# Rat Acaca (Acetyl-CoA carboxylase 1) ELISA Kit

## A Complete ELISA kit for the detection of Rat Acaca

## (Cat. #IT6892)

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## INTRODUCTION

The Rat Acaca (Acetyl-CoA carboxylase 1) ELISA Kit is based on a sandwich enzyme-linked immunosorbent assay (ELISA) technology. A 96-well plate is coated with a specific antibody against the protein of interest (Acaca), which will bind the protein and this is detected by a biotin conjugated antibody against the protein of interest. The biotin moiety is subsequently detected, following washing, by the addition of streptavidin coupled horseradish peroxidase (HRP). The supplied 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 directly proportional to the concentration of protein of interest captured on the plate.

## ITEMS SUPPLIED

| Description | Size |
|---|---|
| Coated Microtiter Plate | 1 |
| ELISA Standard (Lyophilized) | 2 vials |
| ELISA Standard Diluent | 20ml |
| Wash Buffer [25X] | 30ml |
| Biotinylated Detection Antibody | 120µl |
| Detection Antibody Diluent | 10ml |
| ELISA Detection Reagent | 120µl |
| ELISA Detection Reagent Diluent | 10ml |
| ELISA Detection Substrate (TMB) | 10ml |
| ELISA Stop Solution | 10ml |
| Microplate Sealing Tape | 5 |

***NOTE:*** *ELISA Detection Reagent is a HRP-Streptavidin Conjugate.*

## STORAGE CONDITIONS

The kit is shipped on blue ice. Upon arrival, store kit at 4˚C for up to 6 months.

## SPECIFICATIONS

- **Reactivity:** Rat
- **Range:** 15.6-1000pg/ml
- **Sensitivity:** 9.375pg/ml

## ADDITIONAL ITEMS NEEDED

- Microplate reader (wavelength: 450nm)
- 37°C incubator
- Automated plate washer (Optional)
- Precision single and multi-channel pipette and disposable tips
- Clean tubes and Eppendorf tubes
- Deionized or distilled water

## PRECAUTIONS

- We recommend performing pilot experiments using standards and a small number of samples.
- After opening and before using, keep plate dry.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- ELISA Detection Substrate (TMB) must be protected from light.
- False positives may arise if washing steps are not completed.
- The use of duplicate well assays ire recommended for both standard and sample testing.
- Do not let the plate dry out during the assay as this may inactivate active components.
- Do not reuse tips and tubes to avoid cross contamination.

## PREPARATION BEFORE USE

Bring all reagents to room temperature before use.

***Wash Buffer***

Dilute 30mL Wash Buffer [25X] into 750 mL of Wash Buffer with deionized or distilled water. If crystals have formed in the concentrate, you can warm in a 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use. Store diluted wash buffer at 4°C.

***ELISA Standard***

1. ***1000pg/ml of ELISA Standard:*** Add 1 ml of ELISA Standard Diluent into an ELISA Standard tube, keep the tube at room temperature for 10 min and mix thoroughly.
2. ***Dilute ELISA Standard:*** Label 6 Eppendorf tubes (1-6) and aliquot 0.3 ml of the ELISA Standard Diluent into each tube. Add 0.3 ml of the above 1000pg/ml standard solution into 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on. The resulting standard will be repeating 1:1 dilutions of the starting standard
   ***NOTE:*** *The standard solutions are best used within 2 hours. The standard solution should be at 4°C for up to 12 hours. Or store at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.*

***Preparation of Biotinylated Detection Antibody***

Prepare within 1 hour before starting the experiment.

1. Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)
2. Dilute the Biotinylated Detection Antibody with the Detection Antibody Diluent at 1:100 and mix thoroughly.(i.e. Add 1µl of Biotinylated Detection Antibody into 99µl of Diluent.)

***Preparation of ELISA Detection Reagent***

Prepare within 30 minutes before starting the experiment.

1. Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)
2. Dilute the ELISA Detection Reagent with ELISA Detection Reagent Diluent at 1:100 and mix thoroughly.(i.e. Add 1µl of ELISA Detection Reagent into 99µl of ELISA Detection Reagent Diluent.)

