ab222881
Human Carbonic Anhydrase 2 (CA2) ELISA Kit

For the quantitative measurement of human CA2 in plasma, serum, and cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.
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1. Overview

The Human CA2 ELISA (Enzyme-Linked Immunosorbent Assay) kit (ab222881) is designed for detection of human CA2 in plasma, serum, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human CA2 in approximately 4 hours. A polyclonal antibody specific for human CA2 has been pre-coated onto a 96-well microplate with removable strips. CA2 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human CA2, which is recognized by a Streptavidin-Peroxidase (SP) conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Carbonic anhydrase 2 (CA2, CAC), also known as carbonate dehydratase II and carbonic anhydrase II, belongs to the alpha-carbonic anhydrase family. It contains 259 amino acid residues and has a molecular mass of 29 kDa. CA2 catalyzes reversible hydration of carbon dioxide and is expressed at high levels in osteoclasts during bone resorption. CA2 supports the transport of bicarbonate ions, sodium ions, and water from the blood to the cerebrospinal fluid.
2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

↓

Add 50 µL standard or sample to appropriate wells and incubate for 2 hours

↓

Wash wells, add 50 µL Biotinylated Antibody to each well and incubate for 1 hour

↓

Wash wells, add 50 µL Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes

↓

Wash wells, add 50 µL Chromogen Substrate to each well and incubate for 25 minutes

↓

Add 50 µL Stop Solution to each well and read OD at 450 nm
3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C immediately upon receipt, apart from Biotinylated Human CA2, Human CA2 Standard and 100X Streptavidin-Peroxidase Conjugate which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.
5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Human CA2 coated Microplate (12 x 8 wells)</td>
<td>96 wells</td>
<td>+4°C</td>
</tr>
<tr>
<td>Human CA2 Standard</td>
<td>1 Vial</td>
<td>-20°C</td>
</tr>
<tr>
<td>Biotinylated Human CA2</td>
<td>120 µL</td>
<td>-20°C</td>
</tr>
<tr>
<td>10X Diluent N Concentrate</td>
<td>30 mL</td>
<td>+4°C</td>
</tr>
<tr>
<td>1X Standard Diluent</td>
<td>2 mL</td>
<td>+4°C</td>
</tr>
<tr>
<td>20X Wash Buffer Concentrate</td>
<td>2 x 30 mL</td>
<td>+4°C</td>
</tr>
<tr>
<td>100X Streptavidin-Peroxidase Conjugate</td>
<td>80 µL</td>
<td>-20°C</td>
</tr>
<tr>
<td>Chromogen Substrate</td>
<td>8 mL</td>
<td>+4°C</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>12 mL</td>
<td>+4°C</td>
</tr>
<tr>
<td>Sealing Tapes</td>
<td>3 units</td>
<td>+4°C</td>
</tr>
</tbody>
</table>
7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µL, 20-200 µL, 200-1000 µL, and multiple channel).
- Deionized or distilled reagent grade water.
8. Technical Hints

- This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.
9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment

9.1 10X Diluent N Concentrate:
If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Prepare 1X Diluent N by diluting Diluent N Concentrate 1 in 10 with reagent grade water to produce a 1X solution. Store for up to 30 days at 2-8°C.

9.2 20X Wash Buffer Concentrate:
If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Prepare 1X Wash Buffer by diluting Wash Buffer Concentrate 1 in 20 with reagent grade water.

9.3 Biotinylated Human CA2:
Spin down the antibody briefly and dilute the desired amount of the antibody 1 in 50 with 1X Diluent N to produce a 1X solution. The undiluted antibody should be stored at -20°C.

9.4 100X Streptavidin-Peroxidase Conjugate:
Spin down the Streptavidin-Peroxidase Conjugate briefly and dilute the desired amount of the conjugate 1 in 100 with 1X Diluent N. The undiluted conjugate should be stored at -20°C.

