IRS-1 (Total) ELISA Kit

Catalog Number KHO0511 (96 tests)

Pub. No. MAN0014942 **Rev.** 2.0 (30)

CAUTION! 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.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ IRS-1 (Total) ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the total level of IRS-1 in cell lysates from human, mouse, and rat cells. The assay recognizes both natural and recombinant IRS-1 (total).

Insulin receptor substrate-1 (IRS-1), a cytoplasmic adaptor protein of 165 kDa. It plays a key role in mediating metabolic and proliferative signaling arising from stimulation by insulin and various cytokines. IRS-1 protein expression levels increase as preadipocytes mature into adipocytes. Hormones such as estradiol also upregulate the expression of IRS-1.

The kit can also be used to normalize the phosphorylated IRS-1 content of samples when using the IRS-1 [pS312] ELISA Kit (Cat. No. KHO0521).

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KHO0511 (96 tests)
IRS-1 (Total) Standard,lyophilized; contains 0.1% sodium azide	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide and red dye [1]	25 mL
Antibody Coated Wells, 96-well plate	1 plate
IRS-1 (Total) Detection Antibody; contains 0.1% sodium azide and blue dye [1]	11 mL
Anti-Rabbit IgG HRP (100X)	0.125 mL
HRP Diluent; contains 3.3 mM thymol and yellow dye [1]	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

[1] 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.

Required materials not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions
- Cell Extraction Buffer (Cat. No. FNN0011, or see "Prepare Cell Extraction Buffer")

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Prepare Cell Extraction Buffer

Note: See the *ELISA Technical Guide* for detailed information on preparing Cell Extraction Buffer.

1. Prepare 5 mL of Cell Extraction Buffer.

   Cell Extraction Buffer consists of 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, and 0.5% deoxycholate.
2. Immediately before use, add 1 mM PMSF (0.3 M stock in DMSO) and 500 µL protease inhibitor cocktail (e.g., Sigma Cat. No. P-2714).

Prepare cell lysate

1. Collect cells by centrifugation (non-adherent cells) or scraping from culture flasks (adherent cells), then wash cells twice with cold PBS.
2. Remove and discard the supernatant and collect the cell pellet. The pellet can be stored at −80°C and lysed at a later date if desired.
3. Lyse the cell pellet in Cell Extraction Buffer for 30 minutes, on ice. Vortex at 10-minute intervals.

   Note: The volume of Cell Extraction Buffer used depends on the number of cells in the cell pellet, and expression levels of IRS-1 (total). [Researchers must optimize the extraction procedures for their own applications.]
4. Transfer the lysate into microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4°C.
5. Transfer the supernatant into clean microcentrifuge tubes. Samples can be stored at −80°C (avoid multiple freeze-thaw cycles).

Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Perform sample dilutions with Standard Diluent Buffer.
- Dilute samples prepared in Cell Extraction Buffer 1:10 or greater in Standard Diluent Buffer (e.g., 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.

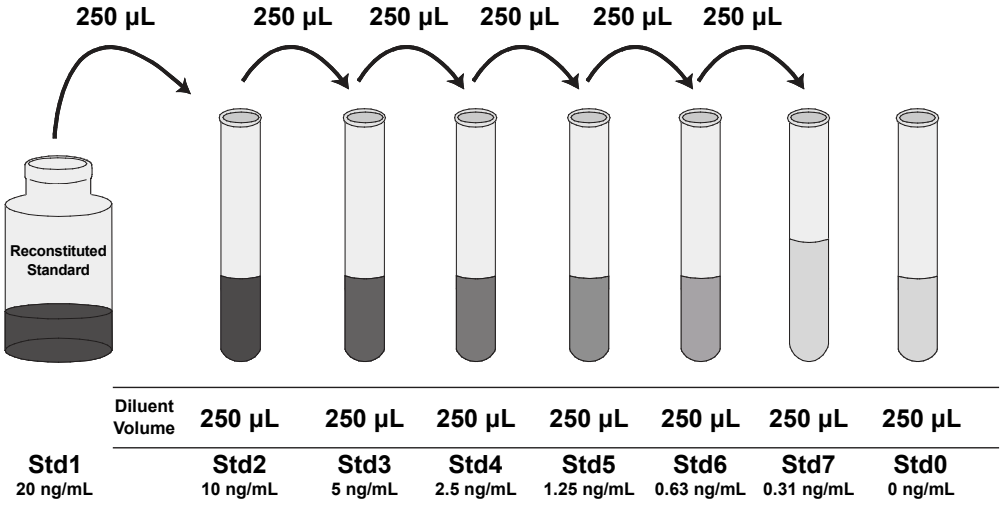
  For 4×10^6 MCF-7 cells use of 1–10 µL of the clarified cell extract diluted to 100 µL in Standard Diluent Buffer for each well.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

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

1. Reconstitute IRS-1 (Total) Standard to 20 ng/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 20 ng/mL IRS-1 (total). **Use the standard within 1 hour of reconstitution.**
2. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 10, 5, 2.5, 1.25, 0.63, 0.31, and 0 ng/mL IRS-1 (total).
3. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
4. 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.



Prepare 1X Anti-Rabbit IgG HRP solution

Note: Prepare 1X Anti-Rabbit IgG HRP solution within 15 minutes of usage.

