Insulin Receptor (ß-Subunit) ELISA Kit

Catalog Number KHR9111 (96 tests)

Pub. No. MAN0014914 **Rev.** 1.0

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 Insulin Receptor (β-Subunit) ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of insulin receptor (β-subunit) in cell lysates and can be used to normalize the sample IR content when examining quantities of phosphorylated IR at tyrosine. The assay recognizes both natural and recombinant insulin receptor (β-subunit).

Insulin receptor (IR), a cell surface receptor, binds insulin and mediates its action on target cells. IR belongs to the superfamily of the growth factor receptor tyrosine kinases that regulate multiple signaling pathways through activation of a series of phosphorylation cascades. IR is a heterotetrameric membrane glycoprotein consisting of disulfide-linked subunits in a β-α-α-β configuration. The α-subunit (135 kDa) is completely extracellular, whereas the β-subunit (95 kDa) possesses a single transmembrane domain with tyrosine kinase activity. Insulin binding to the extracellular domain leads to autophosphorylation of the receptor and activation of the intrinsic tyrosine kinase activity, which allows appropriate substrates to be phosphorylated.

Contents and storage

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

Contents	Cat. No. KH91111 (96 tests)
IR (β-subunit) Standard, lyophilized; contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide	25 mL
IR (ß-subunit) Antibody Coated Wells, 96-well strip-well plate	1 plate
IR (ß-subunit) Detection Antibody; contains 0.1% sodium azide	11 mL
Anti-Rabbit IgG HRP (100X); contains 3.3 mM thymol	0.125 mL
HRP Diluent; contains 3.3 mM thymol	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

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 250 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 insulin receptor (β-subunit). [FOR EXAMPLE, X × 10^6 Jurkat cells can be extracted in X mL of Cell Extraction Buffer. 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.

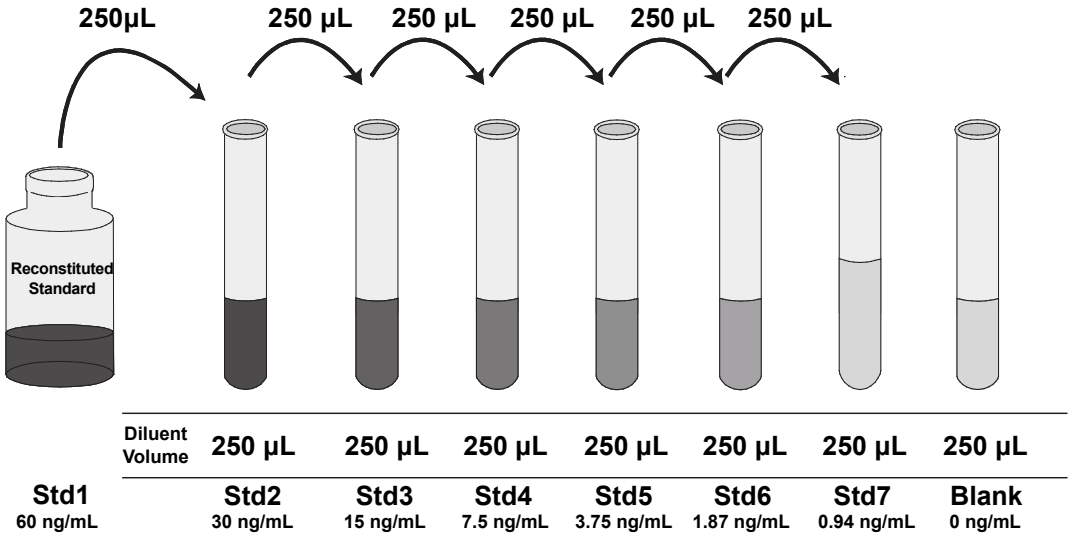
- Perform sample dilutions with Standard Diluent Buffer.
- Dilute samples containing IR (β-subunit) protein extracted from cells at least 1:10 with Standard Diluent Buffer. This dilution is necessary to reduce the matrix effect of the cell lysis buffer.
- Dilute samples prepared in Cell Extraction Buffer at 1:10 or greater with 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 (e.g., 1:50 or 1:100) may be optimal.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: This IR (β-subunit) Standard is a lyophilized lysate from human IR-transfected CHO cells (CHO-T). The standard is calibrated against the mass of purified recombinant IR (β-subunit) expressed in E. coli.

1. Reconstitute IR (β-subunit) Standard to 60 ng/mL with Standard Dilution 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 60 ng/mL IR (β-subunit). **Use the standard within 1 hour of reconstitution.**
2. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 30, 15, 7.5, 3.75, 1.87, 0.94, and 0 ng/mL insulin receptor (β-subunit).
3. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
4. Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Anti-Rabbit IgG HRP solution

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

The Anti-Rabbit IgG HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

1. For each 8-well strip used in the assay, pipet 10 µL Anti-Rabbit IgG HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess 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.

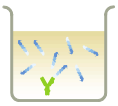
Perform ELISA (Total assay time: 4 hours)

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.

