ELISA Kit

Catalog # KHO1071 (96 tests)

Human

ACC1 (Total)

www.invitrogen.com
Invitrogen Corporation
542 Flynn Road, Camarillo, CA 93012
Tel: 800-955-6288
E-mail: techsupport@invitrogen.com

Table of Contents

Contents and Storage

Storage

Store at 2 to 8°C.

Contents

Reagents Provided	*96 Test Kit*
Human ACC1 (Total) Standard: Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials
Standard Diluent Buffer. Contains 0.1% sodium azide; red dye**; 25 mL per bottle.	1 bottle
*ACC1 Antibody Coated Well*s, 12x8 Well Strips	1 plate
Hu ACC1 (Total) Detection Antibody. Contains 0.1% sodium azide; blue dye**; 6 mL per bottle.	1 bottle
Anti-Rabbit IgG HRP Concentrate, (100X). Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial
HRP Diluent. Contains 3.3 mM thymol; yellow dye**; 25 mL per bottle.	1 bottle
Wash Buffer Concentrate (25X); 100 mL per bottle.	1 bottle
Stabilized Chromogen, *Tetramethylbenzidine (TMB)*; 25 mL per bottle.	1 bottle
Stop Solution; 25 mL per bottle.	1 bottle
Plate Covers, adhesive strips.	3
** In order to help our customers avoid any mistakes in pipetting the ELISAs, we provide colored *Standard Diluent Buffer*, *Detection Antibody*, and *HRP Diluent* to help monitor the addition of solution to the reaction well. This does not in any way interfere with the test results.	

Disposal Note

This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

Safety

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

Introduction

Purpose

The Invitrogen Human ACC1 (Total) ELISA is designed to detect and quantify the level of Human ACC1 protein. This assay is intended for the detection of ACC1 from lysates of human cells. This kit can be used to normalize the phosphorylated ACC1 content of samples when using the Invitrogen Human ACC1 [pS79] ELISA kit (Cat. # KHO1061).

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Principle of the Method

The Invitrogen Human ACC1 [Total] kit is a solid phase sandwich Enzyme Linked Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Human ACC1 (regardless of phosphorylation state) has been coated onto the wells of the microtiter strips provided. Samples, including a standard containing Human ACC1, control specimens, and unknowns, are pipetted into these wells and then a rabbit antibody specific for Human ACC1 is added to the wells. During the first incubation, the Human ACC1 protein binds to the immobilized (capture) antibody and the rabbit ACC1 antibody serves as a detection antibody by binding to the immobilized ACC1 protein. After the first incubation step and washing to remove excess protein and detection antibody, a horseradish peroxidase-labeled Anti-Rabbit IgG (Anti-Rabbit IgG HRP) is added. This binds to the detection antibody to complete the four-member sandwich. After a third incubation and washing to remove all the excess Anti-Rabbit IgG HRP, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Human ACC1 present in the original specimen.

Background Information

Acetyl-CoA carboxylase (ACC) is a biotin-dependent enzyme that catalyses the ATP-dependent carboxylation of acetyl-CoA to produce malonyl-CoA, the pivotal step in the fatty acid synthesis pathway, through its two catalytic activities, biotin carboxylase (BC) and carboxyltransferase (CT). Human genome contains the genes coding for two different ACCs – ACC1 and ACC2, they share 75% overall amino acid sequence identity. ACC2 has an extra 114 amino acids in the N-terminus comparing to ACC1, the first 20 of which are thought to direct ACC2 to the mitochondrial membrane. The 265 kDa ACC1 (2346 aa) is the predominant isoform in liver, adipose and mammary gland, while the 280 kDa ACC2 (2483 aa) is the major isoform in skeletal muscle and heart.

Both ACC1 and ACC2 convert acetyl-CoA, generated from the catabolism of proteins, carbohydrates, and fatty acids, into malonyl-CoA. In the liver, which is both oxidative and lipogenic, the malonyl-CoA formed in the cytoplasm through the actions of ACC1 is utilized for formation of fatty acids that can be stored or converted to triglycerides and phospholipids, and secreted as triglyceride-rich lipoproteins (e.g., VLDL) for transport to extra-hepatic tissues, whereas the malonyl-CoA formed at the mitochondrial surface through the actions of ACC2 acts as an allosteric inhibitor of CPT-I to prevent entry of fatty acids into the mitochondria for oxidation.

