DuoSet® IC

Human Total IGF-I R

Catalog Number DYC305-2
DYC305-5
DYC305E

For the development of sandwich ELISAs to measure insulin-like growth factor I receptor (IGF-I R) in cell lysates.

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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PRINCIPLE OF THE ASSAY

This DuoSet® IC ELISA contains the basic components required for the development of sandwich ELISAs to measure IGF-I R in cell lysates. An immobilized capture antibody specific for IGF-I R binds both tyrosine-phosphorylated and unphosphorylated IGF-I R. After washing away unbound material, a biotinylated detection antibody specific for IGF-I R is used to detect captured receptor, utilizing a standard Streptavidin-HRP format.

MATERIALS PROVIDED

Store the unopened kit at 2-8° C. Do not use past kit expiration date.

			Vials Provided	
Description	**Part #**	**Storage Conditions**	**Cat. # DYC305-2**	**Cat. # DYC305-5**
Total IGF-I R Capture Antibody	841597	2-8° C	1	2
Total IGF-I R Detection Antibody	841598	2-8° C	1	2
Total IGF-I R Standard	841599	2-8° C	3	5
Streptavidin-HRP	890803	2-8° C	1	1

DYC305-2 contains sufficient materials to run ELISAs on at least two 96 well plates.*
DYC305-5 contains sufficient materials to run ELISAs on at least five 96 well plates.*

This kit is also available in an Economy Pack (R&D Systems, Catalog # DYC305E). Economy Packs contain sufficient materials to run ELISAs on 15 microplates.* Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

*Provided the following conditions are met:

- The reagents are prepared as described in this package insert.
- The assay is run as described in the General ELISA Protocol on page 6.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

OTHER MATERIALS REQUIRED

- Aprotinin (Sigma # A6279)
- Leupeptin (Tocris # 1167)
- NP-40 Alternative (EMD/Calbiochem # 492016)
- Sodium Azide (NaN_3) (Sigma # S2002)
- Sodium Orthovanadate (Na_3VO_4) (Sigma # S6508), activated
- Normal Goat Serum (R&D Systems, Catalog # DY005) (heat inactivated in a 56° C water bath for 30 minutes)
- Pipettes and pipette tips
- 96 well microplates [Costar EIA Plates (Catalog # 2592 or R&D Systems Catalog # DY990) are suggested]
- Plate sealers (R&D Systems, Catalog # DY992)
- Squirt bottle, manifold dispenser, or automated microplate washer

SOLUTIONS REQUIRED

PBS - 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2-7.4, 0.2 μm filtered.

Wash Buffer - 0.05% Tween® 20 in PBS, pH 7.2-7.4 (R&D Systems, Catalog # WA126).

Block Buffer - 1% BSA*, 0.05% NaN_3 in PBS, pH 7.2-7.4.

IC Diluent #12** - 1% NP-40 Alternative, 20 mM Tris (pH 8.0), 137 mM NaCl, 10% glycerol, 2 mM EDTA, 1 mM activated sodium orthovanadate.

IC Diluent #14 - 20 mM Tris, 137 mM NaCl, 0.05% Tween 20, 0.1% BSA*, pH 7.2-7.4.

Lysis Buffer #9** - 1% NP-40 Alternative, 20 mM Tris (pH 8.0), 137 mM NaCl, 10% glycerol, 2 mM EDTA, 1 mM activated sodium orthovanadate, 10 μg/mL Aprotinin, 10 μg/mL Leupeptin.

Note: *Lysis Buffer #9 consists of IC Diluent #12 plus 10 μg/mL Aprotinin and 10 μg/mL Leupeptin. Approximately 50 mL of IC Diluent #12 is required to run the assay on one plate.*

Substrate Solution - 1:1 mixture of Color Reagent A (H_2O_2) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY999).

Stop Solution - 2 N H_2SO_4 (R&D Systems, Catalog # DY994).

*The use of R&D Systems Reagent Diluent Concentrate 2 (Catalog # DY995) or Millipore Bovine Serum Albumin, Fraction V, Protease free (Catalog # 82-045) is recommended. All buffers containing BSA must be stored at 2-8° C.