9.5 Anti-Human CA2 coated Microplate (12 x 8 wells):
Ready to use. Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.

9.6 Chromogen Substrate (8 mL):
Ready to use. Store at +4°C.

9.7 Sealing Tapes (3 units):
Ready to use. Store at +4°C.

9.8 Stop Solution (12 mL):
Ready to use. Store at +4°C.
10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Reconstitute the CA2 to generate a 16 ng/mL Stock.

10.1.1 First consult the CA2 Standard vial to determine the mass of protein in the vial.

10.1.2 Calculate the appropriate volume of 1X Diluent N to add when resuspending the CA2 Standard vial to produce a 16 ng/mL CA2 Stock by using the following equation:

\[
\text{CS} = \frac{\text{Starting mass of CA2 Standard stock (see vial label)} \text{ (ng)}}{
\text{CF} = 16 \text{ ng/mL CA2 Standard Stock final required concentration}}
\]

\[
\text{VD} = \text{Required volume of 1X Diluent N for reconstitution (µL)}
\]

Calculate total required volume 1X Diluent N for resuspension:

\[
\left(\frac{\text{CS}}{\text{CS}}\right) \times 1,000 = V_D
\]

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

\[
\text{CS} = 12 \text{ ng of CA2 Standard in vial}
\]

\[
\text{CF} = 16 \text{ ng/mL CA2 stock final concentration}
\]

\[
\text{VD} = \text{Required volume of 1X Diluent N for reconstitution}
\]

\[
(12 \text{ ng} / 16 \text{ ng/mL}) \times 1,000 = 750 \mu\text{L}
\]
10.1.3 First briefly centrifuge the CA2 Standard Vial to collect the contents on the bottom of the tube.

10.1.4 Reconstitute the CA2 Standard vial by adding the appropriate calculated amount VD of 1X Diluent N to the vial to generate the 16 ng/mL CA2 Stock. Mix gently and thoroughly.

10.2 Allow the reconstituted 16 ng/mL CA2 Stock to sit for 10 minutes with gentle agitation prior to making subsequent dilutions.

10.3 Label seven tubes #1 – 8.

10.4 Add 180 µL Diluent N to tube #1, and 120 µL Diluent N to tubes #2- #8.

10.5 Prepare duplicate or triplicate standard points by serially diluting the standard working solution (4 ng/ml) 1 in 2 with 1X Diluent N to produce 2, 1, 0.5, 0.25, 0.125, and 0.063 ng/ml solutions. 1X Diluent N serves as the zero standard (0 ng/ml).

10.6 To prepare Standard #1, add 60 µL of the Stock into tube #1 and mix gently.

10.7 To prepare Standard #2, add 120 µL of the Standard #1 into tube #2 and mix gently.

10.8 Using the table below as a guide, prepare subsequent serial dilutions.

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Volume to dilute (µL)</th>
<th>Volume Diluent N (µL)</th>
<th>CA2 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60 µL Stock from Step 10.1</td>
<td>180</td>
<td>4.0</td>
</tr>
<tr>
<td>2</td>
<td>120 µL Standard #1</td>
<td>120</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>120 µL Standard #2</td>
<td>120</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>120 µL Standard #3</td>
<td>120</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>120 µL Standard #4</td>
<td>120</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>120 µL Standard #5</td>
<td>120</td>
<td>0.125</td>
</tr>
<tr>
<td>7</td>
<td>120 µL Standard #6</td>
<td>120</td>
<td>0.063</td>
</tr>
<tr>
<td>8 (Blank)</td>
<td>N/A</td>
<td>120</td>
<td>0</td>
</tr>
</tbody>
</table>
11. Sample Preparation

11.1 Plasma:
Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. The sample is suggested for use at 1X; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

11.2 Serum:
Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. The sample is suggested for use at 1X; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants:
Centrifuge cell culture media at 3000 x g for 10 minutes at 4°C to remove debris and collect supernatants. Samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.
12. **Assay Procedure**

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

12.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

12.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and store in a vacuum desiccator.

