ELISA Kit
Catalog #KHO0511

IRS-1
(Total)

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

TABLE OF CONTENTS

INTRODUCTION

Insulin receptor substrate-1 (IRS-1), a cytoplasmic adaptor protein with M_r=165 kDa, plays a key role in mediating metabolic and proliferative signaling arising from stimulation by insulin, IGF-1, IGF-2, and cytokines such as IL-4. Human IRS-1 is encoded by a single gene located on chromosome 2 (2q36-37). IRS-1 bears homology with other insulin receptor substrates, including IRS-2, IRS-3 (a mouse homolog absent from humans), and IRS-4. IRS-1 is characterized by the presence of a pleckstrin homology domain that mediates its association with the plasma membrane located at its N-terminus, followed by a phosphotyrosine binding domain that mediates interaction with other proteins. IRS-1 is also characterized by the presence of PEST sequences which may play a role in targeting the protein for rapid degradation, and 21 putative tyrosine phosphorylation sites, many of which are contained within SH2-binding motifs.

IRS-1 protein expression levels increase as preadipocytes mature into adipocytes. Hormones such as estradiol also upregulate the expression of IRS-1.

In response to insulin or IGF-1 stimulation, IRS-1 is phosphorylated at numerous sites by the activated receptor protein tyrosine kinase. IRS-1 phosphorylated in response to receptor activation then binds to numerous other proteins. IRS-1's binding to the 85 kDa regulatory subunit of phosphatidylinositol 3-kinase (PI3-K) results in activation of Akt, which in turn exerts a strong proliferative signal, and possibly regulation of the glucose transporter GLUT4. IRS-1's binding to Grb-2 results in activation of MAPK pathways and changes in gene expression. Other proteins that associate with IRS-1 following insulin or IGF-1 stimulation include SHP-2, Fyn, and Nck.

IRS-1 is also phosphorylated on several serine residues, including serine 312 and 616 of the human IRS-1 sequence (corresponding to serine 307 and 612 in the mouse). These phosphorylation events appear to be dependent on pathways that include PI3-K, JNK, and mTOR, and function to negatively regulate metabolic insulin signaling pathways.

Knock-out studies in mice have revealed several interesting functions of IRS-1. IRS-1 is important for growth. Mice that are homozygous for the targeted disruption of IRS-1 are born alive, but their growth is negatively impacted, both as embryos and after birth. An analysis of the organ systems of these mice revealed that skeletal muscle and liver growth require IRS-1, while the growth of brain, small intestine, and spleen do not require this protein. IRS-1 is critical to the anti-apoptotic function of IGF-1. A brown pre-adipocyte cell line derived from the knock-out mice are less protected from the adverse effect of serum withdrawal, as assessed by the percentage of cells undergoing apoptosis. Studies with these knock-out mice have also indicated that IRS-1 is important for mediating glucose-lowering effect of insulin, IGF-1 and IGF-2.

The Invitrogen IRS-1 (Total) ELISA is designed to detect and quantify the level of IRS-1 protein. This assay is intended for the detection of IRS-1 from lysates of human, mouse, and rat cells. This kit can be used to normalize the phosphorylated IRS-1 content of samples when using the Invitrogen IRS-1 [pS312] ELISA kit (Cat. # KHO0521).

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

Read entire protocol before use.

PRINCIPLE OF THE METHOD

The Invitrogen IRS-1 (Total) kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for IRS-1 (regardless of phosphorylation state) has been coated onto the wells of the microtiter strips provided. Samples, including a standard containing IRS-1, control specimens, and unknowns, are pipetted into these wells. During the first incubation, the IRS-1 antigen binds to the immobilized (capture) antibody. After washing, a rabbit antibody specific for IRS-1 is added to the wells. During the second incubation, this antibody serves as a detection antibody by binding to the immobilized IRS-1 protein captured during the first incubation. After removal of excess 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 IRS-1 present in the original specimen.

REAGENTS PROVIDED

Note: *Store all reagents at 2 to 8°C.*

Reagent	***96 Test Kit***
IRS-1 (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
*IRS-1 (Total) Antibody Coated Well*s. 12 x 8 Well Strips.	1 plate
IRS-1 (Total) Detection Antibody. Contains 0.1% sodium azide; blue dye*; 11 mL per bottle.	1 bottle
Anti-Rabbit IgG HRP (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 Chromoge*n, *Tetramethylbenzidine (TMB);* 25 mL per bottle.	1 bottle
*Stop Solutio*n; 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.