**Sample Diluent Concentrate 2 (2X) (R&D Systems, Catalog # DYC002), prepared as described in the DYC002 insert.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Total IGF-I R Capture Antibody (Part 841597) - Each vial contains 720 μg/mL of mouse anti-human IGF-I R antibody when reconstituted with 200 μL of PBS. After reconstitution, store at 2-8° C for up to 30 days or aliquot and store at ≤ -20° C in a manual defrost freezer or at ≤ -70° C for up to 3 months.*

Total IGF-I R Detection Antibody (Part 841598) - Each vial contains 14.4 μg/mL of biotinylated goat anti-human IGF-I R antibody when reconstituted with 1.0 mL of IC Diluent #14. After reconstitution, store at 2-8° C for up to 30 days or aliquot and store at ≤ -20° C in a manual defrost freezer or at ≤ -70° C for up to 3 months.*

Note: *Prior to assay, dilute the Detection Antibody to a working concentration of 400 ng/mL in IC Diluent #14 containing 2% heat-inactivated normal goat serum. Prepare 1-2 hours before use.*

Total IGF-I R Standard (Part 841599) - Each vial contains 200 ng/mL of recombinant human IGF-I R when reconstituted with 500 μL of IC Diluent #12. **Use within one hour of reconstitution. Use a fresh standard for each assay.** A seven point standard curve using 2-fold serial dilutions and a high standard of 10,000 pg/mL is recommended.

Streptavidin-HRP (Part 890803) - 1 mL of Streptavidin conjugated to horseradish-peroxidase. Store at 2-8° C. **DO NOT FREEZE.**

*Provided this is within the expiration date of the kit.

PREPARATION OF SAMPLES

Cell Lysates - Rinse cells two times with PBS, making sure to remove any remaining PBS after the second rinse. Solubilize cells at 1 x 10^7 cells/mL in Lysis Buffer #9, and allow samples to sit on ice for 15 minutes. Assay immediately or store at ≤ -70° C. Before use, centrifuge samples at 2000 x g for 5 minutes and transfer the supernate to a clean test tube. Sample protein concentration may be quantified using a total protein assay. If needed, further dilutions should be made in IC Diluent #12.

PRECAUTION

The Stop Solution suggested for use with this kit is an acidic solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

TECHNICAL HINTS AND LIMITATIONS

- This DuoSet IC ELISA should not be used beyond the expiration date on the kit label.
- Individual results may vary due to differences in technique, plasticware and water sources.
- It is important that the diluents selected for reconstitution and for dilution of the standard reflect the environment of the samples being measured. The diluents suggested in this protocol should be suitable for most cell lysates.
- The type of enzyme and substrate and the concentrations of capture/detection antibodies used can be varied to create an immunoassay with a different sensitivity and dynamic range. A basic understanding of immunoassay development is required for the successful use of these reagents in immunoassays.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Use a fresh reagent reservoir and pipette tips for each step.
- It is recommended that all standards and samples be assayed in duplicate.
- Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay. Buffers containing protein should be made under aseptic conditions and stored at 2-8° C or be prepared fresh daily.

GENERAL ELISA PROTOCOL

A plate layout is provided to record standards and samples assayed.

Plate Preparation

1. Dilute the Capture Antibody to the working concentration of 4.0 μg/mL in PBS without carrier protein. Immediately coat a 96 well microplate with 100 μL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of 3 washes. Wash by filling each well with Wash Buffer (400 μL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 μL of Block Buffer to each well. Incubate at room temperature for 1-2 hours.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Dilute the Detection Antibody to a working conentration of 400 ng/mL in IC Diluent #14 containing 2% heat-inactivated normal goat serum. Allow the diluted Detection Antibody to sit at least 1-2 hours before use.
2. Add 100 μL of sample or standards in IC Diluent #12 per well. Use IC Diluent #12 as the zero standard. Cover with a plate sealer and incubate 2 hours at room temperature.

   Note: *A seven point standard curve using 2-fold serial dilutions and a high standard of 10,000 pg/mL is recommended.*
3. Repeat the aspiration/wash as in step 2 of Plate Preparation.
4. Add 100 μL of the diluted Detection Antibody to each well. Cover with a new plate sealer and incubate 2 hours at room temperature.
5. Repeat the aspiration/wash as in step 2 of Plate Preparation.
6. Immediately before use, dilute the Streptavidin-HRP to the working concentration specified on the vial label using IC Diluent #14. Add 100 μL of the diluted Streptavidin-HRP to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
7. Repeat the aspiration/wash as in step 2 of Plate Preparation.
8. Add 100 μL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
9. Add 50 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