12.3 Add 50 µL of all sample or standard to appropriate wells. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.

12.4 Wash five times with 200 µl of Wash Buffer manually. Wash by aspirating or decanting from wells then dispensing 200 µL Wash Buffer into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid. If using a machine, wash six times with 300 µl of Wash Buffer.

12.5 Add 50 µl of 1X Biotinylated CA2 to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed and incubate for 1 hour.

12.6 Wash the microplate as described above.

12.7 Add 50 µl of 1X Streptavidin-Peroxidase Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes.

12.8 Turn on the microplate reader and set up the program in advance.

12.9 Wash the microplate as described above.
12.10 Add 50 µl of Chromogen Substrate per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed and incubate for 25 minutes or till the optimal blue color density develops.

12.11 Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.

12.12 Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only.

\textbf{Note:} that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings
13. Calculations

13.1 Calculate the mean value of the duplicate or triplicate readings for each standard and sample.

13.2 To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using four-parameter or log-log logistic curve-fit.

13.3 Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.
14. Typical Data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

![Typical standard curve graph](image)

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>O.D 450 nm</th>
<th>Mean O.D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>0.098</td>
<td>0.101</td>
</tr>
<tr>
<td>0.063</td>
<td>0.128</td>
<td>0.133</td>
</tr>
<tr>
<td>0.125</td>
<td>0.141</td>
<td>0.154</td>
</tr>
<tr>
<td>0.25</td>
<td>0.211</td>
<td>0.235</td>
</tr>
<tr>
<td>0.5</td>
<td>0.357</td>
<td>0.383</td>
</tr>
<tr>
<td>1</td>
<td>0.624</td>
<td>0.662</td>
</tr>
<tr>
<td>2</td>
<td>1.239</td>
<td>1.282</td>
</tr>
<tr>
<td>4</td>
<td>2.014</td>
<td>2.070</td>
</tr>
</tbody>
</table>

**Figure 1.** Example of human CA2 standard curve in 1X Diluent N. The CA2 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.
15. Typical Sample Values

SENSITIVITY –
The calculated minimal detectable dose (MDD) is 0.05 ng/ml. The MDD was determined by calculating the mean of zero standard and adding 2 standard deviations then extrapolating the corresponding concentration.

PRECISION –
Intra-assay precision was determined by testing three plasma samples twenty times in one assay.
Inter-assay precision was determined by testing three plasma samples in twenty assays.

<table>
<thead>
<tr>
<th></th>
<th>Intra-Assay Precision</th>
<th>Inter-Assay Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>CV (%)</td>
<td>4.9%</td>
<td>5.5%</td>
</tr>
<tr>
<td>Average CV (%)</td>
<td>5.3%</td>
<td></td>
</tr>
</tbody>
</table>

RECOVERY –

<table>
<thead>
<tr>
<th>Standard Added Value</th>
<th>0.25-2 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery (%)</td>
<td>87 – 112 %</td>
</tr>
<tr>
<td>Average Recovery (%)</td>
<td>96%</td>
</tr>
</tbody>
</table>

Linearity of Dilution
Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.
Plasma and serum samples were serially-diluted to test for linearity.

<table>
<thead>
<tr>
<th>Dilution Factor</th>
<th>Serum</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
<td>99%</td>
<td>98%</td>
</tr>
<tr>
<td>2</td>
<td>106%</td>
<td>104%</td>
</tr>
<tr>
<td>4</td>
<td>104%</td>
<td>108%</td>
</tr>
</tbody>
</table>
16. Assay Specificity

This kit recognizes human CA2 protein in plasma, serum, and cell culture samples.

