ABIN6975325

Competition ELISA Kit for Complement 4

Dog

96 tests

For research use only
Not for use in clinical diagnostic procedures
Version Aug 2024

Intended use

For the quantitative determination of dog complement 4 (C4) concentrations in serum, plasma, tissue homogenates.

Reagents and materials provided

- Assay plate
- Standard
- HRP-conjugate (100 x concentrate)
- Sample Diluent
- HRP-conjugate Diluent
- Wash Buffer (25 x concentrate)
- TMB Substrate
- Stop Solution
- Adhesive Strip
- Stop Solution
- Adhesive Strip

Materials required but not supplied

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- An incubator which can provide stable incubation conditions up to 37 °C±0.5 °C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100 mL and 500 mL graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.

Storage of the kit

Unopened kit Store at 2 - 8°C. Do not use the kit beyond the expiration date. May be stored for up to 1 month at 2 - 8°C. Coated assay Try to keep it in a sealed aluminum foil bag, plate and avoid the damp. Standard May be stored for up to 1 month at 2 - 8° C. If don't make recent use, better keep it store at HRP-conjugate -20°C. Opened kit HRP-conjugate Diluent Sample Diluent May be stored for up to 1 month at 2 - 8°C. Wash Buffer TMB Substrate Stop Solution *Provided this is within the expiration date of the kit.

Note:

It is strongly recommended to use the remaining reagents within 1 month, if this is done before the expiry date of the kit. Please refer to the label on the kit packaging for the expiration date of the kit. All components are stable until the expiration date.

Sample collection and storage

Sample type	Collection procedure
Plasma	Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8°C within 30 min of collection. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles. Hemolysed samples are not suitable for ELISA assay!
Serum	Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 min at 1000×g. Collect the supernatant and assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.
Tissue homogenates	The preparation of tissue homogenates will vary depending upon tissue type. For general information, hemolysed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. Centrifuge the homogenates for 5 min at 5000×g to get the supernatant and assay immediately or aliquot and store at ≤-20°C.

Note:

1. Samples to be used within 5 days may be stored at 4 °C, otherwise samples must be stored at -20 °C (≤ 1 month) or -80 °C (≤ 2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.

Reagent preparation

1. HRP-conjugate (1x) - Centrifuge the vial before opening. HRP-conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 µL of HRP-conjugate + 990 µL of HRP-conjugate Diluent.
2. Wash Buffer(1x)- If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 mL of Wash Buffer (1 x).
3. Standard Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the Standard with 1.0 mL of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 150 µL of Sample Diluent into each tube (S0-S6). Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (2000 ng/mL). Sample Diluent serves as the zero standard (0 ng/mL).

Note:

- Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.
- Bring all reagents to room temperature (18-25 °C) before use for 30 min.
- Prepare fresh standard for each assay. Use within 4 hours and discard after use.
- Making serial dilution in the wells directly is not permitted.
- Please carefully reconstitute Standards according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10 µL for once pipetting.
- Distilled water is recommended to be used to make the preparation for reagents or samples. Contaminated water or container for reagent preparation will influence the detection result.

Sample preparation

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturation may occur in these samples, leading to false results. Samples should therefore be stored for a short period at 2 - 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thaw cycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to determine compatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance. The recommended dilution factor is for reference only.
- Please estimate the concentration of the samples before performing the test. If the values are not in the range of the standard curve, the optimal sample dilution for the particular experiment has to be determined.

Note:

Serum and plasma samples require a 1000-fold dilution into Sample Diluent. The suggested 1000-fold dilution can be achieved by adding 5 µL sample to 95 µL of Sample Diluent first, then complete the 1000-fold dilution by adding 5 µL of this solution to 245 µL of Sample Diluent. The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments. 6

Assay procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4 °C.
3. Set a Blank well without any solution.
4. Add 50 µL of standard and sample per well. Add 50 µL HRP-conjugate (1x) to each well immediately (not to Blank well). Mix well with the pipette or shake the plate gently for 60 seconds. A plate layout is provided to record standards and samples assayed.
5. Cover with the adhesive strip provided. Incubate for 40 minutes at 37 °C.

6. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (200 µL) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 90 µL of TMB Substrate to each well. Incubate for 20 minutes at 37 °C. Protect from light.
8. Add 50 µL of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate. *Samples may require dilution. Please refer to Sample Preparation section.

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 1 minute soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), TMB Substrate should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. TMB Substrate is easily contaminated. TMB Substrate should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

Test principle

This assay employs the competitive inhibition enzyme immunoassay technique.
The microtiter plate provided in this kit has been pre-coated with the target.
Standards or samples are added to the appropriate microtiter plate wells with Horseradish Peroxidase (HRP) conjugated target.
The competitive inhibition reaction is launched between with HRP-conjugated target and the target in samples.
A substrate solution is added to the wells and the color develops in opposite to the amount of the target in the sample.
The color development is stopped and the intensity of the color is measured.

