C4c FITC

In Vitro Diagnostic Use (IVD)

Product identification

C-FC043-10	1 ml	Concentrate
C-FC043-20	2 ml	Concentrate
P-FC043-30	3 ml	Ready-to-use
P-FC043-70	7 ml	Ready-to-use

Intended use

C4c-FITC is a polyclonal anti-human rabbit antibody coupled with the fluorescent dye fluorescein isothiocyanate (FITC). It is intended for immunohistochemical applications and other immunofluorescence techniques.
The detection with the antibody must be performed by qualified personnel only. The results must be evaluated by qualified pathologists, taking into account the patient's medical history and other diagnostic tests.

Summary and explanation

C4 and its fission products C4b and C4c are components of the complement system of plasma proteins. They serve to defend against infections by eliminating cellular antigens (e.g. bacteria).
The classical activation can be triggered by DNA, collagen and CRP (C-reactive protein), among others.

After its activation, the serine protease C1s catalyzes both start reactions of the classical pathway. One cleaves C2 into C2a and C2b and another cleaves C4 into C4a and C4b. C2a and C4b combine to form the C4b2a complex and thus form the "C3 convertase of the classical pathway". C4b2a3b forms the C5-convertase, which cleaves C5 into C5a and C5b.

Principle of the procedure

The antibody is intended for the qualitative detection of human C4, C4b and C4c complement in tissue, but not C4d. Within the procedure, exact incubation times and temperatures must be observed and washing steps performed. Finally, the result can be evaluated under the fluorescence microscope.
The antibody can also be used for other immunofluorescence techniques.

Materials provided

Primary antibody	Anti-C4c
Host	Rabbit
Clone	Polyclonal
Immunogen	Human C4c
Antibody concentrate	Concentrated antibody in TRIS (pH 7.4) with < 0.1% sodium azide and sera
Recommended working dilution range	1:20
Ready-to-use antibody	Prediluted antibody in TRIS (pH 7.4) with < 0.1% sodium azide and sera
molar FITC/Protein-ratio	2.5

Product label shows the specific lot number.

Prediluted antibody is ready-to-use and optimized for staining. No further dilution, reconstitution, mixing, or titration is needed.

Antibody concentrate is optimized for dilution within dilution range using Q Diluent for IHC (Cat. No. AD-001-xxxx). Indicated dilution range should be considered as recommendation and depends on different factors (tissue, fixation, incubation conditions, etc.). Optimum titration to be determined in the user's own system.

Materials required but not provided

- Positive and negative controls
- Microscope slides (positively charged) and cover slips
- Staining jars
- Timer
- Xylene or xylene alternative, e.g. Q Dewax Solution (Cat. No. DW-001-xxxx)
- Ethanol
- Deionized or distilled water
- Heating equipment for tissue pretreatment step
- Antibody diluent, e.g. Q Diluent for IHC (Cat. No. AD-001-xxxx)
- Antigen retrieval reagent, e.g. Q Retrieval Low pH 6.0 (Cat. No. AR-001-0120) or Q Retrieval High pH 9.0 (Cat. No. AR-002-0120)
- Detection system, e.g. PolyQ Stain kits and appropriate chromogen
- Wash buffer: TBS (Cat. No. BU-006-xxxx) or TBS-Tween20 (Cat. No. BU-007-xxxx)
- Blocking reagent
- Hematoxylin
- Mounting medium
- Light microscope

Storage and handling

Store in the dark at 2 – 8 °C.

When stored correctly, the antibody is stable to the expiration date indicated on the vial. Do not use after expiration date.
To ensure proper reagent delivery and stability of the antibody, replace the dispenser cap after every use and immediately place the bottle into the fridge in an upright position.

Specimen preparation

Routinely processed, FFPE tissues are suitable for use with this primary antibody when used with PolyQ Stain detection kits (see section "Materials required but not provided"). 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. Thickness of tissue sections should be 2 – 5 µm. Slides should be stained as soon as possible, as antigenicity of cut tissue sections may diminish over time.
It is recommended to stain positive and negative controls simultaneously with unknown specimens.
The optimum pretreatment protocol must be determined in the user's own system.

Warnings and precautions

1. Authorized and skilled personnel may only use the product.
2. There are no estimated health risks, if the product is used as directed. MSDS is available on request.
3. Product contains sodium azide as preservative. Pure sodium azide is toxic. The concentration of sodium azide in this reagent is < 0.1 % which is not classified as hazardous.
4. Do not use reagents after expiration date.
5. Take reasonable precautions when handling reagents. Use protective clothing and gloves.

6. All hazardous materials should be disposed according to guidelines for hazardous waste disposal. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
7. Avoid microbial contamination of reagents as it may cause incorrect results.

