FITC Anti-C3 Primary Antibody

Catalog Number 760-2686

INDICATIONS AND USE

Intended Use

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

Ventana® Medical Systems' (Ventana) FITC anti-C3 (complement 3) Primary Antibody is a goat derived polyclonal antibody labeled with fluorescein and specifically directed against human C3. This reagent should be used in conjunction with a panel of antibodies to aid in the identification of complement 3 in C3 target tissue (e.g., in the diagnosis of renal or dermal pathologies). FITC anti-C3 is intended for laboratory use to qualitatively stain sections of frozen tissue on a Ventana automated slide stainer.

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.

Summary and Explanation

Fluorescent antibodies have been used to detect specific antigens in cells or tissue for over 40 years.[1] An informative overview of the use of FITC conjugated antibodies as effective and specific immunofluorescent markers for cellular antigens may be found in Faulk and Humans.[2] Use of the immunofluorescence technique has resulted in an increased overall understanding of renal,[3] and dermal[4] pathologies. The significance of the involvement of C3 in the complement system is reviewed by Ruddy.[5] Human complement C3 has been previously visualized in skin using immunofluorescence.[6] Observation of the presence or absence of C3 in conjugation with immunoglobulins is instrumental in the diagnosis of renal pathologies.[7]

FITC anti-C3 contains a goat polyclonal antibody raised against purified human C3. The antibody is obtained through purification of the goat gamma globulin fraction, followed by reaction with fluorescein isothiocyanate to produce a fluorescein to protein ratio of approximately 3–6 mol/mol. The excess dye is then removed by dialysis and the conjugated globulin is further fractionated on DEAE cellulose to remove over and under labeled protein.[8] Anti-C3 binds specifically with human C3 and exhibits no reactivity with complement components C4-C9 or serum proteins.

Principles and Procedures

FITC anti-C3 may be used as the primary antibody for immunohistochemical staining of frozen tissue sections. 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. For FITC direct labeled antibodies, the fluorochrome is linked to the primary antibody and therefore no secondary antibody or chromogenic detection step is required. The primary antibody binds specifically to the target antigen and can then be visualized. Results are interpreted using a fluorescent microscope with the appropriate filter set and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

FITC anti-C3 is optimally diluted for use with Ventana automated slide stainers. Each step in the staining protocol includes incubation for a precise time at a specific temperature. At the end of each incubation step, the sections are rinsed by the Ventana automated slide stainer to stop the reaction and remove unbound material that would hinder the desired reaction in subsequent steps. To minimize evaporation of the aqueous reagents from the specimen-containing slide a coverslip solution is applied in the slide stainer. For more detailed information on instrument operation, refer to the appropriate Ventana automated slide stainer Operator's Manual.

MATERIALS AND METHODS

Reagents Provided

FITC anti-C3 contains sufficient reagent for 50 tests.

1 – 5 mL dispenser of FITC anti-C3; contains approximately 440 µg (88 µg/mL) of a goat polyclonal antibody directed against human C3. The antibody is diluted in a tris based buffer containing carrier protein and preservative.

Total protein concentration of the reagent is approximately 0.5 mg/mL.

Reconstitution, Mixing, Dilution, Titration

This antibody is optimized for use on a Ventana automated slide stainer. No reconstitution, mixing, dilution, or titration is required.

Further dilution may result in loss of antigen staining. The user must validate any such changes. Differences in tissue processing and technical procedures in the laboratory may produce significant variability in results and require regular use of controls (See 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:

1. Microscope slides, positively charged
2. Positive and negative tissue controls
3. Drying oven capable of maintaining a temperature of 70 °C ± 5 °C
4. Bar code labels (appropriate for negative control and primary antibody being tested)
5. Acetone
6. Staining jars or baths
7. Timer
8. Deionized or distilled water
9. ES®, NexES® IHC, BenchMark® and BenchMark XT automated slide stainers
10. Detection specific software (ES automated slide stainer only)
11. Ventana APK Wash Solution Concentrate (10X) (ES and NexES IHC automated slide stainers)
12. Ventana Low Temperature Liquid Coverslip™ Solution Pre-dilute (ES and NexES IHC automated slide stainers)
13. Ventana Reaction Buffer Solution Concentrate (10X) (BenchMark and BenchMark XT automated slide stainers)
14. Ventana High Temperature Liquid Coverslip Solution Pre-dilute (BenchMark and BenchMark XT automated slide stainers)
15. Aqueous mounting medium, suitable for fluorescence
16. Cover glass
17. Epifluorescence microscope (20-80X) equipped with a FITC filter

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.

To ensure proper reagent delivery and stability of the antibody after every run, the cap must be replaced and the dispenser must be immediately placed in the refrigerator in an upright position.

Every antibody dispenser 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

Routinely processed, frozen tissues are suitable for use with this primary antibody when used with a Ventana automated slide stainer (see Materials and Reagents Needed, But Not Provided section). The recommended tissue fixation is 10 minutes in cold acetone. 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.

