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User Manual

Catalog Number: EKC31182

Product Name: Human carbohydrate antigen 50(CA50) ELISA Kit

Detection Range:10 U/mL- 120 U/mL

Intended Use: For quantitative determination of Human carbohydrate antigen 5 (CA50) concentrations in serum, plasma, cell culture supernates, ascitic fluid, tissue homogenates, cell lysates.

Precautions: For research use only. Not for use in diagnostic procedures.

Manual Version: 202301V1

Storage:

Unopened kit	6 months when stored at 2 - 8°C.
Opened Kit	May be stored up to 1 month at 2 - 8°C. Keep it in sealed aluminum foil bag and avoid moisture.

The product manual may be updated as a result of continuous improvements.

Always refer to the hard copy manual included in the kit for your experiment.

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Kit Components

Reagents	Quantity
Assay plate (12 x 8 coated Microwells)	1(96 wells)
Standard	5x 0.5 ml
HRP-conjugate	1 x 6 ml
Antibody	1 x 6 ml
Wash Buffer (20 x concentrate)	1 x 15 ml
Substrate A	1 x 7 ml
Substrate B	1 x 7 ml
Stop Solution	1 x 7 ml
Adhesive Strip (For 96 wells)	4
Instruction manual	1

Working Principle

This assay adopts quantitative sandwich enzyme immunoassay technique. Antibody specific to CA50 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells with a Horseradish Peroxidase (HRP) conjugated antibody specific to CA50. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of CA50 bound in the initial step. At last, measure the intensity of color after stopping color development.

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Sensitivity

The minimum detectable dose of mouse CA50 is less than 2.5 U/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 the mean O.D value of 20 replicates of the zero standard added by their three standard deviations.

Specificity

This assay has high sensitivity and excellent specificity. No significant cross-reactivity or interference between Human CA50 and analogues was observed. Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between Human CA50 and all the analogues, therefore, cross reaction may still exist.

Precision

Intra-assay Precision (Precision within an assay): CV%<15%

Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<20%

Three samples of known concentration were tested in twenty assays to assess.

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Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 600 nm or 630 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.
- 100ml and 500ml graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.

Sample Collection & Storage

- **Serum** Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation at 1000 ×g for 15 mins. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

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- **Plasma** Collect plasma using EDTA or heparin as an anticoagulant. Within 30 mins after collecting samples, centrifuge samples at 1000 x g, 2 - 8°C, for 15 mins. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernates** Remove particulates by centrifugation for 15 minutes at 1000 x g, 2 - 8°C and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Ascitic Fluid** Remove particulates by centrifugation for 10 minutes at 4000x g at 2-8°C and assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.
- **Tissue Homogenates** Rinse 100mg tissue with 1X PBS, homogenize it in 1 ml of 1X PBS and store it overnight at -20°C. The cell membranes break after two freeze-thaw cycles, then centrifuge homogenates at 5000 x g, 2 - 8°C for 5 mins. Take the supernate for assay. Alternatively, aliquot and store the supernate at -20°C or -80°C. Centrifuge samples again after thawing. Avoid repeated freeze-thaw cycles.
- **Cell Lysates (1) Adherent Cell:** Remove media and rinse cells once with ice-cold PBS (PH7.2-7.4). Scrape cells off the plate and transfer to an appropriate tube. Dilute cell suspension with 1xPBS (PH7.2-7.4), until cell concentration reached 100 million/ml. Then store

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overnight at -20°C. After two freeze-thaw cycles to break up the cell membranes, the cell lysates were centrifuged for 5 minutes at 5000 g, 2 - 8°C. Collect the supernatant. Cell lysates should be assayed immediately or aliquotted and stored at -20°C. Centrifuge the sample again after thawing before the assay. Avoid 7 repeated freeze-thaw cycles. **(2) Suspension Cell:** Collect cells with appropriate tube, centrifuge for 5 minutes at 1000 g, 2 - 8°C. Remove the supernatant and resuspend cells with 1xPBS (PH7.2-7.4). Centrifuge for 5 minutes at 1000 g, 2 - 8°C. Remove the supernatant. Dilute cell with 1xPBS (PH7.2-7.4), until cell concentration reached 100 million/ml. Then store overnight at -20°C. After two freeze-thaw cycles to break up the cell membranes, the cell lysates were centrifuged for 5 minutes at 5000 g, 2 - 8°C. Collect the supernatant. Cell lysates should be assayed immediately or aliquotted and stored at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Notes:

1. Biomatik is only responsible for the kit itself, not for the samples consumed during the assay. The user need to calculate the possible amount of the samples to be used in the whole test. Please reserve sufficient samples in advance.

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2. Samples to be used within 5 days may be stored at 2-8°C, otherwise, samples must be stored at -20°C (≤1month) or -80°C (≤2month) to avoid contamination and loss of bioactivity.

3. Grossly hemolyzed samples are not suitable to use.

4. It would be necessary to run a preliminary experiment for validation, if the samples are not indicated in the manual.

5. Please predict the concentration before assaying. If results were not within the range of the standard curve, users would need to estimate the optimal sample dilutions for their particular experiments.

6. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.

7. Considering the possibility of mismatch between antigen from other resources and antibody in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.

8. Due to factors including cell viability, cell number and sampling time, samples from cell culture supernatant may not be detected by the kit.

9. Recommend to use fresh samples for the test. If you store samples for long time, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

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Reagents Preparation

- **Kindly use graduated containers to prepare the reagent.**
- Bring all reagents to room temperature (18-25°C) before use for 30 mins.
- Distilled Water is recommended. Contaminated water or container for reagents preparation will affect the test result.

HRP-conjugate mixed solution - $V_{Antibody}$ -$V_{HRP\text{-}conjugate}$ =1:1 (eg: 1ml Antibody + 1ml HRP-conjugate, shake well).

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 15 ml of Wash Buffer Concentrate (20 x) into deionized or distilled water to prepare 300 ml of Wash Buffer (1 x)

Standard Concentration

Standard	S0	S1	S2	S3	S4
Concentration (U/ml)	0	10	30	60	120

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Key Notes

- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate higher values than the highest standard, dilute the samples with Sample Diluent and repeat the assay.
- Any variation in Sample Diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

Precautions

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

Assay Procedures

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

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1. Prepare all reagents 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 20µl of standard or sample per well.

5. Add 100µl of HRP-conjugate mixed solution to each well (not to Blank well). Mix well and then incubate at 37°C for one hour.

6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 10 seconds, 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 50µl of **Substrate A** and 50µl of **Substrate B** to each well, mix well. Incubate at 37°C for 15 minutes. Keeping the plate away from drafts and other temperature fluctuations in the dark.

8.Add 50µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.

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9. Determine the optical density of each well within 10 mins, using a microplate reader set to 450 nm.

Notes:

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 mins. 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

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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 30 second 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 mins), 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

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yellow upon addition of the Stop Solution. Wells that are green in color indicate that Stop Solution has not mixed thoroughly with TMB Substrate.

Calculation of Results

Using the professional soft "Curve Expert" to make a standard curve is recommended, which can be downloaded from our web.

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density.

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 CA50 concentrations 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.