FITC Anti-C1q Primary Antibody

REF 760-2688

05267994001

IVD Σ 50

INTENDED USE

FITC Anti-C1q Primary Antibody is a polyclonal antibody labeled with fluorescein. It is intended for laboratory use in the qualitative immunofluorescent detection of C1q by fluorescence microscopy in sections of frozen tissue stained on a BenchMark IHC/ISH instrument.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

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

SUMMARY AND EXPLANATION

Immune complexes (ICs) are macromolecules consisting of antibodies bound to different antigens.[1,2] ICs are formed as part of the normal immune response and can accumulate if not efficiently cleared by normal cellular mechanisms.[2] Excess ICs can circulate in body fluids or deposit in various tissues resulting in inflammation and tissue damage at a local or systemic level.[1,2] The kidneys remove toxic metabolic waste products from blood and some renal diseases are characterized by the deposition of ICs which may contain immunoglobulins (Igs).[2]

C1q (complement 1q) is a hexavalent molecule that when bound to either antibody or antigen, initiates the complement cascade.[3,4] It is a pattern recognition molecule that can identify and bind to various structures and ligands.[5]

Abnormal amounts of ICs and depositions of ICs in tissues and organs can be detected in certain diseases.[2] Renal conditions, including, but not limited to, glomerulonephritis, may be characterized by deposits of ICs formed by various Igs and complement proteins.[6] Collectively, immune-mediated renal diseases, including glomerulonephritis, are a broad group of conditions where a dysregulated autoimmune process is the predominant driving force behind the onset of renal inflammation and injury.[6-8] These diseases may be characterized by IC deposits composed of one or more of the following components: Igs (e.g., IgG, IgM, IgA), kappa and lambda light chains, fibrinogen, C3 and/or C1q.[6-8]

FITC anti-C1q Primary Antibody may be used to aid the pathologist in the identification of immune complex deposition associated with various renal pathologies.

PRINCIPLE OF THE PROCEDURE

FITC Anti-C1q Primary Antibody binds to human C1q in frozen tissue sections. In general, immunohistochemical staining allows the visualization of antigens via the sequential application of a specific antibody and various detection components, where enzymatic activation of a chromogen results in a visible reaction product at the antigen site. For FITC-labeled antibodies, the fluorochrome is linked directly to the primary antibody, and therefore no additional detection cascade 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.

MATERIAL PROVIDED

FITC Anti-C1q Primary Antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of FITC Anti-C1q Primary Antibody contains approximately 333 µg of a FITC-labeled goat polyclonal antibody.

The antibody is diluted in Tris-HCl buffer with carrier protein and 0.10% ProClin 300, a preservative.

Specific antibody concentration is approximately 66.6 µg/mL.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
4. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
5. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
6. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
7. General purpose laboratory equipment
8. BenchMark IHC/ISH instrument
9. Aqueous mounting media, suitable for Flourescence
10. Cover glass
11. Epifluorescence microscope (20-80X) equipped with a FITC filter

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser 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.

SPECIMEN PREPARATION

Routinely processed, frozen tissues are suitable for use with this primary antibody when used with BenchMark IHC/ISH instruments. The recommended tissue fixative 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.

Storage of frozen tissues at -80°C for extended periods of time, up to 5 years, preserves protein quality.[9]

Each sections should be cut at approximately 4 µm thickness and mounted on a positively charged slide.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. CAUTION: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. ProClin 300 solution is used as a preservative in this reagent. 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.
6. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
7. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[10,11]
8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
11. Consult local and/or state authorities with regard to recommended method of disposal.
12. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Warning 	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P261	Avoid breathing mist or vapours.
	P273	Avoid release to the environment.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362 + P364	Take off contaminated clothing and wash it before reuse.
	P501	Dispose of contents/ container to an approved waste disposal plant.

