human IGFBP-3

Catalog Number: DY675

This DuoSet ELISA Development kit contains the basic components required for the development of sandwich ELISAs to measure natural and recombinant human Insulin-like Growth Factor Binding Protein 3 (IGFBP-3) in cell culture supernates.[1] Each kit contains sufficient materials to run ELISAs on approximately fifteen 96-well plates, provided that the following conditions are met:[2]

- The assay is run as summarized in the General ELISA protocol.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

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

MATERIALS PROVIDED

Bring all reagents to room temperature before use.

Capture Antibody (Part 840261, 1 vial) - 720 µg/mL of mouse anti-human IGFBP-3 when reconstituted with 1.0 mL of PBS. After reconstitution, store at 2-8° C for up to 60 days or aliquot and store at -20° C to -70° C in a manual defrost freezer for up to 6 months.[3] Dilute to a working concentration of 4.0 µg/mL in PBS,[4] without carrier protein.

Detection Antibody (Part 840262, 1 vial) - 36 µg/mL of biotinylated goat anti-human IGFBP-3 when reconstituted with 1.0 mL of Reagent Diluent (see Solutions Required section). After reconstitution, store at 2-8° C for up to 60 days or aliquot and store at -20° C to -70° C in a manual defrost freezer for up to 6 months.[3] Dilute to a working concentration of 200 ng/mL in Reagent Diluent.[4]

Standard (Part 840263, 3 vials) - Each vial contains 310 ng/mL of recombinant human IGFBP-3 when reconstituted with 0.5 mL of Reagent Diluent (see Solutions Required section). Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Aliquot and store reconstituted standard at -70° C for up to 2 months.[3] A seven point standard curve using 2-fold serial dilutions in Reagent Diluent, and a high standard of 8000 pg/mL is recommended.

Streptavidin-HRP (Part 890803, 1 vial) - 1.0 mL of streptavidin conjugated to horseradish-peroxidase. Store at 2-8° C for up to 6 months after initial use.[3] **DO NOT FREEZE.** Dilute to the working concentration specified on the vial label using Reagent Diluent (see Solutions Required section).[4]

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 Catalog # DY006).

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

Block Buffer - 5% Tween 20 in PBS with 0.05% NaN_3.

Reagent Diluent[1] - 5% Tween 20, 2% heat inactivated Normal Goat Serum in PBS, pH 7.2-7.4, 0.2 µm filtered.

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).

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GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate[5] 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 three 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 a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Add 100 µL of sample or standards in Reagent Diluent, or an appropriate diluent, per well. Cover with an adhesive strip and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Add 100 µL of the Detection Antibody, diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 100 µL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. 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.

TECHNICAL HINTS AND LIMITATIONS

- This DuoSet should not be used beyond the expiration date on the label.
- It is important that the diluents selected for reconstitution and for dilution of the standard reflect the environment of the samples being measured. The diluent suggested in this protocol should be suitable for most cell culture supernate samples. Validate diluents for specific sample types prior to use.
- 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 a large quantity of protein should be made under sterile conditions and stored at 2-8° C or be prepared fresh daily.

PRECAUTION

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

CALCULATION OF RESULTS

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

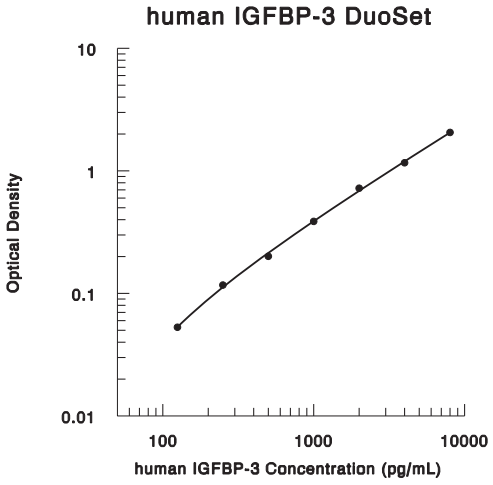
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the IGFBP-3 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. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is only for demonstration purposes.

A standard curve should be generated for each set of samples assayed.

The graph below represents typical data generated when using this human IGFBP-3 DuoSet. The standard curve was calculated using a computer generated 4-PL curve-fit.



SPECIFICITY

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:		
IGF-I R	IGFBP-2	IGFBP-5
IGFBP-1	IGFBP-4	IGFBP-6

Recombinant human IGF-I and recombinant human IGF-II do not cross-react but do interfere in this assay.

A sample containing 50 ng/mL of rhIGF-I and 1 ng/mL of rhIGFBP-3 reads as 0.56 ng/mL.

A sample containing 50 ng/mL of rhIGF-II and 1 ng/mL of rhIGFBP-3 reads as 0.6 ng/mL.

CALIBRATION

This DuoSet is calibrated against a highly purified NS0-expressed recombinant human IGFBP-3 produced at R&D Systems.

[1]For measuring IGFBP-3 in serum samples, we recommend using the R&D Systems Human IGFBP-3 Quantikine ELISA Kit (Catalog # DGB300).

[2]Individual results may vary due to differences in technique, plasticware and water sources.

[3]Provided this is within the expiration date of the kit.

[4]Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Working dilutions should be prepared and used immediately.

[5]Costar EIA Plate (Costar Catalog # 2592 or R&D Systems Catalog # DY990) is suggested. R&D Systems ELISA Plate Sealers (Catalog # DY992) are also available.

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

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