

Version 7b Last updated 10 March 2026

# **ab222881**

# **Human Carbonic Anhydrase 2 (CA2) ELISA Kit**

For the quantitative measurement of human CA2 in Human plasma, serum, cell culture supernatant, cell lysate and tissue samples.

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, cell culture supernatant, cell lysate and tissue 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  $\mu$ L standard or sample to appropriate wells and incubate for 2 hours



Wash wells, add 50  $\mu$ L Biotinylated Antibody to each well and incubate for 1 hour



Wash wells, add 50  $\mu$ L Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes



Wash wells, add 50  $\mu$ L Chromogen Substrate to each well and incubate for 20 minutes



Add 50  $\mu$ L 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.
- 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.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website [www.abcam.com](http://www.abcam.com).

### 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

<b>Item</b>	<b>Quantity</b>	<b>Storage Condition</b>
Anti-Human CA2 coated Microplate (12 x 8 wells)	96 wells	+4°C
Human CA2 Standard	1 Vial	-20°C
Biotinylated Human CA2	1 Vial	-20°C
10X Diluent N Concentrate	30 mL	+4°C
1X Standard Diluent	2 mL	+4°C
20X Wash Buffer Concentrate	2 x 30 mL	+4°C
100X Streptavidin-Peroxidase Conjugate	80 µL	-20°C
Chromogen Substrate	7 mL	+4°C
Stop Solution	11 mL	+4°C
Sealing Tapes	3 units	+4°C

## **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  $\mu\text{L}$ , 20-200  $\mu\text{L}$ , 200-1000  $\mu\text{L}$ , 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:

Dilute the Diluent N Concentrate 10-fold with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1X solution gently until the crystals have completely dissolved. Store for up to 30 days at 4°C.

### 9.2 20X Wash Buffer Concentrate:

Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1X solution gently until the crystals have completely dissolved.

### 9.3 Biotinylated Human CA2:

Spin down the Biotinylated antibody briefly. The stock Biotinylated Human CA2 must be diluted to a 1x working solution using 1X Diluent N, referring to the Biotinylated antibody label for the initial concentration. Any remaining 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 (7 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 (11 mL):

Ready to use. Store at +4°C.

### 9.9 1X Standard Diluent (2 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 4 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 Standard Diluent to add when resuspending the CA2 Standard vial to produce a 4 ng/mL CA2 **Stock** by using the following equation:

$C_S$  = Starting mass of CA2 Standard stock (see vial label) (ng)

$C_F$  = 16 ng/mL CA2 Standard Stock final required concentration

$V_D$  = Required volume of 1X Standard Diluent for reconstitution ( $\mu$ L)

Calculate total required volume 1X Standard Diluent for resuspension:

$$(C_S / C_F) * 1,000 = V_D$$

Example:

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

$C_S$  = 2.6 ng of CA2 Standard in vial

$C_F$  = 16 ng/mL CA2 stock final concentration

$V_D$  = Required volume of 1X Standard Diluent for reconstitution

$$(2.6 \text{ ng} / 16 \text{ ng/mL}) * 1,000 = 162.5 \mu\text{L}$$

- 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 Standard Diluent to the vial to generate the 4 ng/mL CA2 **Stock**. Mix gently and thoroughly.
- 10.2** Allow the reconstituted 4 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.

<b>Standard #</b>	<b>Volume to dilute (µL)</b>	<b>Volume Diluent N (µL)</b>	<b>CA2 (ng/mL)</b>
1	60 µL Stock from Step 10.1	180	2.0
2	120 µL Standard #1	120	1.0
3	120 µL Standard #2	120	0.5
4	120 µL Standard #3	120	0.25
5	120 µL Standard #4	120	0.125
6	120 µL Standard #5	120	0.063
7	120 µL Standard #6	120	0.031
8 (Blank)	N/A	120	0

## 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 Supernatant:

Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. If necessary, dilute samples into Diluent N; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

