Biomatik
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INSTRUCTION MANUAL

Last Updated: Aug 2026

Human C4d (Complement Fragment 4d) Micro-Volume ELISA Kit

Catalog No.: EKR71566

This ELISA kit applies to the in vitro quantitative determination of Human C4d concentrations in serum, plasma, and other biological fluids. The hard copy manual provided with this kit must be read completely and followed strictly before kit use.

About Biomatik

Founded in 2002, Biomatik provides a comprehensive range of integrated products and custom services designed for life science research and drug discovery. To date, we have proudly served over 20,000 unique researchers worldwide, delivering 72,000+ custom-made products — including genes, peptides, proteins, and antibodies — in addition to our extensive catalog of ELISA kits, antibodies, proteins, and biochemicals. With over 6,000 citations in peer-reviewed publications and hundreds of 5-star Google reviews, Biomatik is a trusted partner in the life science and drug discovery community.

This is a sample manual for reference only.

Always use the hard copy manual included with the kit for your assay.

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Principle of the Procedure

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human C4d. Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody and biotinylated detection antibody specific for Human C4d. Then Avidin-Horseradish Peroxidase (HRP) conjugate is added successively to each microplate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human C4d, biotinylated detection antibody, and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a stop solution, and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of Human C4d. You can calculate the concentration of Human C4d in the samples by comparing the OD of the samples to the standard curve.

Performance

- ★ Sensitivity: 0.23ng/mL.
- ★ Detection Range: 0.63-40ng/mL.
- ★ No significant cross-reactivity or interference between Human C4d and analogues was observed.
- ★ Repeatability: The coefficient of variation is 10%.

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Limitations of the Procedure

1. This kit is for laboratory scientific research only. We will not be responsible for any consequences if this kit is used for clinical diagnosis or any other procedures.
2. Due to the uncertainty of its validity, this kit may not be suitable for testing some special experimental samples, such as gene knockout experiments.
3. This kit should be used before its expiration date, and please strictly follow the instructions for storage.
4. Different manufacturers' kits or testing the same analyte by other methods may produce inconsistent results because we do not compare our products with those of other manufacturers.
5. Since the antibodies used in the kit are usually prepared from recombinant proteins as immunogens, and recombinant proteins can be limited by different fragmentation, expression, and purification systems, it is not recommended to use this kit to detect recombinant proteins.
6. In order to get the best experimental results, please use only the reagents provided by the manufacturer, and do not mix reagents from different batches.
7. Due to the existing conditions and the limitations of science and technology, we cannot fully identify and analyze the raw materials provided by the supplier comprehensively. Therefore, the kit may have some quality and technical risks.
8. The possibility of interference cannot be excluded before all factors are tested in the ELISA immunoassay.
9. In order to obtain reproducible results, each step in the experiment should be controlled, and variations in sample collection, handling, and storage may also lead to differences in sample measurements.
10. Although each kit passes rigorous quality testing, differences in measured values between batches of kits can still be caused by factors such as shipping conditions and different laboratory equipment.

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Kit Components & Storage

An unopened kit can be stored at 2-8℃ for 24 months. If the opened kit is not used up, store the items separately according to the following conditions.

Item	Specifications	Storage
Micro ELISA Plate	96T: 8 wells×12 strips 48T: 8 wells×6 strips 24T: 8 wells×3 strips	2-8℃, 24 months
Reference Standard	96T: 2 vials 48T: 1 vial 24T: 1 vial	
Concentrated Biotinylated Detection Ab (100X)	96T:1 vial,120μL 48T:1 vial, 60μL 24T:1 vial, 60μL	
Concentrated HRP Conjugate (100X)	96T:1 vial, 120μL 48T:1 vial, 60μL 24T:1 vial, 60μL	
Reference Standard & Sample Diluent	1 vial, 20 mL	2-8℃, 24 months
Biotinylated Detection Ab Diluent	1 vial, 13 mL	
HRP Conjugate Diluent	1 vial, 13 mL	
Concentrated Wash Buffer (25X)	1 vial, 30 mL	
Substrate Reagent	1 vial, 10 mL	2-8℃ (Protect from light)
Stop Solution	1 vial, 10 mL	2-8℃, 24 months
Plate Sealer	5 pieces	RT
Product Description	1 copy	
Certificate of Analysis	1 copy	

