. One bottle of 10X SSC provides a final volume of 10 liters.
3. If large quantities of bubbles form during the filling procedure, allow the solution to sit until the bubbles have dissipated.
4. Place the cap on the container, mix the solution thoroughly for 30 minutes. The diluted 2X SSC solution is ready to use on the automated stainer.

#### Materials and Reagents Needed But Not Provided

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

1. Positive and negative tissue controls
2. RNA Positive Control or ISH DNA Negative Control Probe
3. AutoCyte® Prep System specimen collection system, TriPath Imaging, Inc.
4. ThinPrep® Pap Test™ specimen collection system, Cytyc Corporation
5. Microscope slides, positively charged
6. Drying oven capable of maintaining a temperature of 70° C ± 5° C
7. Bar code labels (appropriate for negative control and 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. BenchMark® and BenchMark® XT automated slide stainers
15. ISH /VIEW<sub>Blue</sub>™ solution
16. ISH Signal Clarifier\*
17. Probe
18. EZ Prep™\*

19. LCS, coverslip solution
20. Reaction Buffer
21. Cell Conditioning 1 (CC1) or Cell Conditioning 2 (CC2)\*
22. ISH Protease 1, 2 or 3\*
23. ISH Red Counterstain
24. Mounting medium
25. Cover glass
26. Light microscope (20-80X)
27. 20 L carboy
28. 1 or 2 L graduated cylinder

\* As needed for specific applications.

#### Storage and Handling

Store at room temperature (15° to 30°C) 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.

There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be run simultaneously with unknown specimens. Your local Ventana office should be contacted immediately if there is an indication of reagent instability.

#### Specimen Collection and Preparation for Analysis

For tissue specimens, 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.

For cytology specimens, use either TriPath Imaging, Inc.'s AutoCyte Prep System or Cytyc Corporations' ThinPrep Pap Test specimen collection systems which are collection systems specifically designed for certain cytology specimens. Cytology specimens must be collected and placed in the appropriate preservative and slide preparation must be performed according to the manufactures' instructions.

### 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 1:5 (2X) 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, containing the active ingredients 5-chloro-2-methyl-4-isothiazine-3-one and 2-methyl-4-isothiazolin-3-one. Symptoms of overexposure to ProClin 300 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. This reagent may be harmful if swallowed or inhaled. This reagent may cause short term skin or eye irritation and may be irritating to mucous membranes and the respiratory tract. Inhalation of high vapor or aerosol concentrations may result in headaches, dizziness, anesthesia, drowsiness, unconsciousness and other central nervous system symptoms. OSHA's permissible exposure limit is 5 mg/m<sup>3</sup> (oil mist).
9. 10X SSC is not flammable.

## INSTRUCTIONS FOR USE

The 2X SSC solution is poured into the appropriate position (SSC) of the automated fluidics module on the Ventana automated slide stainer. The 2X SSC solution is applied automatically as required by the procedure being run to rinse slides between staining steps and maintain a stable aqueous environment on the slide.

Prior to initial use of the 2X SSC 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 sample. Variable results may occur due to sample 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 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 your Operator's Manual.

### BenchMark or BenchMark XT Automated Slide Stainers

1. Apply slide bar code label which corresponds to the probe protocol to be performed.
2. Load the probe, 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 ISH MIEW<sup>Blue</sup> detection kit, wash in a mild dishwashing detergent or alcohol to remove the coverslip solution.
7. Transfer the slides into a bath of distilled water for approximately 1 to 3 minutes. Shake off excess water.
8. 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.
9. Transfer the slides to a 90% ethanol for approximately 1 to 3 minutes.
10. Transfer the slides to a 100% ethanol for approximately 1 to 3 minutes.
11. Transfer the slides into a second bath of 100% ethanol for approximately 1 to 3 minutes.
12. Transfer the slides into the first xylene bath for approximately 1 to 3 minutes.
13. 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.
14. Return all covers to dishes and turn off the fume hood.

### Quality Control Procedures

#### Positive Tissue Control

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

The positive staining sample components are used to confirm that the reagents were applied and the instrument functioned properly. This sample may contain both positive and negative staining cells or tissue components and serve as both the positive and negative control. Control samples should be fresh autopsy, biopsy or surgical specimens prepared or fixed as soon as possible in a manner identical to the test sections. Such samples may monitor all steps of the procedure, from sample 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 sample with weak positive staining is more suitable for optimal quality control and for detecting minor levels of reagent degradation.

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

#### Negative Tissue Control

The same sample used for the positive sample control may be used as the negative sample control. The variety of cell types present in most sample 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 sample 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 *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 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 experienced must evaluate positive and negative controls before interpreting results.

#### Positive Tissue Control

The stained positive sample 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 *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.

#### Patient Tissue

Patient specimens should be examined last. Positive staining intensity should be assessed within the context of any background staining. As with any probe test, 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 immunohistochemical, *in situ* or special stains result. The patient's morphologic findings and pertinent clinical data must be interpreted by a qualified pathologist.

## LIMITATIONS

### General Limitations

1. ISH 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. Sample staining is dependent on the handling and processing of the sample prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other samples 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 sample.
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 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 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 samples. The possibility of unexpected reactions even in tested sample groups cannot be completely eliminated because of biological variability of antigen expression in neoplasms, or other pathological samples. Contact your local Ventana office with documented unexpected reactions.

#### Specific Limitations

1. This reagent 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 1:5 (2X) 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

Refer to the appropriate Ventana probe package insert for expected patient sample results. Appropriate sample control results verify the reagents and system are working properly.

#### 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 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 the 10X SSC was too dilute, other reagents in the automated fluidics module may have been transposed or samples may have been improperly collected, fixed or deparaffinized.
3. If excessive background staining occurs, high levels of endogenous biotin may be present. Call your local Ventana office.
4. If all of the paraffin has not been removed, the deparaffinization procedure should be repeated.
5. If specific probe 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 sample 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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Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6<sup>th</sup> edition. (NR Rose Ed.) ASM Press, 2002.

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