Capture antibody | Antigen | Detector antibody | HRP Secondary antibody

1 Bind antigen



a. 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.
b. Cover the plate with a plate cover and incubate 2 hours at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add detector antibody

a. Add 100 µL of IR (β-subunit) 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

Standard curve example

The following data were obtained for the various standards over the range of 0 to 60 ng/mL IR.

Std Insulin Receptor (ß-Subunit) (ng/mL)	Optical Density (450 nm)
60	2.55
30	1.43
15	0.86
7.5	0.60
3.75	0.39
1.87	0.34
0.94	0.27
0	0.17

Recovery

The recovery of IR (β-subunit) added to Jurkat cells lysate, adjusted to 200 µg/mL, averaged 102% when diluted in Standard Diluent Buffer.

Cross-reactivity

This ELISA is not cross-reactive with IGF-1R. Although this ELISA kit is developed using cells containing human insulin receptor, mouse and rat insulin receptor will cross react.

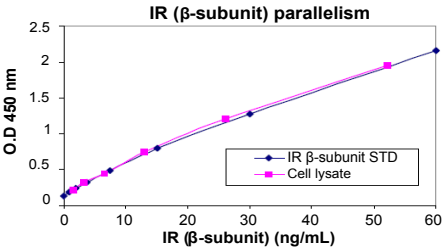
Linearity of dilution

Human IR transfected CHO cells were grown in tissue culture medium containing 10% fetal bovine serum and lysed with Cell Extraction Buffer. This lysate was adjusted to 60 ng/mL IR (β-subunit) and serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

Dilution	Cell Lysate		
	Measured (ng/mL)	Expected	
		(ng/mL)	%
Neat	52.5	—	—
1/2	28.7	26.2	109
1/4	14.2	13.1	108
1/8	6.3	6.5	97
1/16	3.5	3.3	106

Parallelism

Natural IR was prepared from a lysate of hu IR-transfected CHO cells and serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the IR standard curve. The Standard accurately reflects IR (β-subunit) content in samples.



Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	38.52	10.04	1.32
Standard Deviation	2.60	0.84	0.13
% Coefficient of Variation	6.75	8.41	9.49

Intra-assay precision

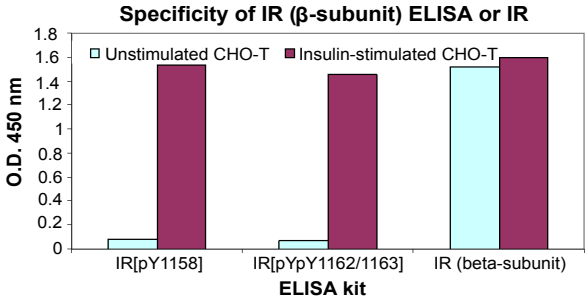
Samples of known IR (β-subunit) concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	38.19	10.79	1.36
Standard Deviation	1.29	0.45	0.12
% Coefficient of Variation	3.37	4.17	8.69

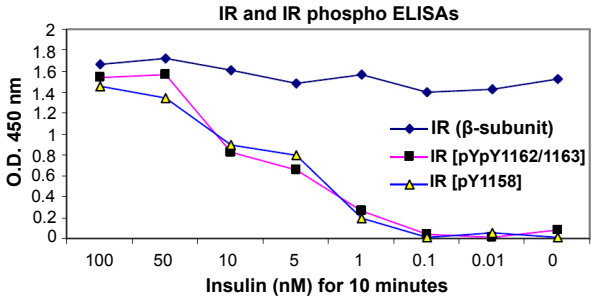
Specificity

This IR (β-subunit) ELISA Kit is specific for measurement of IR (β-subunit) protein, regardless of phosphorylation state of IR. The assay is designed to allow normalization of IR (β-subunit) content among samples to permit interpretation of results from other Phosphorylation-Site-Specific IR kits.

In the experiments presented, IR CHO-T cells were stimulated using 100 nM insulin for 10 minutes. Unstimulated cells were used as control. Cell lysates from the cells were measured for the levels of IR and phosphorylated IR. The results show that IR (β-subunit) ELISA Kit detects phosphorylated IR in insulin-stimulated CHO-T and non-phosphorylated IR in unstimulated control cells.



IR phosphorylation in CHO-T cells is dependent on the levels of insulin stimulation. CHO-T cells were treated with insulin at varying concentrations (0–100 nM), lysed and quantitated in parallel for contents of IR (β-subunit) and phosphorylated IR. The amount of IR (β-subunit) remains relatively constant, while levels of IR phosphorylation at tyrosine 1158, 1162 and 1163 decrease with diminishing insulin dose.



Sensitivity

The analytical sensitivity of IR (β-subunit) is 0.5 ng/mL. This was determined by adding two standard deviations to the mean O.D. obtained from 30 assays of the zero standard.

In addition, the sensitivity of the ELISA is ~2 fold greater than that of western blotting when tested against known quantities of IR (β-subunit).

ELISA: OD 450 nm	0.150	0.226	0.292	0.406	0.602	1.009	1.760	3.1
IR (β-subunit) (ng/test)	0	0.09	0.19	0.38	0.75	1.5	3	6

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: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

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