In humans and other animals, ACC activity is tightly regulated through a variety of dietary, hormonal, and other physiological responses, feedback inhibition by long-chain fatty acids, reversible phosphorylation, and modulation of enzyme production through altered gene expression. The activities of ACC is inhibited if phosphorylated; the phosphorylation takes place when the hormones, glucagon

or epinephrine bind to the receptors or the energy status of the cell is low, leading to the activation of the AMP-activated protein kinase. When insulin binds to its receptors of the cell, it activates a phosphatase to dephosphorylate the enzyme; the activity of ACC is thus enhanced. It has been determined that phosphorylation by AMPK at Ser79 inhibits the enzymatic activity of ACC.

Methods

Materials Needed But Not Provided

- Microtiter plate reader (at or near 450 nm) with software
- Calibrated adjustable precision pipettes
- Distilled or deionized water
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
- Glass or plastic tubes for diluting solutions
- Absorbent paper towels
- Calibrated beakers and graduated cylinders

Procedural Notes

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. Samples above the highest standard should be diluted with *Standard Diluent Buffer*.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. **Do not mix or interchange different reagent lots from various kit lots**.
10. Do not use reagents after the kit expiration date.
11. Absorbances should be read immediately, but can be read up to 2 hours of assay completion. For best results, keep plate covered in the dark.
12. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
13. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
14. Because Stabilized *Chromogen* is light sensitive, avoid prolonged exposure to light. Avoid contact between chromogen and metal, or color may develop.

Directions for Washing

- **Incomplete washing will adversely affect the test outcome**. All washing must be performed with *Wash Buffer Concentrate (25X)* provided.
- Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well. After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- If using an automated washer, follow the operating washing instructions carefully.

Procedure For Extraction Of Proteins From Cells Or Tissues

Recommended Formulation of Cell and Tissue Extraction Buffer:

- 10 mM Tris, pH 7.4
- 100 mM NaCl
- 1 mM EDTA
- 1 mM EGTA
- 1 mM NaF
- 20 mM $Na_4P_2O_7$
- 2 mM Na_3VO_4
- 1% Triton X-100
- 10% glycerol
- 0.1% SDS
- 0.5% deoxycholate

This Cell Extraction Buffer (Invitrogen, Cat. # FNN0011) needs the following items to be added:

- 1 mM PMSF (stock is 0.3 M in DMSO)
- Protease inhibitor cocktail (e.g., Sigma Cat. # P-2714) (reconstituted according to manufacturer's guideline). Add 500 µL per 5 mL Cell Extraction Buffer.

The Cell Extraction Buffer is stable for 2 to 3 weeks at 4°C or for up to 6 months when aliquoted (without protease inhibitors and PMSF added) and stored at -20°C. When stored frozen, the buffer should be thawed on ice. **Important:** add the protease inhibitors just before using. The stability of protease inhibitor supplemented Cell Extraction Buffer is 24 hours at 4°C. PMSF is very unstable and must be added prior to use, even if added previously.

The following protocol has been applied to several cell lines using this Cell Extraction Buffer. Researchers may optimize the cell extraction procedures that work best in their hands.

1. Collect cells in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent).
2. Wash cells twice with cold PBS.
3. Remove and discard the supernatant and collect the cell pellet. (At this point the cell pellet can be frozen at -80°C and lysed at a later date).
4. Lyse the cell pellet in Cell Extraction Buffer for 30 minutes on ice with vortexing at 10 minute intervals. The volume of Cell Extraction Buffer depends on the cell number in cell pellet and expression of ACC1. For example, $1x10^7$ HepG2 cells grown in DMEM (Invitrogen Cat. # P104-500) plus 10% FBS can be extracted in 1 mL of Extraction Buffer. Under these conditions, use of 1-10 mL of the clarified cell extract diluted to a volume of 50 µL/well in *Standard Diluent Buffer* (See **Assay Procedure**) is sufficient for the detection of ACC1.
5. Transfer extracts to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4^oC.
6. Aliquot the clear lysate to clean microcentrifuge tubes. These samples are ready for assay. Lysates can be stored at -80^oC. Avoid multiple freeze-thaw cycles.