WARNINGS AND PRECAUTIONS

1. Take reasonable precautions when handling reagents. Use disposable gloves when handling suspected carcinogens or toxic materials (example: xylene or formaldehyde).
2. Do not smoke, eat or drink in areas where specimens or reagents are being handled.
3. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
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. Incubation times and temperatures other than those specified may give erroneous results. The user must validate any such change.
7. The reagents have been optimally diluted, and further dilution may result in loss of antigen staining. The user must validate any such change.
8. 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 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.
9. Consult local or state authorities with regard to recommended method of disposal.

INSTRUCTIONS FOR USE

Step by Step Procedure

Ventana primary antibodies 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 listed below in Table 1. 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.

Table 1. Recommended Staining Protocols for FITC anti-C3

Procedure Type	Platform/Method	
	ES or NexES IHC	BenchMark or BenchMark XT
Protocol Selection	Frozen	Frozen
Deparaffinization	N/A	N/A
Cell Conditioning (Antigen Unmasking)	None Required	None Required
Enzyme (Protease)	None Required	None Required
Antibody (Primary)	FITC anti-C3, 8 minutes	FITC anti-C3, 8 minutes
Counterstain	N/A	N/A

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.

For All Instruments

1. Apply slide bar code label which corresponds to the antibody protocol to be performed.
2. Load the primary antibody onto the reagent tray and place 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 FITC chromogen, do not dehydrate and clear. Mount the FITC primary antibody stained slides with aqueous mounting medium. Efficient removal of the Liquid Coverslip Solution from the slides following removal from the instrument will greatly reduce background autofluorescence. To accomplish this, rinse the slides thoroughly in APK Wash or Reaction Buffer. The slides may then be rinsed in distilled water and coverslipped. The stained slides should be read the same day as staining, and should be stored in the dark in a cold environment (–20 °C). Slides stained with the FITC primary antibodies are not stable, but staining can be preserved if slides are stored at –20 °C or –80 °C in the dark. Slides stained with the FITC primary antibodies can quench over time or with prolonged light exposure. Avoid exposure to light.

Quality Control Procedures

Positive Tissue Control

A positive tissue control must be run with every staining procedure performed. An example of a positive control with FITC anti-C3 kidney or skin from Lupus cases containing C3 deposits. 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.

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.

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 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,[9] and the NCCLS Approved Guideline[10]). 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 Summary of Expected Results section are suitable for assay verification.

Interpretation of Results

The Ventana automated immunostaining procedure causes the target antigen to be visualized with the tagged primary antibody and linked fluorochrome. FITC labeled antibodies give an apple green colored reaction product. 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. 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 appear bright yellow to greenish in color. Sporadic light staining of connective tissue may also be observed in sections from excessively fixed tissues. Autofluorescense may be present in stained tissue specimens. 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 reactions (see Summary of Expected Results section). 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. 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. 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.
4. 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.
5. This product is not intended for use in flow cytometry, performance characteristics have not been determined.
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.[11] Contact your local Ventana office with documented unexpected reactions.
7. False positive results may be seen because of nonimmunological binding of proteins.
8. 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

1. The antibody has been optimized for an 8 minute incubation time in combination with Ventana automated slide stainers. Because of variation in tissue fixation, it may be necessary to increase or decrease the primary antibody incubation time on individual specimens. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".[12]
2. The antibody detects antigen that survives routine fixation and sectioning. Users who deviate from recommended test procedures are responsible for interpretation and validation of patient results.

SUMMARY OF EXPECTED RESULTS

1. Specificity of FITC anti-C3 was determined by a study that showed appropriate staining of normal and diseased tissues. Ten skin biopsies and 5 diseased kidney samples were stained with FITC anti-C3. The 15 clinical samples gave expected clinical results. Eleven of the 15 tissues stained gave positive staining outcomes. A variety of normal tissues were stained with FITC anti-C3 and the following tissues tested negative: lung, brain, thyroid, diaphragm, and uterus. For both nerve and small intestine, 3 out of 3 cases were negative, but exhibited some nonspecific background staining.
2. Sensitivity is dependent upon the preservation of the antigen. Any improper tissue handling during fixation, sectioning, embedding or storage which alters antigenicity weakens C3 detection by FITC anti-C3 and may generate false negative results.
3. Intra run reproducibility of staining with FITC anti-C3 was determined by staining 5 slides containing the same tissue on the same instrument run. Five of 5 slides stained positively. All slides stained with the same staining intensity. Users should verify within run reproducibility results by staining several sets of serial sections with low, medium and high antigen density in a single run.
4. Inter run reproducibility of staining with FITC anti-C3 was determined by staining slides containing the same tissue on 5 different instrument runs. Five of 5 slides stained positively. All slides stained with similar staining intensity. Users should verify between run reproducibility results by staining several sets of serial sections with low, medium and high antigen density on different days.

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 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.
4. If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged.
5. For corrective action, refer to the Step By Step Procedure section, the automated slide stainer Operator's Manual or contact your local Ventana office.

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