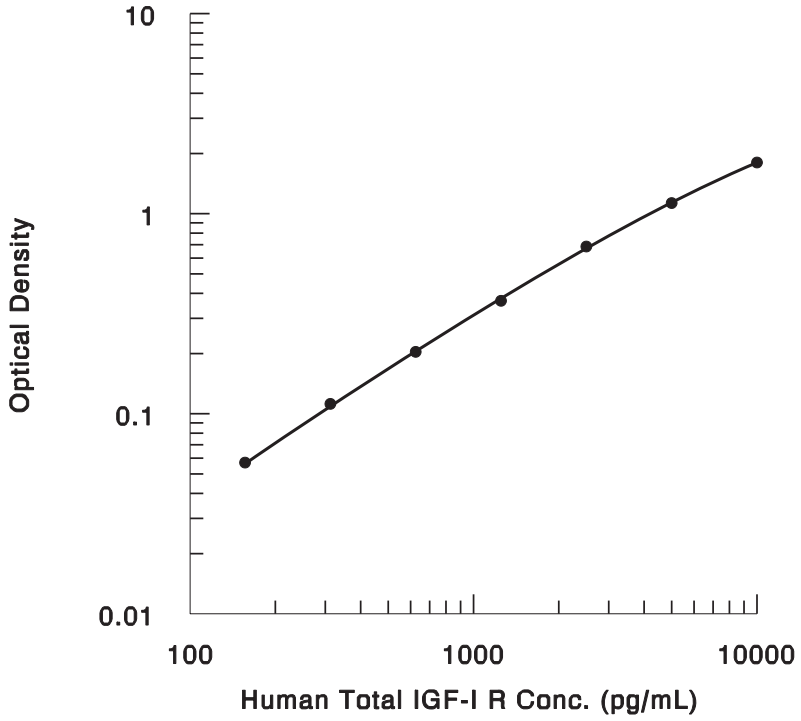
CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample, then subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the IGF-I R concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

A standard curve should be generated for each set of samples assayed. The graph below represents typical data generated when using the Human Total IGF-I R DuoSet IC ELISA. The standard curve was calculated using a computer generated 4-PL curve-fit. This standard curve is for demonstration purposes only.



CALIBRATION

The Human Total IGF-I R DuoSet IC ELISA is calibrated against a highly purified *E. coli*-expressed recombinant human IGF-I R produced at R&D Systems. Samples containing natural IGF-I R showed linear dilution parallel to the standard curve obtained using the Total IGF-I R Standard. These results indicate that O.D. values from this DuoSet IC ELISA can be used to determine the concentration of IGF-I R in natural samples.