### 11.4 Cell Lysate:

Rinse cell with cold PBS and then scrape the cell into a tube with 5 ml of cold PBS and 0.5 M EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4°C and aspirate supernatant. Resuspend pellet in ice-cold Lysis Buffer (PBS, 1% Triton X-100, protease inhibitor cocktail). For every  $1 \times 10^6$  cells, add approximately 100  $\mu$ l of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4°C and collect supernatant. If necessary, dilute samples into Diluent N; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

### 11.5 Tissue:

Extract tissue samples with 0.1 M phosphate-buffered saline (pH 7.4) containing 1% Triton X-100 and centrifuge at 14000 x g for 20 minutes. Collect the supernatant and measure the protein concentration. If necessary, dilute samples into Diluent N; user should determine optimal dilution factor depending on

application needs. Store remaining extract at -80°C. 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  $\mu\text{L}$  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  $\mu\text{L}$  of Wash Buffer manually. Wash by aspirating or decanting from wells then dispensing 200  $\mu\text{L}$  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  $\mu\text{L}$  of Wash Buffer.
  - 12.5** Add 50  $\mu\text{L}$  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  $\mu\text{L}$  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 35 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  $\mu$ l of Chromogen Substrate per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed and incubate in ambient light for 20 minutes or until the optimal blue color density develops.
- 12.11** Add 50  $\mu$ L 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.

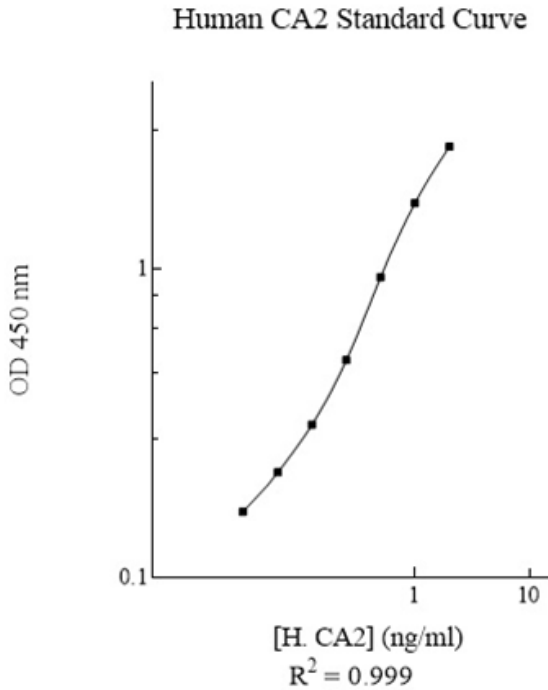
$\Delta$  **Note:** that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings

## 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.



**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 21 pg/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.

	<b>Intra-Assay Precision</b>	<b>Inter-Assay Precision</b>
Average CV (%)	4.5	10.9

### RECOVERY –

Standard Added Value	0.063 - 1 ng/ml
Recovery (%)	91 – 113%
<b>Average Recovery (%)</b>	102%

## 16. Assay Specificity

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

### CROSS REACTIVITY

Species	Cross Reactivity (%)
Dog	15%
Cow	None
Equine	15%
Monkey	90%
Mouse	None
Rat	40%
Pig	40%
Rabbit	None
Human CA1 protein	None
Human CA3 protein	None
Human CA8 protein	None
Human CA14 protein	None

- 10% FBS in culture media will not affect the assay

## 17. Species Reactivity

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

Please contact our Technical Support team for more information.

## 18. Troubleshooting

Problem	Reason	Solution
<b>Low Precision</b>	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots.
	Improper wash step	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.
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.
	Improperly sealed microplate	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.
<b>Unexpectedly Low or High Signal Intensity</b>	Microplate was left unattended between steps	Each step of the procedure should be performed uninterrupted.
	Omission of step	Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	Check pipette calibration. Check pipette for proper performance.
	Wash step was skipped	Consult the provided procedure for all wash steps.

	Improper wash buffer	Check that the correct wash buffer is being used.
	Improper reagent preparation	Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	Consult the provided procedure for correct incubation time.
<b>Deficient Standard Curve Fit</b>	Non-optimal sample dilution	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.
	Contamination of reagents	A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.

# 19. Notes

## **Technical Support**

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