**CROSS REACTIVITY**

<table>
<thead>
<tr>
<th>Species</th>
<th>Cross Reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>35%</td>
</tr>
<tr>
<td>Cow</td>
<td>None</td>
</tr>
<tr>
<td>Monkey</td>
<td>90%</td>
</tr>
<tr>
<td>Mouse</td>
<td>None</td>
</tr>
<tr>
<td>Rat</td>
<td>80%</td>
</tr>
<tr>
<td>Pig</td>
<td>100%</td>
</tr>
<tr>
<td>Rabbit</td>
<td>None</td>
</tr>
<tr>
<td>CA1 protein</td>
<td>None</td>
</tr>
<tr>
<td>CA3 protein</td>
<td>None</td>
</tr>
</tbody>
</table>

17. Species Reactivity

This kit recognizes human, dog, monkey, rat and pig CA2 protein.

Please contact our Technical Support team for more information.
### Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Precision</td>
<td>Use of expired components</td>
<td>Check the expiration date listed before use. Do not interchange components from different lots.</td>
</tr>
<tr>
<td></td>
<td>Improper wash step</td>
<td>Check that the correct wash buffer is being used. Check that all wells are dry after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique.</td>
</tr>
<tr>
<td></td>
<td>Splashing of reagents while loading wells</td>
<td>Pipette properly in a controlled and careful manner.</td>
</tr>
<tr>
<td></td>
<td>Inconsistent volumes loaded into wells</td>
<td>Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.</td>
</tr>
<tr>
<td></td>
<td>Insufficient mixing of reagent dilutions</td>
<td>Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.</td>
</tr>
<tr>
<td></td>
<td>Improperly sealed microplate</td>
<td>Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing.</td>
</tr>
<tr>
<td>Unexpectedly Low or High Signal Intensity</td>
<td>Microplate was left unattended between steps</td>
<td>Each step of the procedure should be performed uninterrupted.</td>
</tr>
<tr>
<td></td>
<td>Omission of step</td>
<td>Consult the provided procedure for complete list of steps.</td>
</tr>
<tr>
<td></td>
<td>Steps performed in incorrect order</td>
<td>Consult the provided procedure for the correct order.</td>
</tr>
<tr>
<td></td>
<td>Insufficient amount of reagents added to wells</td>
<td>Check pipette calibration. Check pipette for proper performance.</td>
</tr>
<tr>
<td></td>
<td>Wash step was skipped</td>
<td>Consult the provided procedure for all wash steps.</td>
</tr>
<tr>
<td></td>
<td>Improper wash</td>
<td>Check that the correct wash buffer</td>
</tr>
<tr>
<td>Buffer is being used.</td>
<td>Improper reagent preparation section for the correct dilutions of all reagents.</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Insufficient or prolonged incubation periods</td>
<td>Consult the provided procedure for correct incubation time.</td>
<td></td>
</tr>
<tr>
<td>Non-optimal sample dilution</td>
<td>Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. User should determine the optimal dilution factor for samples.</td>
<td></td>
</tr>
<tr>
<td>Contamination of reagents</td>
<td>A new tip must be used for each addition of different samples or reagents during the assay procedure.</td>
<td></td>
</tr>
<tr>
<td>Contents of wells evaporate</td>
<td>Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.</td>
<td></td>
</tr>
<tr>
<td>Improper pipetting</td>
<td>Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.</td>
<td></td>
</tr>
<tr>
<td>Insufficient mixing of reagent dilutions</td>
<td>Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.</td>
<td></td>
</tr>
</tbody>
</table>
19. Notes
Technical Support

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Austria
wissenschaftlicherdienst@abcam.com | 019-288-259

France
supportscientifique@abcam.com | 01.46.94.62.96

Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154

Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com

UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
can.technical@abcam.com | 877-749-8807

US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823

China
cn.technical@abcam.com | +86-21-5110-5938 | 400-628-6880

Japan
technical@abcam.co.jp | +81-(0)3-6231-0940

Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450

New Zealand
nz.technical@abcam.com | +64-(0)9-909-7829