Calculation of results

Average the duplicate readings for each standard and sample and subtract the average optical density of Blank.
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit.
As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph.
The data may be linearized by plotting the log of the target concentration versus the log of the O.D. and the best fit line can be determined by regression analysis.
This procedure will produce an adequate but less precise fit of the data.
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical data

In order to make the calculation easier, we plot the O.D. value of the standard (X-axis) against the log of concentration of the standard (Y-axis), although concentration is the independent variable and O.D. value is the dependent variable. The O.D. values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects).

Detection range

The detection range of the kit is 31.25 ng/mL - 2000 ng/mL
The standard curve concentrations used for the ELISA's were 2000 ng/mL, 1000 ng/mL, 500 ng/mL, 250 ng/mL, 125 ng/mL, 62.5 ng/mL, 31.25 ng/mL, 0 ng/mL

Sensitivity

The minimum detectable dose of C4 is typically less than 7.8 ng/mL.
The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.
It was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Note:

Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between C4 and all the analogues, therefore, cross reaction may still exist.

Precision

Intra-assay Precision (Precision within an assay): CV%<8% Three samples of known concentration were tested twenty times on one plate to assess.
Inter-assay Precision (Precision between assays): CV%<10% Three samples of known concentration were tested in twenty assays to assess.

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5 % prior to the expiration date under appropriate storage condition. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly monitored. It is also strongly suggested that the assay is performed by the same operator from the beginning to the end.

Assay procedure summary

1. Prepare reagents, samples and standards as instructed.
2. Set a Blank well without any solution.
3. Add 50 µL standard or sample to each well.
4. Add 50 µL HRP-conjugate (1x) to each well (Not to Blank well).
5. Incubate 1 hour at 37 °C
6. Aspirate and wash 5 times.
7. Add 90 µL of TMB Substrate to each well. Incubate for 20 minutes at 37 °C. Protect from light.
8. Add 50 µL Stop Solution to each well. Read at 450 nm within 5 minutes.

Important note

1. The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.
2. Limited by the current conditions and scientific technology, we cannot perform a complete identification and analysis of the raw material used. Therefore, the use of the kit may be associated with some qualitative and technical risks.
3. We are only responsible for the kit itself, not for the samples used in the test. The possible amount of sample used in the whole test should be calculated in advance and sufficient sample material should be provided.
4. Each kit undergoes a very strict QC testing. Nevertheless, end-user results may differ from our internal results due to unexpected transport conditions or different laboratory equipment. Intra-assay deviations between kits from different lots can also be related to this.
5. The test results depend on the validity of the products, so the kit should be used before the expiration date and stored according to the instructions.
6. Even the same user may obtain different results in two separate experiments. To obtain reproducible results, each step of the assay should be controlled.
7. The standard of the kit and immunogen used for antibody preparation are commonly recombinant proteins, as different fragments, expression systems, purification methods might be used in recombinant protein preparation, we can not guarantee the kit could detect recombinant protein from other companies. So, it is not recommended to use the kit for the detection of recombinant protein.

8. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by the manufacturer.
9. Protect all reagents from strong light during storage and incubation. All reagent bottle caps should be tightly closed to prevent evaporation and contamination with microorganisms. The TMB substrate should remain colourless until it reacts with the enzyme.
10. A freshly opened ELISA plate may show a water-like substance, which is normal and does not affect the test results. Return unused wells to the foil pouch and store as before.
11. Mistakes in reagent preparation and application, as well as incorrect parameter setting for the plate reader, can lead to incorrect results. A microplate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. at a wavelength of 450 ± 10 nm is suitable for absorbance measurement. Please read the instructions carefully and set up the instrument before the experiment.
12. Do not reuse the reconstituted standard and the prepared working solutions. The unused stock solutions should be stored according to the storage conditions.

Precaution

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Troubleshooting

Problem	Possible Source	Corrective Action
Poor Standard Curve	Improper standard curve preparation	Ensure accurate operation of the dilution
	Incomplete washing and aspiration	Adequate washing and adequate aspiration
	Inaccurate Pipetting	Check and Calibrate pipettes
Poor Precision	Incomplete washing of wells	Ensure sufficient washing
	Inadequate mixing and aspiration reagents	Adequate aspiration and mixing reagents
	Reused pipette tips, containers and sealers	Change and use new pipette tips, containers and sealers

Problem	Possible Source	Corrective Action
	Inaccurate Pipetting	Check and Calibrate pipettes
Low O.D Values	Inadequate reagent volumes added to wells	Calibrate pipettes and add adequate reagents
	Incorrect incubation times	Ensure sufficient incubation times
	Incorrect incubation temperature	Reagents balanced to room temperature
	Conjugate or substrate reagent failure	Mix conjugate and substrate, color should develop immediately
	No stop solution added	Follow the assay protocol in the kit manual
	Read beyond suggested reading time	Read within the time recommended in the manual
Sample Values	Improper Sample Storage	Store the sample properly and use the fresh sample
	Improper sample collection and preparation	Take proper sample collection and preparation method
	Low quantity of analyte in samples	Resample and repeat assay

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