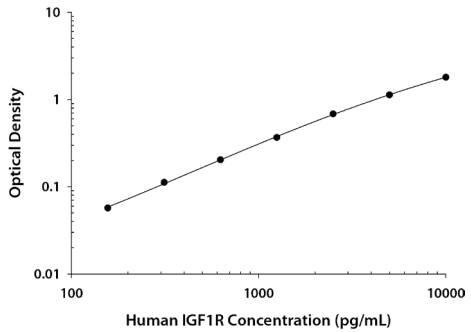
CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density (O.D.). Results may be normalized to total protein or cell number.

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 human IGF1R 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 this Human IGF1R 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 IGF1R DuoSet IC ELISA is calibrated against a highly purified *E. coli*-expressed recombinant human IGF1R produced at R&D Systems®. Samples containing natural IGF1R showed linear dilution parallel to the standard curve obtained using the Total Human IGF1R Standard. These results indicate that O.D. values from this DuoSet IC ELISA can be used to determine the concentration of IGF1R in natural samples.

SPECIFICITY

To determine specificity, recombinant human (rh) Insulin R, rhIGF-I/IGF1, and rhGHR were assayed at 100 ng/mL and did not cross-react or interfere in the assay. Additionally, rhIGF-II/IGF2, rhIL-3 Rα, rhIL-9 R, rhTGF-β RII, 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.

TECHNICAL HINTS & 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 samples and 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.

PRECAUTIONS

The Stop Solution recommended for use with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

DuoSet™ IC ELISA
INTRACELLULAR

Human Total IGF-I R/IGF1R

Catalog Number: DYC305-2 (2 plates)
DYC305-5 (5 plates)

INTENDED USE

For the development of sandwich ELISAs to measure human insulin-like growth factor I receptor (IGF1R) in cell lysates.

PRINCIPLE OF THE ASSAY

This DuoSet IC ELISA contains the basic components required for the development of sandwich ELISAs to measure human IGF1R in cell lysates. An immobilized capture antibody specific for human IGF1R binds both tyrosine-phosphorylated and unphosphorylated IGF1R. After washing away unbound material, a biotinylated detection antibody specific for total human IGF1R is used to detect captured receptor, utilizing a standard Streptavidin-HRP format.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

Manufactured and Distributed by:
USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956 FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:
Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 **FAX:** +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475 **FAX:** +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

MATERIALS PROVIDED & STORAGE CONDITIONS

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

DESCRIPTION	PART #	CATALOG # DYC305-2	CATALOG # DYC305-5	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Total IGF1R Capture Antibody	841597	1 vial	2 vials	Store for up to 1 month at 2-8 °C or aliquot and store at ≤ -20 °C for up to 3 months in a manual defrost freezer.*
Human Total IGF1R Detection Antibody	841598	1 vial	2 vials	Aliquot and store at ≤ -20 °C for up to 3 months in a manual defrost freezer.*
Streptavidin-HRP A	890803	1 vial	1 vial	Store for up to 3 months at 2-8 °C.* **DO NOT FREEZE.**
Human Total IGF1R Standard	841599	3 vials	5 vials	**Use within one hour of reconstitution. Use a fresh standard for each assay.**

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

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.†

† 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.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

OTHER MATERIALS REQUIRED

- Aprotinin (Tocris™, # 4139)
- 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®, # DY005) (heat inactivated in a 56 °C water bath for 30 minutes)
- Pipettes and pipette tips
- Deionized or distilled water
- 96 well microplates (R&D Systems, # DY990)
- Plate sealers (R&D Systems, # 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 (R&D Systems, # DY006)

Wash Buffer - 0.05% Tween® 20 in PBS, pH 7.2-7.4 (R&D Systems, # 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 96 well plate.*

Substrate Solution: ELISA TMB Substrate (R&D Systems, # DY999B)

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

**Alternatively, use Sample Diluent Concentrate 2 (2X) (Catalog # DYC002), prepared as described in the DYC002 insert.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Human Total IGF1R Capture Antibody (Part 841597) - Each vial contains 720 µg/mL of mouse anti-human IGF1R antibody when reconstituted with 200 µL of PBS.

Human Total IGF1R Detection Antibody (Part 841598) - Each vial contains 14.4 µg/mL of biotinylated goat anti-human IGF1R antibody when reconstituted with 1 mL of IC Diluent #14.

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.*

Human Total IGF1R Standard (Part 841599) - **Reconstitute with a recommended volume of 500 µL of IC Diluent #12 to produce a stock solution. Refer to the vial label for the concentration of recombinant human IGF1R.** A seven point standard curve using 2-fold serial dilutions and a high standard of 10,000 pg/mL is recommended.

Streptavidin-HRP A (Part 890803) - 1 mL of Streptavidin conjugated to horseradish- peroxidase. Immediately before use, dilute the Streptavidin-HRP A to the working concentration specified on the vial label using IC Diluent #14.

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.

GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the capture antibody to the working concentration of 4 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 concentration 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 the 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 the Plate Preparation.
6. Add 100 µL of the diluted Streptavidin-HRP A 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 the 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.