SUPPLIES REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Cell Extraction Buffer (see Recommended Formulation, p.12).
4. Distilled or deionized water.
5. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
6. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semilog, as desired.
7. Glass or plastic tubes for diluting and aliquoting standard.
8. Absorbent paper towels.
9. Calibrated beakers and graduated cylinders in various sizes.

PROCEDURAL NOTES/LAB QUALITY CONTROL

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 bag.** 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 frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
4. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
5. It is recommended that all standards, controls and samples be run in duplicate.
6. Extracted cell lysate samples containing IRS-1 protein should be diluted with *Standard Diluent Buffer* at least 1:10. This dilution is necessary to reduce the matrix effect of the Cell Extraction Buffer.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
8. Cover or cap all reagents when not in use.
9. **Do not mix or interchange different reagent lots from various kit lots**.
10. Do not use reagents after the kit expiration date.
11. Read absorbances within 2 hours of assay completion.
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. Also avoid contact between *Stabilized Chromogen* and metal, or color may develop.

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.

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 (aspiration device) 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 METHOD**. 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, the operating instructions for washing equipment should be carefully followed. If your automated washer allows, 30 second soak cycles should be programmed into the wash cycle.

PROCEDURE FOR EXTRACTION OF PROTEINS FROM CELLS OR TISSUES

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

This 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 Cell Extraction Buffer should be thawed on ice. This buffer (minus protease inhibitor cocktail and PMSF) can be obtained from Invitrogen , Cat. # FNN0011. **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.

B. Protocol for Cell Extraction

This protocol has been applied to several cell lines with the Cell Extraction Buffer above. 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^{o}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 IRS-1. For example, 4x10^{6} MCF-7 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 µL of the clarified cell extract diluted to a volume of 100 µL/well in *Standard Diluent Buffer* (See **Assay Method**) is sufficient for the detection of IRS-1.
5. Transfer extracts to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4^{o}C.
6. Aliquot the clear lysate to clean microfuge tubes. These samples are ready for assay. Lysates can be stored at -80^{o}C. Avoid multiple freeze-thaw cycles.

REAGENT PREPARATION AND STORAGE

A. Reconstitution and Dilution of IRS-1 (Total) Standard

Note: The *IRS-1 (Total) Standard* was prepared from recombinant IRS-1 C-terminal region.

1. Reconstitute *IRS-1 (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 20 ng/mL IRS-1. Use the standard within 1 hour of reconstitution.
2. Add 0.25 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 10, 5, 2.5, 1.25, 0.63, and 0.31 ng/mL of IRS-1.
3. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

B. Dilution of IRS-1 (Total) Standard

Standard:	Add:	Into:
20 ng/mL	Prepare as described in step 1	
10 ng/mL	0.25 mL of the 20 ng/mL std.	0.25 mL of the Diluent Buffer
5 ng/mL	0.25 mL of the 10 ng/mL std.	0.25 mL of the Diluent Buffer
2.5 ng/mL	0.25 mL of the 5 ng/mL std.	0.25 mL of the Diluent Buffer
1.25 ng/mL	0.25 mL of the 2.5 ng/mL std.	0.25 mL of the Diluent Buffer
0.63 ng/mL	0.25 mL of the 1.25 ng/mL std.	0.25 mL of the Diluent Buffer
0.31 ng/mL	0.25 mL of the 0.63 ng/mL std.	0.25 mL of the Diluent Buffer
0 ng/mL	0.25 mL of the Diluent Buffer	An empty tube

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

C. Storage and Final Dilution of Anti-Rabbit IgG HRP (100X)

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.

1. 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.

For Example:

# 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

2. Return the unused *Anti-Rabbit IgG HRP (100X)* to the refrigerator.

D. Dilution of Wash Buffer

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.

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

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the *Procedural Notes/Lab Quality Control* 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 100 µL of the *Standard Diluent Buffer* to zero wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 100 µL of standards, 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 1:10 or greater in *Standard Diluent Buffer* (for example, 10 µL sample into 90 µL buffer). While a 1:10 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 thoroughly mix.

4. Cover plate with *plate cover* and incubate for **2 hours at room temperature.**
5. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
6. Pipette 100 µL of *IRS-1 (Total) Detection Antibody* solution into each well except the chromogen blank(s). This solution will have a blue color. Tap gently on the side of the plate to mix.
7. Cover plate with *plate cover* and incubate for **1 hour at room temperature.**
8. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
9. Add 100 µL Anti-Rabbit IgG HRP Working Solution to each well except the chromogen blank(s). This solution will have a yellow color. (Prepare the working dilution as described in **REAGENT PREPARATION AND STORAGE**, Section C.)
10. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.
11. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
12. Add 100 µL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
13. 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 exceed 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.

