

ABIN8065413

Sandwich ELISA Kit for Carbohydrate Antigen 242

Human

96 tests

For research use only

Not for use in clinical diagnostic procedures

Version Sep 2026

Intended use

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti ca242 antibody was pre-coated onto the 96-well plate. The biotin conjugated anti ca242 antibody was used as the detection antibody. The standards and pilot samples were added to the wells subsequently. After incubation, unbound conjugates were removed by wash buffer. Then, biotinylated detection antibody was added to bind with ca242 conjugated on coated antibody. After washing off unbound conjugates, HRP-Streptavidin was added. After a third washing, TMB substrates were added to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that turned yellow after adding a stop solution. Read the O.D. absorbance at 450nm in a microplate reader. The concentration of ca242 in the sample was calculated by drawing a standard curve. The concentration of the target substance is proportional to the OD450 value.

Reagents and materials provided

- Pre-coated, ready to use 96-well strip plate
- Plate sealer for 96 wells
- Standard
- Sample/Standard Dilution Buffer
- Biotin-labeled Antibody (Concentrated)
- Antibody Dilution Buffer
- HRP-Streptavidin Conjugate (SABC)
- SABC Dilution Buffer
- TMB Substrate
- Stop Solution
- Wash Buffer (25 x concentrate)
- Instruction manual

Materials required but not supplied

- Microplate reader (wavelength: 450nm)
- 37 °C incubator
- Automated plate washer

- Precision single and multi-channel pipette and disposable tips
- Clean tubes and Eppendorf tubes
- Deionized or distilled water

Storage of the kit

2-8°C (for sealed box), please do not freeze unopened product!

Store remaining standard and plate strips in a sealed bag with dessicant for 1 month at 4°C or 12 months at -20°C

Store other components at 4°C and avoid direct light exposure.

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
Cell Culture Supernatant	Centrifuge samples for 20 min at 1000×g at 2-8 °C. 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
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

Sample type	Collection procedure
	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

Take the Elisa kit from the fridge around 20 minutes earlier and equilibrate to room temperature(18-25°C). **For repeated assays, please just take the strips and standards required for the current assay, store the rest materials according to the relevant condition.**

Wash Buffer

- Dilute 30ml (15ml for 48T) concentrated wash buffer to 750ml (375ml for 48T) wash buffer with deionized or distilled water and mix well. (The recommended resistivity of ultrapure water is 18MΩ.) Alternatively, take appropriate amount of concentrated wash buffer according to the assay requirement, then create a 25-fold dilution and mix well. Store the rest solution at 2-8°C. **Crystals formed in the concentrated wash buffer can be heated by water bath at 40°C till complete dissolution. (Heating temperature should be below 50°C.)** Mix well for the next step. It's better to use up the prepared wash buffer in one day. Store the rest buffer at 2-8°C within 48h.

Standards

- Centrifuge standards tube for 1min at 10000×g. Label it as Zero tube.
- Add 1ml sample dilution buffer into the standard tube. Tighten the tube cap and Let it stand for 2min at room temperature. Invert the tube several times to mix gently. (Or you can mix it using a low speed vortex mixer for 3-5 seconds.)
- Centrifuge the tubes for 1min at 1000×g, making the liquid towards the bottom of tube and removing possible bubbles.
- Standard dilution: Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank

respectively. Add 0.3ml of the sample dilution buffer into each tube. Add 0.3ml solution from zero tube into 1/2 tube and mix them thoroughly. Transfer 0.3ml from 1/2 tube into 1/4 tube and mix them thoroughly. Transfer 0.3ml from 1/4 tube into 1/8 tube and mix them thoroughly, so on till 1/64 tube. Now blank tube only contain 0.3ml sample dilution buffer. The standard concentration from zero tube to blank tube is 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.25pg/ml, 0pg/ml.

- Notes: Store the zero tube with dissolved standards at 2-8°C and use it within 12h. Other diluted working solutions containing standards should be used in 2h.

Preparation of Biotin-labeled Antibody Working Solution

- The working solution should be prepared within 30min before the assay and can't be stored for a long time.
- Calculate required total volume of the working solution: 100ul/well x quantity of wells. (It's better to prepare additional 100ul-200ul.)
- Centrifuge for 1min at 1000xg in low speed and bring down the concentrated biotin-labeled antibody to the bottom of tube.
- Dilute the biotinylated detection antibody with antibody dilution buffer at 1:99 and mix them thoroughly. (e.g. Add 10ul concentrated biotin-labeled antibody into 990ul antibody dilution buffer.)

Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution

- The working solution should be prepared within 30min before the assay and can't be stored for a long time.
- Calculate required total volume of the working solution: 100ul/well x quantity of wells. (It's better to prepare additional 100ul-200ul.)
- Centrifuge for 1min at 1000xg in low speed and bring down the concentrated SABC to the bottom of tube.
- Dilute the concentrated SABC with SABC dilution buffer at 1:99 and mix them thoroughly. (e.g. Add 10ul concentrated SABC into 990ul SABC dilution buffer.)

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.