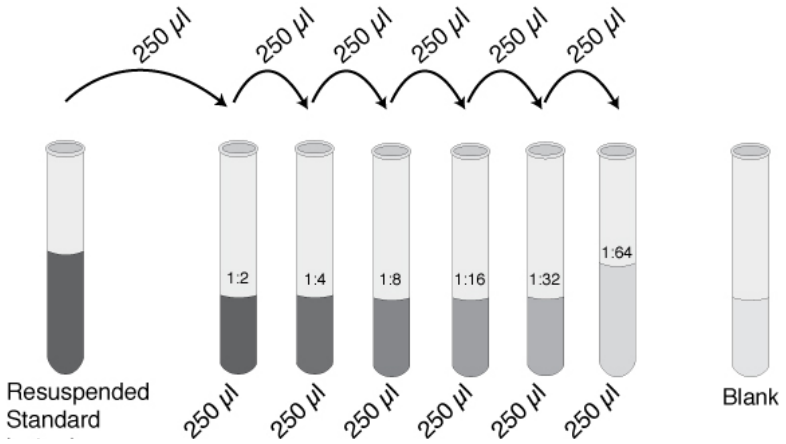
Preparation of Reagents

Dilution of Standard

Note: The *Hu ACC1 [Total] Standard* was prepared from recombinant protein of Human ACC1 c-terminal region expressed in E Coli.

1. Reconstitute *Hu ACC1 [Total] Standard* with *Standard Diluent Buffer*. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Label as 10 ng/mL Human ACC1 [Total]. Use the standard within 1 hour of reconstitution.
2. Add 0.25 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 5, 2.5, 1.25, 0.625, 0.313, and 0.157 ng/mL of Human ACC1 [Total].
3. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

Remaining reconstituted standard should be discarded or frozen in aliquots at -80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



Preparing IgG-HRP

Please Note: The *Anti-Rabbit IgG HRP (100X)* is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow *Anti-Rabbit IgG HRP (100X)* to reach room temperature. Gently mix. Pipette *Anti-Rabbit IgG HRP (100X)* slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

Dilute 10 µL of this 100X concentrated solution with 1 mL of *HRP Diluent* for each 8-well strip used in the assay. Label as Anti-rabbit IgG-HRP Working Solution. Return the unused *Anti-Rabbit IgG HRP (100X)* to the refrigerator. Note: Prepare within 15 minutes of usage, as activity decreases.

# of 8-Well Strips	Volume of Anti-Rabbit IgG HRP (100X)	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

Dilution of Wash Buffer

1. Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

2. Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

Assay Procedure

Be sure to read the *Procedural Notes* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 50 µL of the *Standard Diluent Buffer* to zero wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 50 µL of standards and diluted samples or controls to the appropriate microtiter wells. Standards, samples, and controls will have a red color. Samples prepared in Cell Extraction Buffer must be diluted 1:5 or greater in *Standard Diluent Buffer* (for example, 10 µL sample into 40 µL buffer). While a 1:5 sample dilution has been found to be satisfactory, higher dilutions such as 1:25 or 1:50 may be optimal. The dilution chosen should be optimized for each experimental system. Tap gently on side of plate to mix.
4. Pipette 50 µL *Human ACC1 (Total) Detection Antibody* solution into each well except the chromogen blank(s). Tap gently on the side of the plate to thoroughly mix.
5. **Cover wells with *plate cover* and incubate for 3 hours at room temperature.**
6. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
7. Add 100 µL *Anti-Rabbit IgG HRP* Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in **Preparing IgG-HRP)**.
8. Cover wells with the *plate cover* and incubate for **30 minutes at room temperature**.
9. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
10. Add 100 µL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
11. Incubate for **30 minutes at room temperature and in the dark. *Please Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceeds the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
12. Add 100 µL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.

13. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.
14. Use a curve fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit.
15. Read the concentrations for unknown samples and controls from the standard curve. (Samples producing signals greater than that of the highest standard should be diluted in *Standard Diluent Buffer* and reanalyzed. Multiply value(s) obtained for sample(s) by the appropriate dilution factor to correct for the dilution in step 3.

Typical Data (Example)

The following data were obtained for the various standards over the range of 0 to 10 ng/mL Human ACC1 [Total].

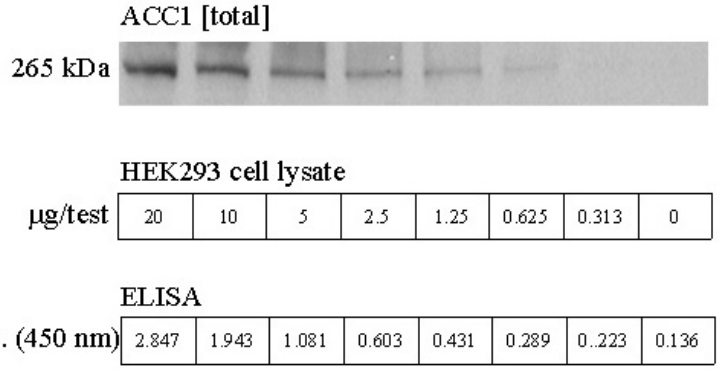
Standard Human ACC1 [Total] (ng/mL)	Optical Density (450 nm)
10	3.067
5	2.027
2.5	1.301
1.25	0.775
0.625	0.459
0.313	0.305
0.157	0.205
0	0.117

Performance Characteristics

Sensitivity

The analytical sensitivity of this assay is < 0.05 ng/mL of Human ACC1 [Total]. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

The sensitivity of this ELISA was compared to Western blotting using known quantities of Human ACC1 [Total]. The data presented below show that the sensitivity of the ELISA is approximately 2x greater than that of Western blotting. The bands shown in the Western blot data were developed using rabbit anti-Human ACC1 [Total], and an alkaline phosphatase conjugated anti-rabbit IgG followed by chemiluminescent substrate and autoradiography.



Precision

1. Intra-Assay Precision

Samples of known Human ACC1 [Total] concentration were assayed in replicates of 12 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	6.99	1.58	0.40
SD	0.25	0.04	0.01
%CV	3.61	2.51	3.77

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 36 times in multiple assays to determine precision between assays

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	6.82	1.65	0.38
SD	0.36	0.08	0.03
%CV	5.29	5.01	7.88

SD = Standard Deviation
CV = Coefficient of Variation

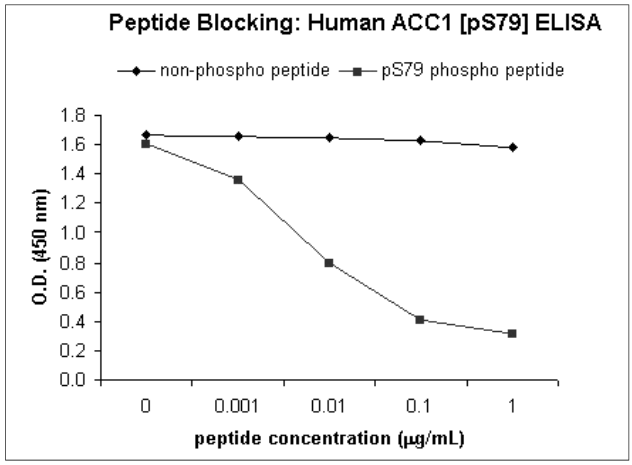
Recovery

To evaluate recovery, Human ACC1 [Total] Standard was spiked at 3 different concentrations into 5% Cell Extraction Buffer. The percent recovery was calculated as an average of 97%.