SENSITIVITY

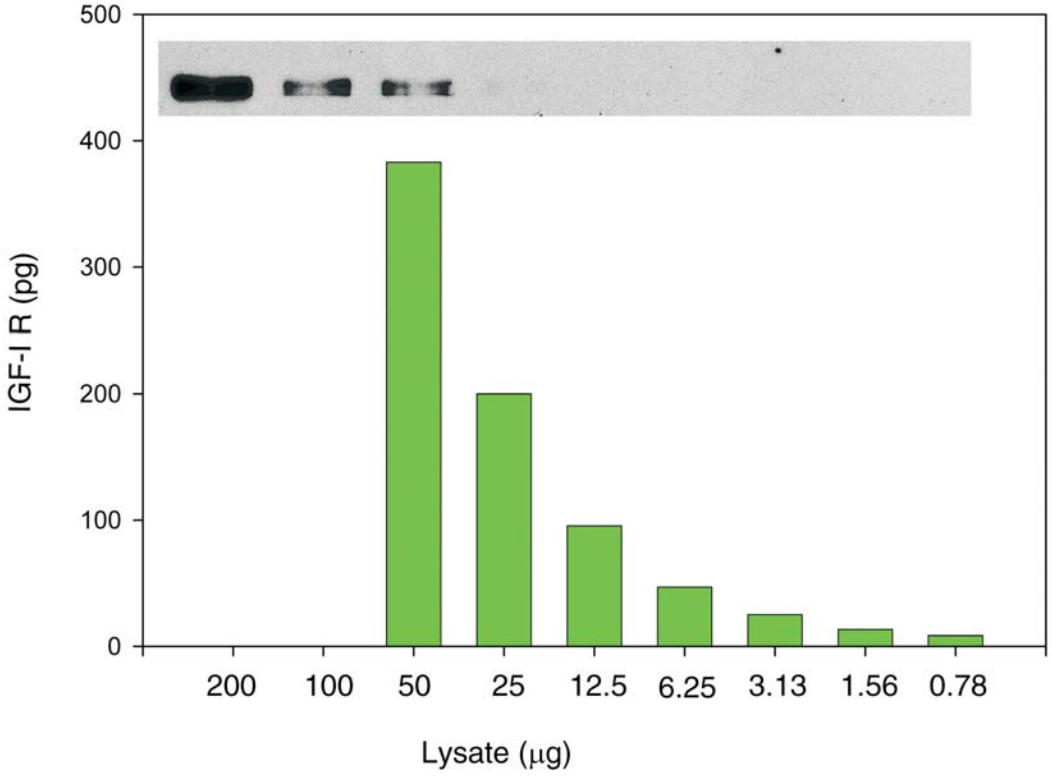


Figure 1: The Human Total IGF-I R DuoSet IC ELISA is more sensitive than immunoprecipitation (IP)-Western blot analysis. Lysates prepared from HepG2 human hepatocellular carcinoma cells were diluted in series and analyzed by this DuoSet IC ELISA and by IP-Western blot (inset). IPs were performed using an anti-IGF-I R monoclonal antibody and goat anti-mouse agarose. Immunoblots were incubated with a biotinylated anti-IGF-I R polyclonal antibody (R&D Systems, Catalog # BAF391) to detect total IGF-I R. Bands were visualized by chemiluminescent detection. Human IGF-I R can be detected by this DuoSet IC ELISA by using approximately 8-20 times less lysate than is needed for a conventional IP-Western blot.