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Materials & Equipment Required But Not Provided

Microplate reader with 450nm wavelength filter

Incubator capable of maintaining 37℃

Single- or multi-channel pipettes with high precision

disposable pipette tips

EP tubes

Container for Wash Solution

Squirt bottle, manifold dispenser, or automated microplate washer

Deionized or distilled water

Absorbent paper for blotting the microplate

Note

1. The detection range of the kit is different from the concentration range of the substance to be measured in the sample. It is recommended to estimate the concentration by looking up the reference before the experiment and determining the actual concentration by pre-experiment. If the concentration is too high or too low, dilute or concentrate the sample appropriately.

2. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

3. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

4. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatants, there is a possibility of causing a deviation due to the introduced chemical substance.

5. Samples should be used within 6 days when stored at 2-8℃, otherwise samples must be stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze-thaw cycles.

6. With the standard product concentration as the horizontal coordinate and the OD value as the vertical coordinate, the standard curve is formed by using the nonlinear four-parameter logistic (4-PL) model, and the sample concentration is calculated by the curve.

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Sample Collection

Serum: Allow blood samples to clot for 2 hours at room temperature or overnight at 2-8℃ before centrifugation for 15 min at 2000×g at 2-8℃. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 2000×g at 2-8℃ within 30 min of collection. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.

Tissue homogenates: Tissues should be rinsed in ice-cold PBS to remove excess blood thoroughly, weighed, and minced into small pieces. Then, tissue pieces were homogenized in PBS (tissue weight (g): PBS (mL) volume = 1:9) with a glass homogenizer on ice. The resulting suspension is sonicated with an ultrasonic cell disrupter till the solution is clarified. The homogenates are then centrifuged for 5 min at 10000×g. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.

Cell lysates: For adherent cells, gently wash the cells with a moderate amount of cool PBS and detach the cells with trypsin. Collect cells by centrifugation for 5 min at 1500×g (suspension cells can be collected by centrifugation directly). Discard the supernatant and wash cells 3 times with cool PBS. Resuspend cells in cool PBS at a concentration of 5×10^6 cells/mL. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 15 min at 2000×g at 2-8℃. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.

Cell culture supernatant and other biological fluids: Centrifuge samples for 15 min at 1500× g at 2-8℃. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.

Reagent Preparation

1. Bring all reagents to room temperature (18-25℃) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for the present experiment, and store the remaining strips and reagents under the required conditions.

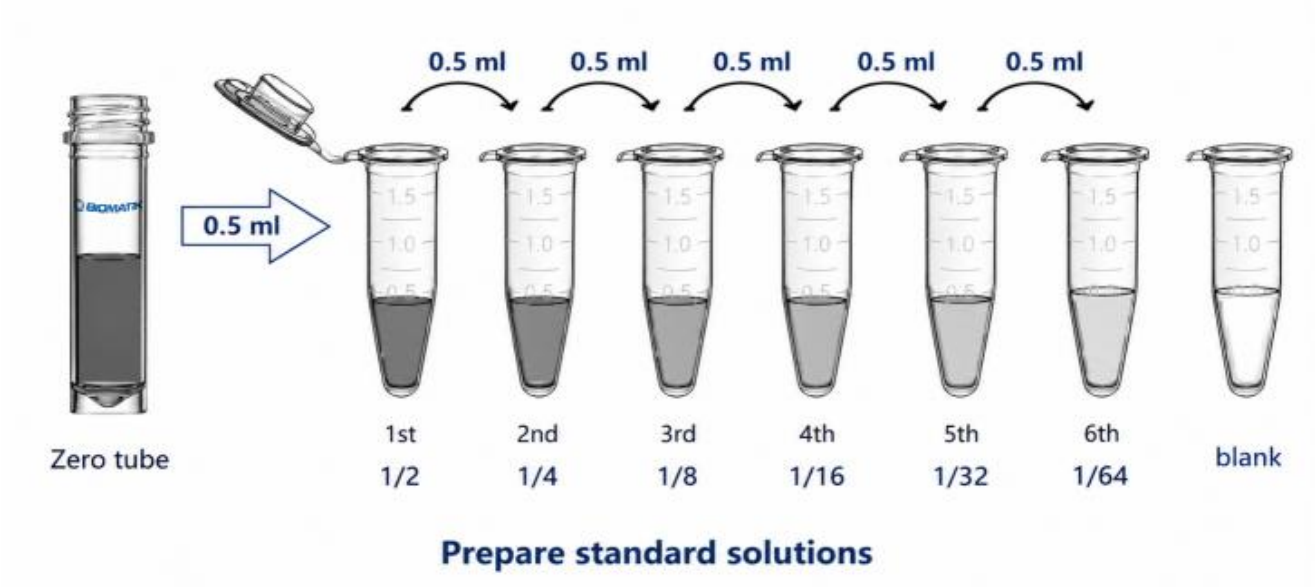
2. **Wash Buffer:** Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: If crystals have formed in the concentrate, warm it in a 40 ℃ water bath and mix it gently until the crystals have completely dissolved.