This product contains CAS # 55965-84-9, reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

STAINING PROCEDURE

VENTANA FITC-labeled primary antibodies have been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA accessories. Refer to the Table 2 below for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 760-2688.

Table 2. Recommended Staining Protocols for FITC Anti-C1q Primary Antibody on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	XT	ULTRA or ULTRA PLUS[a]
Fluorescent Antibody	Selected, 8 minutes	Selected, 8 minutes

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation based on individual specimens used and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances."[12]

At the completion of the run, remove the slides from the BenchMark IHC/ISH instrument. 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 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 or -80° C). Slides stained with the FITC primary antibodies can quench over time or with prolonged light exposure. Avoid exposure to light.

POSITIVE TISSUE CONTROL

A tissue control must be included with each staining run. Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections.

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test specimen should be considered invalid.

An examples of positive control tissue for FITC Anti-C1q antibody is diseased renal tissue.

STAINING INTERPRETATION / EXPECTED RESULTS

The Ventana automated immunostaining procedure causes the target antigen to be visualized with the tagged primary antibody and linked fluorochrome. 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. Autofluorescence may be present in stained tissue specimens.

A qualified pathologist experienced in immunofluorescent procedures must evaluate positive and negative controls before interpreting results.

SPECIFIC LIMITATIONS

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below

Sensitivity and Specificity

The tables below indicate the reactivity of the product on various normal and neoplastic frozen tissue, as well as renal pathology (kidney lupus) tissues. In renal tissue, positive C1q status is suggestive of, but not confirmatory evidence of, the presence of IC deposits.

Table 3. Sensitivity/Specificity of FITC Anti-C1q Primary Antibody was determined by testing frozen normal and indication pathology tissues

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum (brain)	0/4	Myeloid (bone marrow)	0/3
Cerebellum	0/3	Lung	0/4
Adrenal gland	0/3	Heart	0/3
Ovary	0/4	Esophagus	0/3
Pancreas	0/4	Stomach	1/4
Placenta	0/3	Small intestine	1/1
Pituitary gland	0/3	Colon	0/4
Testis	0/3	Liver	0/4
Thyroid	0/3	Ureter	0/3
Breast	0/4	Kidney	0/4
Spleen	0/3	Kidney (Lupus)	5/5
Uterus	0/4	Prostate	3/4
Striated muscle	0/3	Cervix	0/3
Muscle	0/1	Skin	0/4
Spinal Cord	0/3	Lymph Nodes	0/3
Blood vessel (artery)	0/3	Fallopian Tube	0/3
Thymus	0/3		

Table 4. Sensitivity/Specificity of FITC Anti-C1q Primary Antibody was determined by testing a variety of frozen neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/1
Adenocarcinoma (Ovary)	0/1
Adenocarcinoma (Pancreas)	0/1
Invasive ductal carcinoma (Breast)	0/1
Adenocarcinoma (Lung)	0/1
Adenocarcinoma (Stomach)	0/1
Non-Hodgkin Lymphoma (Small Intestine)	0/1
Adenocarcinoma (Colorectal)	0/1
Adenocarcinoma (Liver)	0/1
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	0/1
Adenocarcinoma (Uterus)	0/1
Melanoma (Skin)	0/1
Leiomyosarcoma (Smooth muscle)	0/1

Precision

Precision studies for FITC Anti-C1q Primary Antibody were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark ULTRA instrument.
- Between instrument precision on the BenchMark XT and BenchMark ULTRA instruments.
- Between platform precision between the BenchMark XT and BenchMark ULTRA instruments.

All studies met their acceptance criteria.

Precision on the BenchMark ULTRA PLUS instrument was demonstrated using representative assays. Studies included Within-run Repeatability, Between-day and Between-run Intermediate Precision. All studies met their acceptance criteria.

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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for more information).

GTIN — Global Trade Item Number

UDI — Unique Device Identification

Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
E	Updates Specimen Preparation, and Staining Procedure sections.

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