14. 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.
15. 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.*
16. Plot the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from *all* data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
17. Read the IRS-1 (Total) concentrations for unknown samples and controls from the standard curve plotted in step 16. **Multiply value(s) obtained for sample(s) by the dilution factor to correct for the dilution in step 3.** (Samples still producing signals higher than the highest standard (20 ng/mL) should be further diluted in *Standard Diluent Buffer* and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 20 ng/mL IRS-1 (Total).

Standard IRS-1 (ng/mL)	Optical Density (450 nm)
20	2.67
10	1.34
5	0.74
2.5	0.39
1.25	0.26
0.63	0.17
0.31	0.14
0	0.09

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 20 ng/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >20 ng/mL 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 IRS-1 in various matrices has not been investigated.

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

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The analytical sensitivity of this assay is <260 pg/mL of IRS-1 (Total). This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times. Using MCF-7 cells, this level of sensitivity was equivalent to the detection of IRS-1 in 3000 cells.

The sensitivity of this ELISA was compared to Western blotting using known quantities of IRS-1. The data presented in Figure 1 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-IRS-1, and an alkaline phosphatase conjugated anti-rabbit IgG followed by chemiluminescent substrate and autoradiography.

Figure 1: Detection of IRS-1 (Total) by ELISA vs Western Blot:

IRS-1 (Total)
(165 kDa)

ELISA: OD 450 nm	2.67	1.34	0.74	0.39	0.26	0.17	0.14	0.09
MCF-7 lysate (ug/test)	10	5	2.5	1.25	0.63	0.31	0.15	0

PRECISION

1. Intra-Assay Precision

Samples of known IRS-1 concentration were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	9.17	2.92	1.05
SD	0.43	0.24	0.08
%CV	4.69	8.21	7.62

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 (ng/mL)	9.08	3.23	1.09
SD	0.46	0.30	0.07
%CV	5.07	9.28	6.42

SD = Standard Deviation
CV = Coefficient of Variation

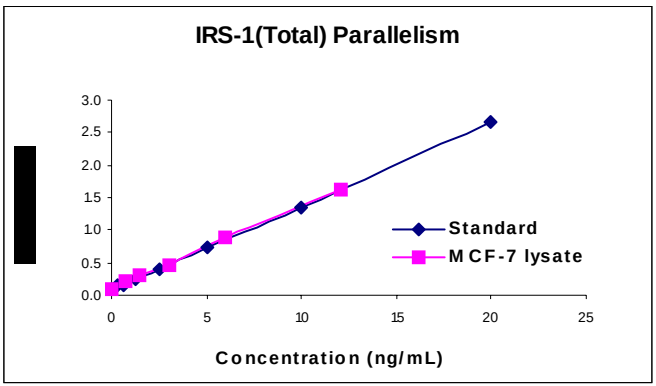
RECOVERY

To evaluate recovery, IRS-1 (Total) Standard was spiked at 3 different concentrations into 20% Cell Extraction Buffer. The percent recovery was calculated as an average of 99%.

PARALLELISM

Natural IRS-1 from MCF-7 cell lysate was serially diluted in *Standard Diluent Buffer*. The optical density of each dilution was plotted against the IRS-1 standard curve. Parallelism was demonstrated by the figure below and indicated that the standard accurately reflects IRS-1 content in samples.

Figure 2



LINEARITY OF DILUTION

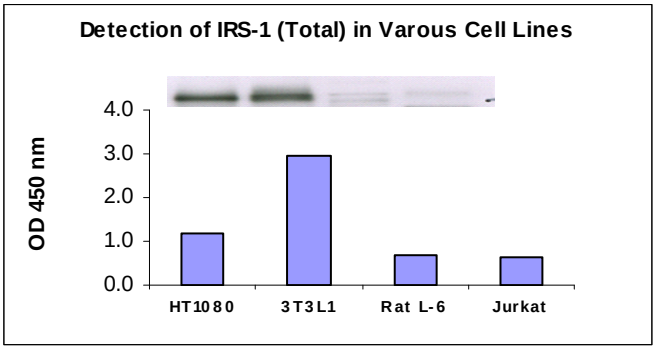
MCF-7 cells were grown in DMEM (Invitrogen Cat. # P104-500) containing 10% fetal bovine serum at 37°C and lysed with Cell Extraction Buffer. This lysate was diluted in *Standard Diluent Buffer* over the range of the assay and measured for IRS-1 content. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