## PROTOCOL

***For Manual Washing***

1. Discard the solution in the plate without touching the side walls.
2. Clap the plate on absorbent filter papers or other absorbent material.
3. Fill each well completely with 350µl wash buffer and soak for 1 to 2 minutes
4. Aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material.
5. Repeat this procedure two more times for a total of THREE washes.

***For Automated Washing***

Aspirate all wells, and then wash plate THREE times with 350µl wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

***Sample Dilution Guideline***

End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to ensure the diluted target protein concentration falls in the optimal detection range of the kit. Dilute the sample with the ELISA Standard Diluent. The test sample must be well mixed with the ELISA Standard Diluent.

- High target protein concentration (10000-100000pg/ml):Dilution: 1:100.(i.e. Add 1µl of sample into 99µl of ELISA Standard Diluent)
- Medium target protein concentration (1000-10000pg/ml):Dilution: 1:10.( i.e. Add 10µl of sample into 90µl of ELISA Standard Diluent)
- Low target protein concentration (15.6-1000pg/ml):Dilution: 1:2.( i.e. Add 50µl of sample into 50µl of ELISA Standard Diluent)
- Very low target protein concentration (outside the lower range of the assay), it is unnecessary to dilute, or dilute at 1:2.

***Assay Procedure***

Before adding to wells, equilibrate the ELISA Detection Reagent working solution and TMB substrate for at least 30 min at room temperature. When diluting samples and reagents, they must be mixed completely and evenly. It is recommended to plot a standard curve for each test.

1. Wash plate 2 times before adding standard, sample and control (zero) well as directed above.
2. Aliquot 0.1ml standard solutions into the standard wells. Perform in duplicate
3. Add 0.1 ml of ELISA Standard Diluent into the control (zero) wells.
4. Add 0.1 ml of properly diluted sample into test sample wells.
   ***NOTE:*** *See Sample Dilution Guideline above*
5. Seal the plate with a cover and incubate at 37°C for 90 min.
6. Remove the cover and discard the plate content, clap the plate on the absorbent filter papers or other absorbent material. Do NOT let the wells completely dry at any time.
   ***NOTE*****:** *Do not wash the plate at this time***.**
7. Add 0.1 ml of Biotinylated Detection Antibody working solution into the above wells (standard, test sample & zero wells). Add the solution at the bottom of each well without touching the side wall.
8. Seal the plate with a cover and incubate at 37°C for 60 min.
9. Remove the cover, and wash plate 3 times with Wash buffer.
10. Add 0.1 ml of ELISA Detection Reagent working solution into each well, cover the plate and incubate at 37°C for 30 min.

11. Remove the cover and wash plate 5 times with Wash buffer, and each time let the wash buffer stay in the wells for 1-2 min.
12. Add 90µl of ELISA Detection Substrate (TMB) into each well, cover the plate and incubate at 37°C in the dark for 15-30 min.
    ***NOTE**: This incubation time is for reference use only; the optimal time should be determined by end user.A blue color should be seen in the first 3-4 wells (with most concentrated standard solutions), the other wells show no obvious color change.*
13. Add 50µl of Stop solution into each well and mix thoroughly. The color changes from blue to yellow immediately.
14. Read the O.D. absorbance at 450 nm in a Microplate reader immediately after adding the stop solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of the samples can be interpolated from the standard curve.