Assay procedure

1. When diluting samples and reagents, they must be mixed completely. It's recommended to plot a standard curve for each test.
2. Set standard, pilot samples, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It's recommended to measure each standard and sample in duplicate to decrease experimental errors.
3. Standards and samples loading: Aliquot 100ul of zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube into each standard well. Also add 100ul sample dilution buffer into the control (blank) well. Then, add 100ul pilot samples into each sample well. Seal the plate and static incubate for 90 minutes at 37°C. (Add the solution to the bottom of each well. Mix gently and without touch the sidewall and foam the sample.)
4. Wash twice: Remove the cover, then absorb the liquid in the plate or tap the plate on a clean absorbent paper two or three times. Add 350ul wash buffer into each well without immersion. Discard the liquid in the well and tap on the absorbent paper again. Repeat the washing step twice.
5. Biotin-labeled Antibody: Add 100ul biotin-labeled antibody working solution into each well. Seal the plate and static incubate for 60 minutes at 37°C.
6. Wash three times: Remove the cover, then absorb the liquid in the plate or tap the plate on a clean absorbent paper two or three times. Add 350ul wash buffer into each well and immerse for 1min. Discard the liquid in the well and tap on the absorbent paper again. Repeat the washing step three times.
7. HRP-Streptavidin Conjugate (SABC): Add 100ul SABC working solution into each well. Seal the plate and static incubate for 30 minutes at 37°C. (Put the whole bottle of TMB into the 37°C incubator to equilibrate for 30min.)
8. Wash five times: Remove the cover, and then wash the plate with wash buffer five times. Read washing method in step 5.
9. TMB Substrate: Add 90ul TMB Substrate into each well, seal the plate and static incubate at 37°C in dark within 10-20 minutes. Run the microplate reader and preheat for 15min.

(Notes: Please do not use the reagent reservoirs used by HRP couplings. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells. Weaker or stronger color intensity is unacceptable.)

10. Stop: Keep the liquid in the well after staining. Add 50ul stop solution into each well. The color will turn yellow immediately. The order for adding stop solution and TMB substrate solution is the same.
11. OD Measurement: Read the O.D. absorbance at 450nm in a microplate reader immediately. (If your microplate reader has a choice of correction wavelength, set it to 570nm or 630nm. Correct the read value to the OD450 value minus the OD570 or OD630 value. In this way, the OD value of non-chromogenic substances can be corrected and removed, thus obtaining more accurate results. If the microplate reader does not have a 570nm or 630nm wavelength, the original OD450 value can be used.)

Test principle

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Capture antibody was pre-coated onto 96- well plates. And the biotin conjugated antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of target can be calculated.

Calculation of results

1. Calculate the mean OD450 value (using the original OD450 value or the corrected OD450 value) of the duplicate readings for each standard, control, and sample. Then, obtain the value of calculation by subtracting the OD450 blank.
2. Create a four parameter logistic curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. (Remove the OD450 blank during plotting.) Alternatively, you can use the curve fitting software offered by the microplate reader (e.g. Thermo SkanIt RE software,).
3. Calculate the sample concentration by substituting OD450 value into the standard curve. Diluted samples should be multiplied by the relevant dilution ratio.

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 pg/mL – 2000 pg/mL

The standard curve concentrations used for the ELISA's were 2000 pg/mL, 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.25 pg/mL, 0 pg/mL

Sensitivity

The minimum detectable dose of CA242 is typically less than 18.75 pg/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 CA242 and all the analogues, therefore, cross reaction may still exist.

Precision

Intra-assay Precision: samples with low, medium and high concentration are tested 20 times on same plate. CV% = <6%

Inter-assay Precision: samples with low, medium and high concentration are tested 20 times on three different plates. CV% = <7%

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

Step 1: Add 100ul standard or sample into each well, seal the plate and static incubate for 90 minutes at 37°C.

Washing: Wash the plate twice without immersion.

Step 2: Add 100ul biotin-labeled antibody working solution into each well, seal the plate and static incubate for 60 minutes at 37°C.

Washing: Wash the plate three times and immerse for 1min each time.

Step 3: Add 100ul SABC working solution into each well, seal the plate and static incubate for 30 minutes at 37°C.

Washing: Wash the plate five times and immerse for 1min each time.

Step 4: Add 90ul TMB substrate solution, seal the plate and static incubate for 10-20 minutes at 37°C. (Accurate TMB visualization control is required.)

Step 5: Add 50ul stop solution. Read at 450nm immediately and calculate.

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
	Inaccurate Pipetting	Check and Calibrate pipettes

Problem	Possible Source	Corrective Action
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

For more information, please contact:

antibodies-online Inc.

PO Box 5201
Limerick, PA 19468
USA

Website: www.antibodies-online.com
Email: info@antibodies-online.com
Phone: +1 877 302 8632
Fax: +1 888 205 9894

antibodies-online GmbH

Schloss-Rahe-Straße 15
52072 Aachen
Deutschland

Website: www.antikoerper-online.de
Email: info@antikoerper-online.de
Phone: +49 (0)241 95 163 153
Fax: +49 (0)241 95 163 155