3. **Standard working solution:**

① Centrifuge the Reference Standard at 10000×g for 1 min.

② Add 1 mL of Reference Standard & Sample Diluent, let it stand for 10 min, and then mix gently; that is 40ng/mL of standard working solution.

③ Prepare 7 EP tubes, add 500μL standard & sample diluent to each tube, take 500μL from 40ng/mL to make 20ng/mL, and so on, the last tube is blank (no more liquid needs to be drawn from the penultimate tube). Use it now!



40	20	10	5	2.5	1.25	0.63	0
ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL

4. **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (75μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before

use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent.

5. **HRP Conjugate working solution:** Calculate the required amount before the experiment (100μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated HRP Conjugate to 1× working solution with HRP Conjugate Diluent.

6. Experimental Operation Tips

① Solutions should be added to the bottom of the micro ELISA plate well, avoiding touching the inside wall and causing foaming as much as possible.

② Make the tested strips usable immediately after the wash step. Do not allow wells to dry.

③ After adding the Substrate Reagent. The reaction time can be shortened or extended according to the actual color change, but not more than 30 min.

④ Adding the stop solution should be done in the same order as the substrate solution.

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Assay Protocol

1. Determine wells for the diluted standard, blank, and sample. Add 25 μL each dilution of standard, blank, and sample into the appropriate wells. Immediately add 75μL of Biotinylated Detection Ab working solution to each well.

2. Cover the plate with a new sealer. Incubate for 90 min at 37°C. Decant the solution from each well and pat it dry against clean absorbent paper.

3. Add 300μL of wash buffer to each well. Soak for 0.5 min and aspirate or decant the solution from each well, and pat it dry against clean absorbent paper. Repeat this wash step 3 times.