***NOTE:** If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.*

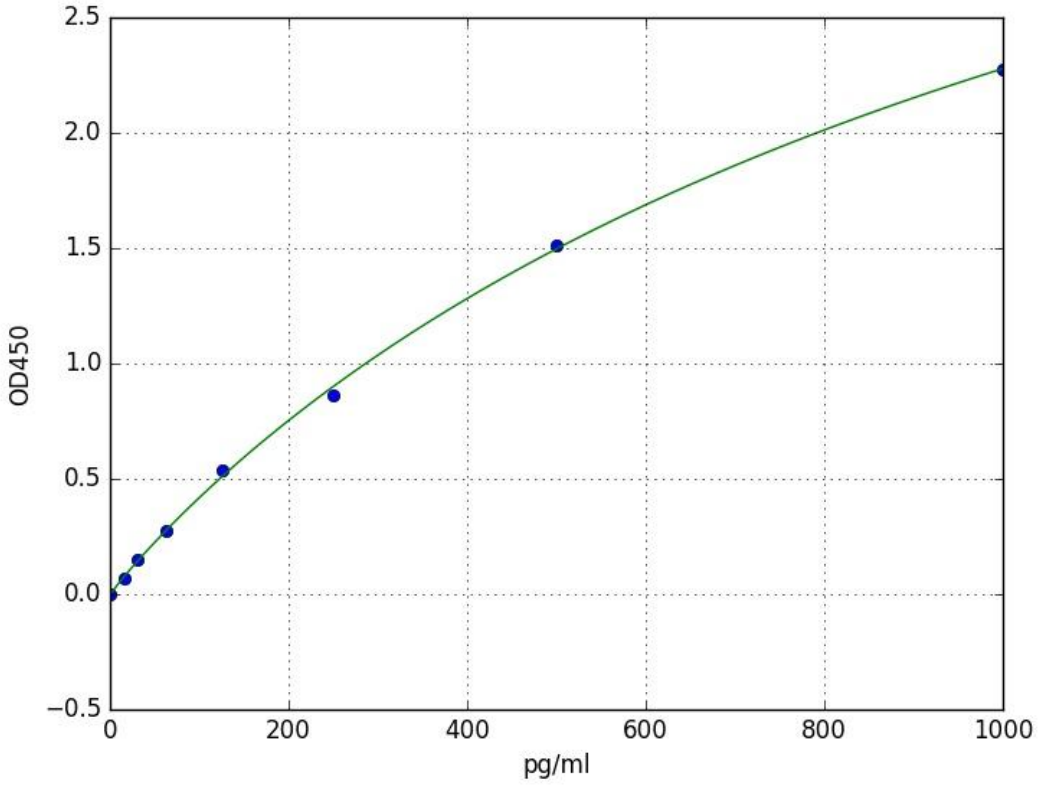
## PROTOCOL SUMMARY

1. Wash plate 2 times before adding standard, sample and control (zero) wells
2. Add 100µL standard or sample to each well for 90 minutes at 37°C
3. Add 100µL Biotinylated Detection Antibody working solution to each well for 60 minutes at 37°C
4. Aspirate and wash 3 times
5. Add 100µL ELISA Detection Reagent working solution to each well. Incubate for 30 minutes at 37°C
6. Aspirate and wash 5 times
7. Add 90µL ELISA Detection Substrate (TMB). Incubate 15 -30 minutes at 37°C
8. Add 50µL Stop Solution. Read at 450nm immediately
9. Calculation of results

## TYPICAL DATA & STANDARD CURVE

Results of a typical standard operation of an Acaca ELISA Kit are listed below. This standard curve was generated at our lab for demonstration purpose only. Users shall obtain standard curve as per experiment by themselves. (N/A=not applicable)

| X | pg/ml | 0 | 15.625 | 31.25 | 62.5 | 125 | 250 | 500 | 1000 |
|---|---|---|---|---|---|---|---|---|---|
| Y | OD450 | 0.0 | 0.066 | 0.148 | 0.272 | 0.536 | 0.865 | 1.514 | 2.275 |




## SPECIFICITY

This assay has high sensitivity and excellent specificity for detection of Acaca. No significant cross-reactivity or interference between Acaca and analogues was observed.

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

## RECOVERY

Matrices listed below were spiked with certain level of Acaca and the recovery rates were calculated by comparing the measured value to the expected amount of Acaca in samples.

| Matrix | Recovery Range (%) | Average (%) |
|---|---|---|
| Serum(n=5) | 85-105 | 93 |
| EDTA Plasma(n=5) | 85-99 | 91 |
| Heparin Plasma(n=5) | 89-102 | 96 |

## LINEARITY

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Acaca and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

| Sample | 1:2 | 1:4 | 1:8 | 1:16 |
|---|---|---|---|---|
| Serum (n=5) | 89-97% | 85-100% | 87-95% | 85-102% |
| EDTA Plasma (n=5) | 83-100% | 82-100% | 86-98% | 82-97% |

| | | | | |
|---|---|---|---|---|
| Heparin Plasma(n=5) | 85-100% | 87-100% | 83-86% | 85-94% |

## PRECISION

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Acaca were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Acaca were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/meanX100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

## STABILITY

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage condition.

| | | |
|---|---|---|
| Standard (n=5) | 37°C for 1 month | 4°C for 6 months |
| Average (%) | 80 | 95-100 |

To minimize extra influence on performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is strongly suggested that the same operator performs the whole assay from the beginning to the end.