Quality control procedures

Positive tissue control
A positive tissue control must be run with every staining procedure performed for monitoring the correct performance of processed tissues and test reagents.
If the positive tissue controls fail to demonstrate appropriate positive staining, results with the test specimens must be considered invalid.

Negative tissue control
Negative tissue controls provide an indication of non-specific background staining. If a strong staining provides the impression of a specific staining, results with the patient specimens must be considered invalid.

Discrepancies
If quality control results do not meet specifications, patient results are invalid. Identify and correct the problem, then repeat the entire procedure with the patient samples.

Negative control reagent
A negative control reagent is used in place of the primary antibody to evaluate non-specific staining. Host species and incubation time should be similar to primary antibody.

Interpretation of results

At the end of the procedure, there is a fluorescence-labeled area at the antigen site localized by the antibody.

A qualified pathologist experienced in immunohistochemistry procedures must evaluate positive and negative tissue controls before interpreting patient specimens.
Positive staining intensity should be assessed within the context of any background staining of the negative reagent control.
Note: A negative result means that the antigen in question was not detected, not that the antigen is absent in the cells or tissue assayed. A panel of antibodies may be used to verify the results. Additionally, the morphology of each tissue sample should be examined utilizing a hematoxylin and eosin stained section. A qualified pathologist must interpret the patient's morphologic findings and pertinent clinical data.

Troubleshooting

1. Only intact cells should be used for interpretation of staining results, as degenerated cells show non-specific staining.
2. If no staining occurs, control application order of reagents. Follow all indications given in the instructions for use.
3. Do not allow the sections to dry out.
4. If weak staining occurs, pay attention during staining steps to freshly prepared chromogen, incubation times and temperatures, as well as accurate draining off of reagents.
5. Avoid surplus background staining by optimal removal of paraffin, washing of slides and dilution of primary antibody. If excessive background staining occurs, high levels of endogenous biotin may be present (unless a biotin-free detection system is being used). A biotin blocking step should be included.
6. Sodium azide inactivates HRP, which may lead to false results. Wash sections in sodium azide free buffer.
7. Contact quartett customer service in case of any uncertainties.

Limitations

1. Errors excepted. This data sheet contains general information.
2. For *in vitro* diagnostic use.
3. For laboratory use only.
4. This reagent is "for professional use only" as immunohistochemistry is a multiple step process that requires specialized training in the selection of the appropriate reagents, tissues, fixation and processing, preparation of the immunohistochemistry slide, choice of detection system, and interpretation of the staining results.
5. Tissue staining is dependent on the handling, processing and storage 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 incorrect results. Optimal performance requires adequate specimen quality as well as appropriate sample preparation.
6. Excessive or incomplete counterstaining may compromise proper interpretation of results.
7. False positive results may be seen because of non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudo peroxidase activity (erythrocytes), endogenous biotin (example: liver, brain, kidney) or endogenous peroxidase activity (cytochrome C).
8. When used in blocking steps, normal sera from the same animal source as the secondary antisera may cause false negative or false positive results because of the effect of autoantibodies or natural antibodies.
9. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen may exhibit nonspecific staining with HRP.
10. Unexpected results may occur due to biological variability of antigen expression in neoplasms or other pathological tissues.
11. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic laboratory test results. Staining must be performed in a certified, licensed laboratory under the supervision of a qualified pathologist who is responsible for evaluation and assuring the adequacy of positive and negative controls. Manufacturer is not liable for incorrect results due to visual evaluation.
12. Prediluted antibodies are ready-to-use and optimized for staining. Further dilution may lead to incorrect results.
13. After successful validation users may dilute antibody concentrates according to requirements. Appropriate controls must be employed and documented.
14. The performance of the product was established using the procedures provided in this package insert only and modifications to these procedures may lead to changes in efficiency. Non-application as prescribed in this data sheet leads to loss of all liability. Any changes in product, composition, implementation, as well as use in combination with any reagents other than recommended herein is not allowed; users are responsible themselves for those changes and have to perform prior validation.
15. Application in combination with diagnostic devices requires prior validation.
16. We do not take responsibility for any possible damage including personal injury, time or effort on economic loss caused by this product. Our warranty is limited to the price paid for the product.

References

[1] Collins AB, Colvin RB, Nousari CH and Anhalt GJ. Immunofluorescence Methods in the Diagnosis of Renal and Skin Diseases. Manual of molecular and clinical laboratory immunology 2006 7th edition.

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Explanation of symbols

REF	Bestellnummer Catalog number		Verwendbar bis Use by
LOT	Chargenbezeichnung Batch code	2 °C 8 °C	Temperaturbegrenzung Temperature limitation
IVD	In Vitro Diagnostika In vitro diagnostic agent		Gebrauchsanweisung beachten Consult instructions for use
	Hersteller Manufacturer		