4. Add 100μL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 60 min at 37℃.

5. Decant the solution from each well, repeat the wash process for 3 times as conducted in step 3.

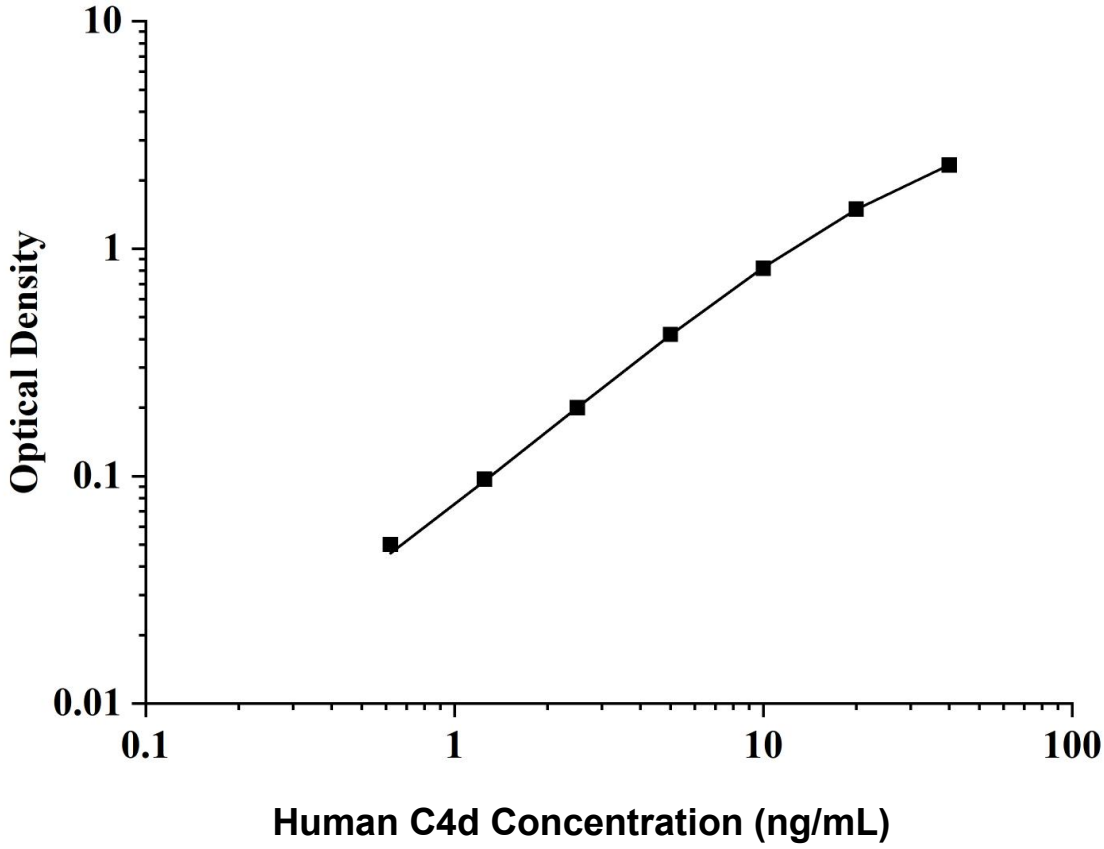
6. Add 100μL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light.

7. Add 50μL of Stop Solution to each well.

8. Determine the optical density of each well at once with a microplate reader set to 450 nm.

Example Data

For each test, a standard curve must be set for each plate. The standard curves below are for example purposes only.



Sensitivity

The minimum detection concentration of the Human C4d detected by this kit is usually less than the 0.23ng/mL. The sensitivity was determined by adding two standard deviations to the mean OD value of twenty zero standard replicates and calculating the corresponding concentration.

Precision

Mean coefficient of variation for Intra-Assay and Inter-Assay: 3 samples with low, medium, and high concentrations were tested multiple times. The results showed that the coefficient of variation of the kits was less than 10%, which met the precision quality control standard.

Specificity

This method has high sensitivity and specificity for the detection of Human C4d. No significant cross-reactivity was observed between the Human C4d and its analogues.

Recovery

The known concentration of Human C4d was added to different samples respectively, and the recovery experiment was conducted. The results showed that the recovery range and average recovery rate of the kit were 80-120%, which met the recovery quality control standard.

Linearity

Dilute linear experiments were performed on samples with Human C4d to evaluate the linearity of the kit. The results showed that the kit's linear range (%) was 80-120%, which met the linear quality control standard.

Troubleshooting & Solutions

Problem	Possible Causes	Subsequent Actions
Poor standard curve	Improper standard dilution	Ensure that standards are dissolved and diluted in the recommended manner
	Inaccurate pipetting	Periodically calibrate pipettes and check the pipette tips
	Evaporate the reaction solution	Seal the enzyme plate with a plate sealer
	Incomplete plate washing	Adequate washing times and the amount of washing solution added
	Foreign matter in the bottom of wells	Clean the bottom of the plate before reading
Weak or no color development	Insufficient reaction of reagents	Ensure incubation time and incubate at the recommended temperature
	Inadequate reagent volumes	Check the pipette and follow the steps strictly to operate
	Improper dilution	Check the reagent dilution process
	Inactivation of enzyme conjugate	Mix conjugate and substrate, check for color development
	No stop solution added	Add an appropriate amount of stop solution
	Waiting too long to read	Read the plate on time
High background	Contaminated chromogenic solution	Replace the chromogenic solution
	Coloring time is too long	Control the coloring time
	Wrong dilution of the reaction reagent	Use the recommended dilution
	Inadequate washing of the plate	Adequate washing times and the amount of washing solution added