## ELISA TROUBLESHOOTING GUIDE

| Problem | Possible Source | Solution |
|---|---|---|
| Poor Standard Curve | Improper standard solution | Confirm dilutions are made correctly |
| | Standard improperly reconstituted | Briefly spin vial before opening, inspect for undissolved material after reconstituting |
| | Standard degraded | Store and handle standard as recommended |
| | Curve does not fit scale | Try plotting using different scales e.g. log-log, 5 parameter logistic curve fit |
| | Pipetting error | Use calibrated pipettes and proper pipetting technique |
| | Standard was incompletely reconstituted or was inappropriately stored | Reconstitute standard according to protocol. Store reconstituted standard in appropriate vials. Store reconstituted standard at -70 °C |
| | Reagents added to wells with incorrect concentrations | Check for pipetting errors and correct reagent volume. |
| | Incubations done at inappropriate temperature, timing or agitation | Assay conditions need to be checked |
| No signal | Incubation time too short | Incubate samples overnight at 4°C or follow the manufacturer guidelines. |
| | Target present below detection limit of assay | Decrease dilution factor or concentrate samples. |
| | Incompatible sample type | Detection may be reduced or absent in untested sample types. Include a sample that the assay is known to detect a positive control. |
| | Recognition of epitope impeded | To enhance detection of a peptide by direct or indirect ELISA, |

| Problem | Possible Source | Solution |
|---|---|---|
| | by absorption to plate | conjugate peptide to a large carrier protein before coating onto the microtiter plate. |
| | Assay buffer compatibility | Ensure assay buffer is compatible with target of interest (e.g. enzymatic activity retained, protein interactions retained) |
| | Incorrect or no Detection Antibody was added | Add appropriate Detection Antibody and continue. |
| | Avidin-HRP was not added | Add Avidin-HRP according to protocol and continue. |
| | Substrate solution was not added | Add substrate solution and continue. |
| | Wash buffer contains sodium azide | Avoid sodium azide in the wash buffer. |
| | Multichannel pipette errors | Calibrate the pipettes. |
| | Plate washing was not adequate or uniform | Make sure pipette tips are tightly secured. Confirm all reagents are removed completely in all wash steps. |
| | Non-homogenous samples | Thoroughly mix samples before pipetting |
| | Samples may have high particular matter | Remove the particular matter by centrifugation. |
| | Cross-well contamination | When reusing plate sealers check that no reagent has touched the sealer. Care should be taken when using the same pipette tips used for reagent additions. Ensure that pipette tips do not touch the reagents on the plate. |
| | Not enough detection reagent | Increase concentration or amount of detection reagent following manufacturer guidelines. |
| | Sample prepared incorrectly | Ensure proper sample preparation/dilution. Samples may be incompatible with microtiter plate assay format. |
| | Insufficient antibody | Try different concentrations/dilutions of antibody |
| | Incubation temperature too low | Ensure the incubations are carried out at the correct temperature. All reagents including plate should be at room temperature or as recommended by the manufacturer before proceeding. |
| | Incorrect wavelength | Verify the wavelength and read plate again |
| | Plate washings too vigorous | Check and ensure correct pressure in automatic wash system. Pipette wash buffer gently if washes are done manually. |
| | Wells dried out | Do not allow wells to become dry once the assay has started. Cover the plate using sealing film or tape for all incubations |
| | Slow color developments of enzymatic reaction | Prepare substrate solution immediately before use. Ensure the stock solution has not expired and is not contaminated. Allow longer incubation. |
| High variation in samples and/or standards | Bubbles in wells | Ensure no bubbles are present prior to reading plate |
| | Wells not washed equally/thoroughly | Check that all ports of the plate washer are not obstructed. Wash wells as recommended. |
| | Incomplete reagent mixing | Ensure all reagents are mixed thoroughly |
| | Inconsistent pipetting | Use calibrated pipettes and proper technique to ensure accurate pipetting |
| | Edge effects | Ensure the plate and all reagents are at room temperature. |
| | Inconsistent sample preparation | Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaw cycles). |
| High background | Wells are insufficiently washed | Wash wells as per protocol recommendations |
| | Contaminated wash buffer | Prepare fresh water buffer |
| | Too much detection reagent | Ensure the reagent has been diluted properly or decrease the recommended concentration of detection reagent. |
| | Blocking buffer ineffective (e.g. | Try different blocking reagent and/or blocking reagent to wash |