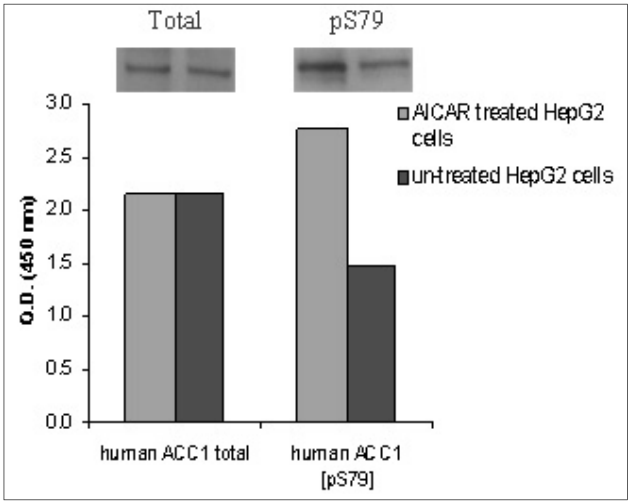
Specificity

The Invitrogen Human ACC1 [pS79] ELISA recognizes Human ACC1 phosphorylated at serine residue 79. It does not cross react with mouse and rat ACC1.

The specificity of this assay for phosphorylated ACC1 [pS79] was confirmed by peptide competition. The data presented below show that only the phospho-peptide containing the phosphorylated serine 79 could block the ELISA signal. The non-phosphorylated peptide did not block the signal.



In the figure below, HepG2 cells were treated with 2µM AICAR for 60 minutes. Untreated HepG2 cells were used as control. Cell extracts were prepared and analyzed with the Human ACC1 [pS79] (Cat. # KHC1061) and Human ACC1 [Total] ELISA. The results show that compared to untreated control, the Ser79 phosphorylation of ACC1 is up-regulated in AICAR treated HepG2 cells, whereas the total level of ACC1 remains constant.



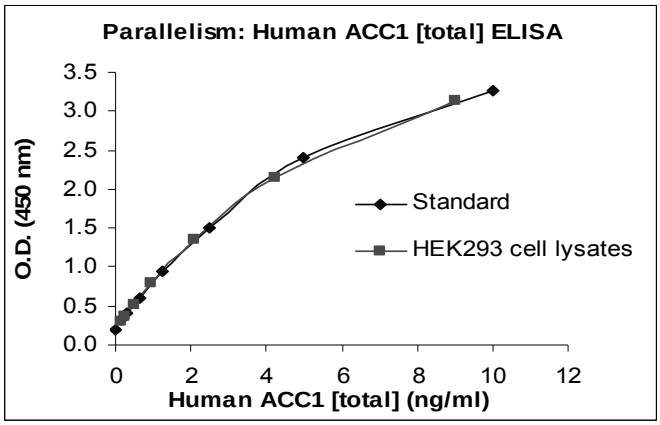
Linearity of Dilution

HEK 293 cells were grown in DMEM (Invitrogen Cat. # P104-500) containing 10% fetal bovine serum at 37°C, lysed with Cell Extraction Buffer. This lysate was diluted in *Standard Diluent Buffer* over the range of the assay and measured for Human ACC1 [Total] content. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

	Cell Lysate		
Dilution	**Measured (ng/mL)**	**Expected (ng/mL)**	**% Expected**
Neat	4.212	4.212	100
1/2	2.072	2.106	98.4
1/4	0.959	1.053	91.1
1/8	0.501	0.527	95.1
1/16	0.251	0.263	95.4

Parallelism

Natural Human ACC1 [Total] from HEK 293 cell lysate was serially diluted in *Standard Diluent Buffer.* The optical density of each dilution was plotted against the Human ACC1 [Total] standard curve. Parallelism was demonstrated by the figure below and indicated that the standard accurately reflects Human ACC1 [Total] content in samples.



Limitations of the Procedure

Do not extrapolate the standard curve beyond top standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute all samples above the highest standard point with *Standard Diluent Buffer*; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various extraction buffers has not been thoroughly investigated. The rate of degradation of native human ACC1 in various matrices has not been investigated.