	Cell Lysate		
Dilution	**Measured (ng/mL)**	**Expected (ng/mL)**	**% Expected**
Neat	12.07	12.07	100
1/2	6.29	6.04	104.3
1/4	3.08	3.02	102.3
1/8	1.69	1.51	112.3
1/16	0.93	0.75	122.9

SPECIFICITY

The IRS-1 ELISA kit is specific for the measurement of IRS-1 (Total) protein. To determine the specificity of this ELISA kit, cell extracts from different cell lines, each at a final concentration of 200 µg/mL were analyzed. The data presented in Figure 3 show that the kit detects IRS-1 in cell lysates from human Jurkat, HT1080, mouse 3T3L1 and rat L6. The levels of IRS-1 protein detected with this ELISA kit are consistent with results obtained by Western blot analysis (see inset in Figure 3).

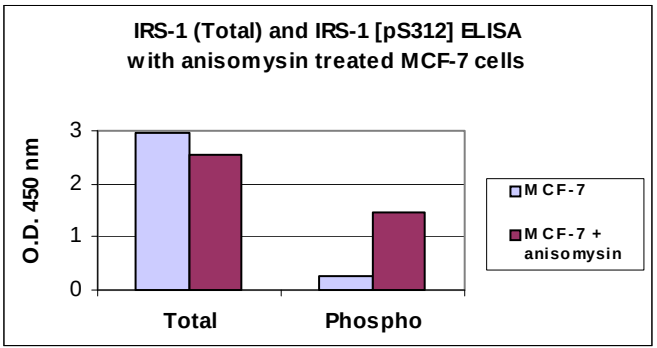
Figure 3



The IRS-1 (Total) ELISA kit is specific for measurement of IRS-1 (Total) protein. The following proteins were tested in the assay and found to have no cross-reactivity: IGF-1R, EGFR, FAK, Src, AKT, ERK.

In Figure 4, MCF-7 cells were treated with anisomysin (100 μM) plus calyculin (50 nM) for 40 minutes. Untreated MCF-7 cells were used as control. Cell extracts were prepared in Cell Extraction Buffer (Cat. # FNN0011). 100 μL of each cell lysate (100 μg/mL) was analyzed with IRS-1 (Total) ELISA and Invitrogen 's IRS-1 [pS312] ELISA (Cat. # KHO0521). The results show that the phosphorylation of IRS-1 [pS312] is upregulated in anisomysin treated MCF-7 cells, whereas the level of IRS-1 (Total) remains approximately the same in anisomysin treated and untreated control.

Figure 4



This "Total" assay is designed to allow normalization of IRS-1(Total) content among samples to permit interpretation of results from the phosphorylation site specific IRS-1 [pS312] ELISA kit available from Invitrogen (Cat. # KHO0521).

REFERENCES

1. Amoui, M., et al. (2001) Differential phosphorylation of IRS-1 by insulin and insulin-like growth factor 1 receptors in Chinese hamster ovary cells. J. Endocrinol. 171:153-162.
2. Federici, M., et al. (2001) The common Arg972 polymorphism in insulin receptor substrate-1 causes apoptosis of human pancreatic islets. FASEB J. 15:22-24.
3. Favelyukis, S., et al. (2001) Structure and autoregulation of the insulin-like growth factor 1 receptor kinase. Nat. Struct. Bio. 8:1058-1063.
4. Kriauciunas, K.M., et al. (2000) Cellular compartmentalization in insulin action: Altered signaling by a lipid-modified IRS-1. Mol. Cell. Biol. 20(18):6849-6859.
5. Molloy, C.A., et al. (2000) Insulin receptor substrate-1 expression is regulated by estrogen in the MCF-7 human breast cancer cell line. J. Biol. Chem. 275:12565-12571.
6. Sesti, G., et al. (2001) Defects of the insulin receptor substrate (IRS) system in human metabolic disorders. FASEB J. 15:2099-2111.
7. Sun, X.J., et al. (1991) Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. Nature 352:73-77.
8. Tamemoto, H., et al. (2002) Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. Nature 272:182-186.
9. Tseng, Y.-H., et al. (2002) Differential roles of insulin receptor substrates in the anti-apoptotic function of insulin-like growth factor-1 and insulin. J. Biol. Chem. 277:31601-31611.

Important 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.		

IRS-1 (Total) Assay Summary

Incubate 100 µL Standard, Cell or Tissue Extract (>1:10) for 2 hours at RT

aspirate and wash 4x

Incubate 100 µL of Detection Antibody for 1 hour 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