QUANTIFICATION

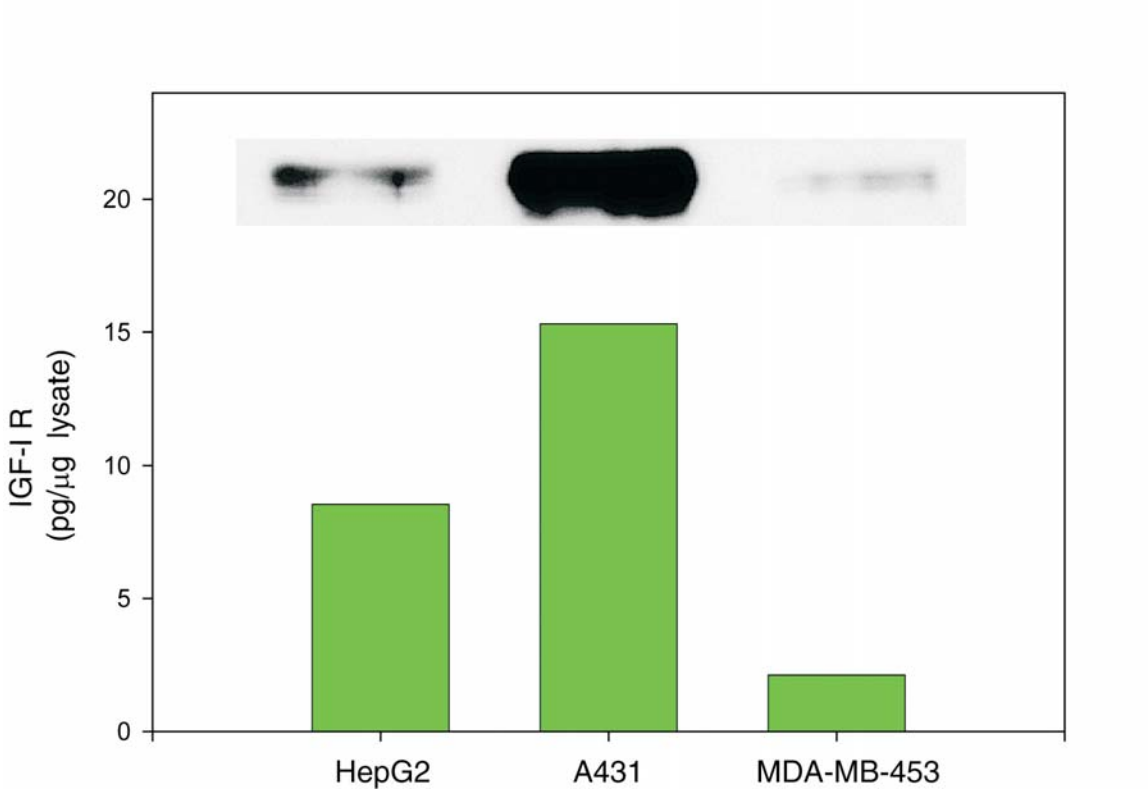


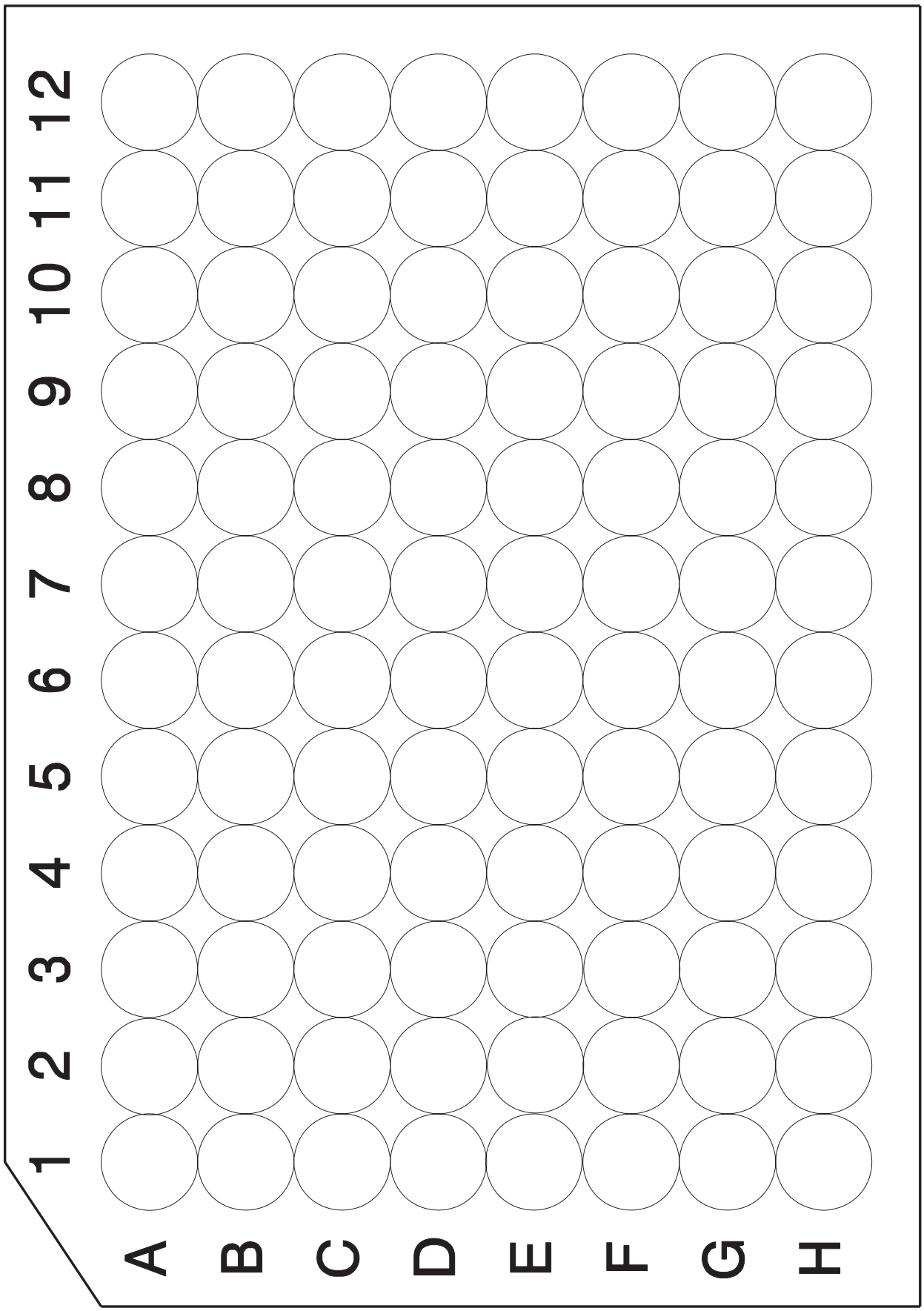
Figure 2: The Human Total IGF-I R DuoSet IC ELISA measures the relative level of IGF-I R. Lysates were prepared from HepG2 human hepatocellular carcinoma cells, A431 human epithelial carcinoma cells, and MDA-MB-453 human breast cancer cells. ELISA and IP-Western blot (inset) analyses were performed using 50 µg and 200 µg of lysate, respectively. The IP-Western blot was performed as described in Figure 1.

SPECIFICITY

To determine specificity, recombinant human (rh) Insulin R, rhIGF-I, and rhGHR were assayed at 100 ng/mL and did not cross-react or interfere in the assay. Additionally, rhIGF-II, rhIL-3 sRα, rhIL-9 sR, rhTGF-β sRII, rhIGFBP-1, rhIGFBP-2, rhIGFBP-3, rhIGFBP-4, rhIGFBP-5, and rhIGFBP-6 were assayed at 50 ng/mL and exhibited no cross-reactivity or interference.

PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.



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