Appendix

Troubleshooting Guide

Standard curve wells develop, but sample wells produce weak or no signal.	*Cause:* Improper sample preparation. *Solution 1:* Make sure to prepare cell extracts in the protease inhibitor-supplemented Cell Extraction Buffer recommended in the protocol booklet. Other buffer formulations have not been evaluated. *Cause:* Samples contain materials that interfere with the assay. *Solution 1:* The Cell Extraction Buffer recommended in the protocol booklet contains SDS. This detergent can potentially interfere with the immunoassay. For this reason, we recommend that all samples be diluted by a factor of at least 1:10 using the Standard Diluent Buffer provided in the kit. This buffer also contains blocking proteins that will reduce background signal. *Cause:* The concentration of the target analyte is too dilute. *Solution 1:* When preparing the cell extracts, increase the number of cells per volume of protease inhibitor supplemented Cell Extraction Buffer. Ideally, the concentration of protein in each cell extract, as determined by the Quant-iT™ protein assay kit, will be between 1 and 10 mg/ml (Method 1) or 1 and 5 mg/ml (Method 2). *Solution 2:* Optimize the stimulation procedure and time. *Cause:* A sample treatment step was not performed. *Solution 1:* Certain analytes (e.g., ERK1/2 [pTpY185/187] and ERK1/2 Total) require a sample treatment step to improve performance with Invitrogen phosphoELISA™ kits. Please see the analyte-specific protocol booklet for information on sample treatment procedures. *Cause:* Samples deteriorated during storage. *Solution 1:* Make sure that the Cell Extraction Buffer is supplemented with phosphatase inhibitors and protease inhibitors just prior to use. *Solution 2:* All samples should be stored frozen at –80°C. *Solution 3:* Samples should be subjected to only one freeze-thaw cycle. *Solution 4:* Some proteins can be lost by absorption when stored in containers made of polystyrene or certain kinds or glass. Polypropylene tubes are best for storing samples.
Sample wells develop, but standard wells produce weak or no signal.	*Cause:* Improper dilution of standards. *Solution 1:* Check reconstitution volume of standard. *Solution 2:* Standard curves are generated by serially diluting the reconstituted standard. Check the serial dilution method. *Solution 3:* Standards should be used within an hour of reconstitution and serial dilution. *Cause:* Improper storage of standards. *Solution 1:* Standards are provided as lyophilized powders that should be stored at 2–8°C.

Neither the standard curve wells nor the sample wells develop.	*Cause:* Insufficient Anti-Rabbit IgG HRP secondary antibody activity. *Solution 1:* Check the dilution of the Anti-Rabbit IgG HRP Working Solution. *Solution 2:* The Anti-Rabbit IgG HRP (100X) must be freshly diluted for each assay. *Solution 3:* The Anti-Rabbit IgG HRP (100X) must be stored at 2–8°C. *Solution 4:* Sodium azide is an irreversible inhibitor of horseradish peroxidase enzyme activity. Make sure to dilute the Anti-Rabbit IgG HRP (100X) in the correct buffer. A quick test can be performed to determine if the Anti-Rabbit IgG HRP (100X) is active. Into a clean test tube, dispense 200 µl of the TMB substrate solution provided in the kit. This TMB substrate solution should be clear to slightly blue-green tinted. Next, pipette 2 µl of the Anti-Rabbit IgG HRP (100X). The color of the TMB will change to an intense aqua blue instantaneously if the HRP has retained its enzyme activity. *Cause:* Insufficient Detector Antibody. *Solution 1:* The Detector Antibody must be stored at 2–8°C. *Solution 2:* Improper dilution of Detector Antibody. *Cause:* TMB solution lost activity. *Solution 1:* The TMB solution should be clear before it is dispensed into the wells of the microtiter plate. An intense aqua blue color indicates that the product is contaminated. Please contact our Technical Support Department if this problem is noted. To avoid contamination, we recommend that the quantity required for an assay be dispensed into a disposable trough for pipetting. Any TMB solution left in the trough should be discarded. *Solution 2:* Avoid contact of the TMB solution with items containing metal ions.
Standard curves are not developing consistently in between different runs.	*Cause:* Improper dilution of Anti-Rabbit IgG HRP (100X) *Solution 1:* The Anti-Rabbit IgG HRP (100X) is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow Anti-Rabbit IgG HRP (100X) to reach room temperature. Gently mix. Pipette concentrate slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

Technical Support

Contact Us

For more troubleshooting tips, information, or assistance, please call, email, or go online to www.invitrogen.com/ELISA.