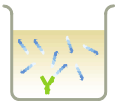
1. For each 8-well strip used in the assay, pipet 10 µL Anti-Rabbit IgG HRP (100X) solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
2. Return the unused Anti-Rabbit IgG HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen



a. Add 100 µL of Standard Diluent Buffer to zero wells except the chromogen blanks.
b. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples"on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
c. Cover the plate with a plate cover and incubate 2 hours at room temperature.
d. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add detector antibody

a. Add 100 µL of IRS-1 (Total) Detection Antibody solution into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate 1 hour at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 Add IgG HRP

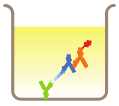
a. Add 100 µL 1X Anti-Rabbit IgG HRP solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

4 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution



Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Inter-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	9.08	3.23	1.09
Standard Deviation	0.46	0.30	0.07
% Coefficient of Variation	5.07	9.28	6.42

Standard curve example

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

Standard IRS-1 (Total) (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

Recovery

To evaluate recovery, IRS-1 (Total) ELISA Kit standard was spiked at 3 different concentrations into 20% Cell Extraction Buffer. The average percent recovery was calculated to be 99%.

Intra-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	9.17	2.92	1.05
Standard Deviation	0.43	0.24	0.08
% Coefficient of Variation	4.69	8.21	7.62

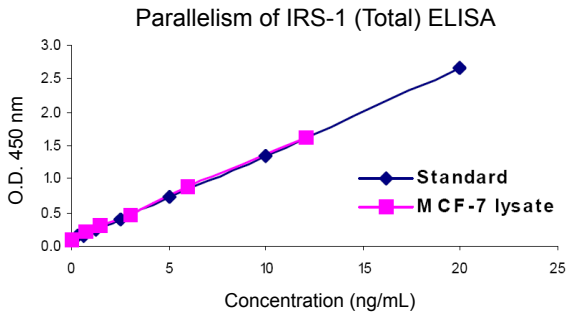
Linearity of dilution

Lysate from MCF-7 cells prepared with Cell Extraction Buffer was diluted in Standard Diluent Buffer over the range of the assay and measured for IRS-1 (total) content. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Dilution	Measured (ng/mL)	Expected	
		(ng/mL)	%
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

Parallelism

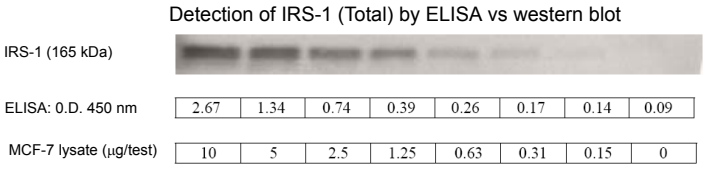
Natural IRS-1 from MCF-7 cell lysate was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the IRS-1 (total) standard curve.



Sensitivity

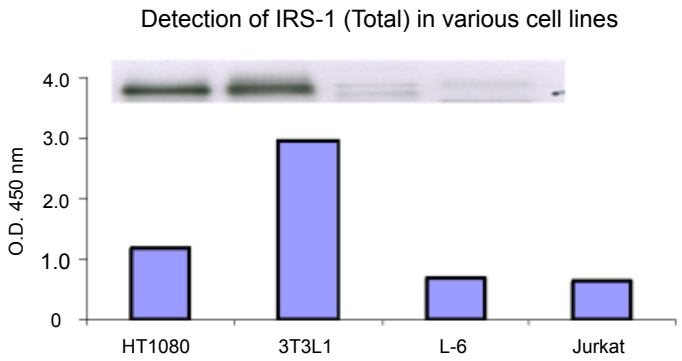
The analytical sensitivity of this assay is <260 ng/mL of IRS-1. This was determined by adding two standard deviations to the mean O.D. obtained from 30 assays of the zero standard. The value corresponds to the amount of IRS-1 extracted from 3,000 MCF-7 cells.

The sensitivity of the ELISA is approximately 2-fold greater than that of western blot.



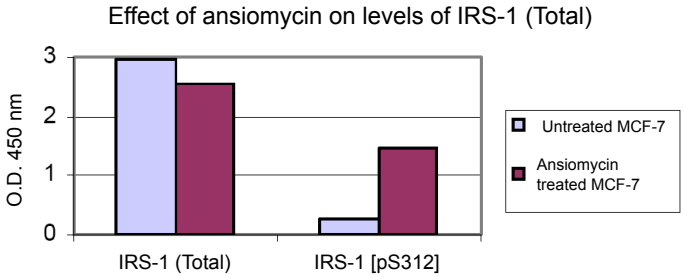
Specificity

Specificity of the assay was analyzed by measuring IRS-1 (total) in cell lysates from human Jurkat, HT1080, mouse 3T3L1, and rat L6 cells, each at a final concentration of 200 µg/mL. The data show that levels of IRS-1 (total) protein detected with this assay are consistent with results obtained by western blot (see inset).



The following proteins were tested with the assay and found to have no cross-reactivity: IGF-1R, EGFR, FAK, Src, AKT, ERK.

Specificity for total IRS-1 content was analyzed by treating MCF-7 cells with anisomysin (100 µM) and calyculin (50 nM) for 40 minutes. Untreated MCF-7 cells were used as control. Cell extracts were analyzed with the IRS-1 (Total) ELISA Kit and the IRS-1 [pS312] ELISA Kit (Cat. No. KHO0521). The results show that phosphorylated IRS-1 [pS312] is upregulated in treated MCF-7 cells, but the total level of IRS-1 remains approximately the same in treated and untreated cells.



Limited product warranty

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.