| Problem | Possible Source | Solution |
|---|---|---|
| | detection reagent binds blocker; wells not completely blocked) | buffer. |
| | Salt concentration of incubation/wash buffers | Increasing salt concentrations may reduce non-specific and/or weak off-target interactions. |
| | Waiting too long to read plate after adding stop solution. | Read plate immediately after adding stop solution. |
| | Non-specific binding of antibody | Use suitable blocking buffers e.g. BSA or 5010% normal serum-species same as primary antibody if using a directly conjugated detection antibody or same as secondary if using conjugated. |
| | Background wells were contaminated | Avoid cross-well contamination by using the sealer appropriately. Use multichannel pipettes without touching the reagents on the plate. |
| | Matrix used has endogenous analyte or interference | Check the matrix ingredients for cross reacting components (e.g. interleukin modified tissue culture medium). |
| | TMB Substrate Solution was contaminated | TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells. |
| | High antibody concentration | Try different dilutions for optimal results |
| | Substrate incubation carried out in light | Substrate incubations should be carried out in the dark or as recommended by manufacturer. |
| | Precipitate formed in wells upon substrate addition. | Increase dilution factor of sample or decrease concentration of substrate |
| | Dirty plate | Clean the plate bottom. |
| Low Sensitivity | Improper storage of ELISA kit | Store all reagents as recommended. Please note that all reagents may not have identical storage requirements. |
| | Not enough target | Concentrate sample or reduce sample dilution |
| | Inactive detection reagent | Ensure reporter enzyme has the expected activity. |
| | Plate reader settings incorrect | Ensure plate reader is set to read the correct absorbance wavelength or excitation/emission wavelengths for fluorescent detection. |
| | Assay format not sensitive enough | Switch to a more sensitive detection system (e.g. colorimetric to chemiluminescence / fluorescence) Switch to a more sensitive assay type (e.g. direct ELISA to sandwich ELISA). Lengthen incubation times or increase temperature. |
| | Target poorly absorbs to microtiter plate | Covalently link target to microtiter plate. |
| | Not enough substrate | Add more substrate |
| | Incompatible sample type (e.g. serum vs. cell extract) | Detection may be reduced or absent in untested sample types. Include a sample that the assay is known to detect as a positive control. |
| | Interfering buffers or sample ingredients | Check reagents for any interfering chemicals. For example, sodium azide in antibodies inhibit HRP enzyme and EDTA used as anticoagulant for plasma collection inhibits enzymatic reactions. |
| | Mixing or substituting reagents from different kits | Avoid mixing components from different kits. |

## APPENDIX: SAMPLE COLLECTION AND STORAGE

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

- Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.
- Plasma: Collect plasma using EDTA-Na2 as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- Tissue homogenates: For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) With a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernatant. We recommend including ProteaseARREST™ (Cat. # 786-108), a protease inhibitor cocktail, during the homogenization.
- Cell culture supernatant: Centrifuge supernatant for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernatant and carry out the assay immediately. We recommend including TCM-ProteaseARREST™ (Cat. # 786-238), a tissue culture media protease inhibitor cocktail, during the homogenization.
- Other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2 - 8°C. Collect the supernatant and carry out the assay immediately.
- Sample preparation: Samples should be clear and transparent and be centrifuged to remove suspended solids.

***NOTE:*** *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. Hemolyzed samples are not suitable for use in this assay.*

## RELATED PRODUCTS

Download our Protein Assay Development or Bioassay Handbook

https://info2.gbiosciences.com/complete-assay-development-handbooke

https://info2.gbiosciences.com/complete-bioassay-handbook

*For other related products, visit our website at www.GBiosciences.com or contact us.*

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