US:

Invitrogen Corporation
542 Flynn Rd
Camarillo, CA 93012

Tel: 800-955-6288

E-mail: techsupport@invitrogen.com

Europe:

Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK

Tel: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6117

E-mail: eurotech@invitrogen.com

References

1. Levert, K., et al. (2002) A biotin analog inhibits acetyl-CoA carboxylase activity and adipogenesis. J. Biol. Chem. 277:16347-16350.
2. Sinilnikova, O.M., et al. (2004) Acetyl-CoA carboxylase alpha gene and breast cancer susceptibility. Carcinigenesis 25:2417-2424.
1. Moreau, K., et al. (2006) BRCA1 affects lipid synthesis through its interaction with acetyl-CoA carboxylase. J. Biol. Chem. 281:3172-3181.
2. Tong, L., et al. (2006) Acetyl-coenzyme A carboxylases: versatile targets for drug discovery. J Cell Biochem. 99:1476-1488.
3. Harada, N., et al. (2007) Hepatic De Novo lipogenesis is present in liver-specific ACC1-deficient mice. Mol. Cell. Biol. 27:1881-1888.
4. Brownsey, R.W., et al. (2006) Regulation of acetyl-CoA carboxylase. Bioch. Society Trans. 34:223-227.
5. Abu-Elheiga, L., et al. (2001) Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2. Science 291:2613-1616.
6. Abu-Elheiga, L., et al. (2005) Mutant mice lacking acetyl-CoA carboxylase 1 are embryonically lethal. Proc Natl. Acad. Sci. USA 102:12011–12016.
7. Brusselmans, K., et al. (2005) RNA interference-mediated silencing of the acetylCoA-carboxylase-alpha gene induces growth inhibition and apoptosis of prostate cancer cells. Cancer Res. 65:6719-6725.
8. Freiberg, C., et al. (2004) Identification and characterization of the first class of bacterial acetyl-CoA carboxylase inhibitors with antibacterial activity. J. Biol. Chem. 279:26066–26073.
9. Furler, S.M., et al. (2006) The ACC inhibitor CP-640186 acutely increases muscle fatty acid clearance independent of glucose clearance and cellular .energy demand. Diabetes 55:A333. Nature 272:182-186.
10. Harwood, H.J. Jr., et al. (2005) Treating the metabolic syndrome: Acetyl-CoA carboxylase inhibition. Expert Opin. Ther. Targets 9:267-281.
11. Savage, D.B., (2006) Reversal of diet-induced hepatic steatosis and hepatic insulin resistance by antisense oligonucleotide inhibitors of acetyl-CoA carboxylases 1 and2. J. Clin. Invest. 116:817-824.

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, please contact our Technical Support Representatives. Invitrogen warrants that all of its products will perform according to the specifications stated on the Certificate of Analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order. Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Support Representatives. **Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.**

Licensing Information

These products may be covered by one or more Limited Use Label Licenses (see the Invitrogen Catalog or our website, www.invitrogen.com). By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.

Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

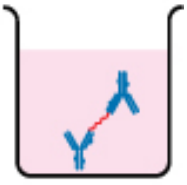
Human ACC1 [Total] Assay Summary

Sample type:
Standard

Sample type:
Cell Extract/Control
(>1:5 dilution)

Add 50 µl of Standard or sample

Add 50 µl of Detection Antibody and incubate for 3 hours at RT

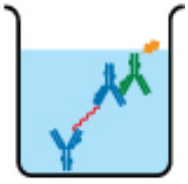


aspirate and wash 4x

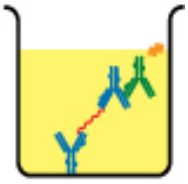
Incubate 100 µl of HRP Anti-Rabbit Antibody for 30 minutes at RT

aspirate and wash 4x

Incubate 100 µl of Stabilized Chromogen for 30 minutes at RT



Add 100 µl of Stop Solution and read at 450 nm



Total time: 4 hours