

Human IRAb ELISA Kit

(Catalog Number: 31C170)

For the quantitative determination of human Anti-insulin
receptor antibodies concentrations in serum or plasma
samples

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INTRODUCTION

Anti-insulin receptor antibodies (IRAb) are autoantibodies that target the insulin receptor, a key transmembrane protein responsible for mediating insulin's metabolic effects. By binding to the receptor's extracellular domain, these antibodies can either block insulin signaling—leading to severe insulin resistance—or, less commonly, activate the receptor and cause hypoglycemia¹. IRAb is most often associated with a rare autoimmune condition known as type B insulin resistance syndrome, which is frequently linked to underlying disorders such as systemic lupus erythematosus or other autoimmune diseases². Their presence disrupts normal glucose homeostasis, resulting in extreme metabolic instability that can be difficult to manage clinically. Understanding the structure, binding characteristics, and physiological impact of IRAb is essential for diagnosing autoimmune insulin resistance and guiding targeted therapies, including immunosuppression and metabolic management strategies.

PRINCIPLE OF THE ASSAY

This assay is a sandwich enzyme-linked immunosorbent assay (ELISA). The microtiter plate is pre-coated with a human insulin receptor protein. Controls and samples are pipetted into the wells and any human IRAb present is bound by the immobilized protein. After washing away any unbound substances, a biotin labelled human insulin receptor protein is added to the wells. After wash step to remove any unbound reagents, streptavidin-horseradish peroxidase conjugate (STP-HRP) is added. After the last wash step, an HRP substrate solution is added and color develops in proportion to the amount of human IRAb bound initially. The assay is stopped, and the optical density of the wells is determined using a microplate reader. The increases in absorbance are directly proportional to the amount of captured insulin receptor autoantibody.

INTENDED USE

This Human IRAb ELISA kit is designed for quantification of human IRAb in serum and plasma samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Microtiter Strips (96 wells), coated with a human insulin receptor protein, sealed
2. 10x Wash buffer, 40 mL
3. 5x Assay buffer, 20 mL
4. 100x Detection solution, a biotin labelled human insulin receptor protein, 0.12 mL
5. Negative control, 0.15 mL
6. Positive control, 0.15 mL
7. 200x STP-HRP solution, 0.06 mL
8. Substrate solution, 12 mL, ready for use
9. Stop solution, 12 mL, ready for use

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OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips
2. 96-well plate or manual strip washer
3. Buffer and reagent reservoirs
4. Paper towels or absorbent paper
5. Plate reader capable of reading absorbency at 450 nm
6. Distilled water or deionized water
7. Horizontal micro-plate shaker capable of 120 rpm

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the human Leptin microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1x Assay buffer

Prepare 1×Assay buffer by mixing the 5×Assay buffer (20 mL) with 80 mL of distilled water or deionized water. If precipitates are observed in the 5×Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1x Wash buffer

Prepare 1x Wash buffer by mixing the 10x Wash buffer (40 mL) with 360 mL of distilled water or deionized water. If precipitates are observed in the 10x Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1x Wash buffer may be stored at 2-8°C for up to one month.

C. 1xDetection solution

Spin down the 100x Detection solution briefly and dilute the desired amount of the antibody 1:100 with 1x Assay buffer, 100 µL of the 1x Detection solution is required per well. Prepare only as much 1x Detection solution as needed. Return the 100x Detection solution to 2-8°C immediately after the necessary volume is removed.

D. 1x STP-HRP solution

Spin down the 200x STP-HRP solution briefly and dilute the desired amount of the 200x STP-HRP solution 1:200 with 1x Assay buffer, 100 µL of the 1x STP-HRP solution is required per well. Prepare only as much 1x STP-HRP solution as needed. Return the 200x STP-HRP solution to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF SAMPLES**Sample Preparation:**

Serum or plasma sample and controls generally requires a dilution in the FBS (5 µL FBS added to 20 µL sample).

ASSAY PROCEDURE

It is recommended that all standards and samples be assayed in duplicate.

1. Add 25 µL of diluted controls or sample per well, incubate at room temperature for 2 hours, shaking the plate at 120 rpm on a horizontal micro-plate shaker.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 250 µL of 1x Wash buffer to each well and incubate for 1 minute. Discard the 1x Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
3. Add 100 µL of 1x Detection antibody solution to each well, incubate at room temperature for 1 hour, shaking the plate at 120 rpm on a horizontal micro-plate shaker.
4. Wash each well 3 times as in step 2.
5. Add 100 µL of 1x STP-HRP solution to each well, incubate at room temperature for 20 minutes, shaking the plate at 120 rpm on a horizontal micro-plate shaker.
6. Wash each well 4 times as described in step 2.
7. Add 100 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
8. Add 100 µL of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
9. Measure absorbance of each well at 450 nm immediately.

CALCULATION

1. Subtract the absorbance of the negative control from that of positive control and samples.
2. Compare human IReb concentration of samples.

TYPICAL ASSAY DATA

The following assay data is provided for demonstration only. A dataset should be generated for each set of sample assay.

Sample	Absorbance (450 nm)
Negative control	0.080
Positive control	2.567

ASSAY CHARACTERISTICS

A. Precision

Intra-assay Precision (Precision within an assay) C.V. <8%.

Inter-assay Precision (Precision between assays) C.V. <10%.

REFERENCES

1. Brown N, Elston MS. (2024) J Clin Endocrinol Metab., 109(4):936–943.
2. Jachiet V, Vuillaume P, Hadjadj J, et al. (2025) Diabetes Care., 48(4):e51–e53.

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SUMMARY OF ASSAY PROCEDURE

