



ELEGANCE

IGFBP-2 ELISA KIT

REF 40 BP296

96



WARRANTY

The manufacturer makes no express warranty other than the diagnostic kit will measure the designated analyte when used in accordance with the manufacturer's printed instructions. The use of the diagnostic kit for any other purpose is outside the intended use of this product and is done at the user's own risk.

The manufacturer disclaims any and all implied warranties of merchantability, fitness for use or implied utility for any other purposes. Any and all damages for failure of the diagnostic kit to perform according to its instructions are limited to the replacement value of the kit.

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FOR PROFESSIONAL USE ONLY

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INTENDED USE

The ELEGANCE IGFBP-2 ELISA has been designed for the quantitative *in vitro* diagnostic measurement of Insulin-like Growth Factor Binding Protein-2 (IGFBP-2) in serum.

PRINCIPLES OF THE ELEGANCE ELISA

The ELEGANCE IGFBP-2 ELISA is an enzyme immunoassay incorporating a rabbit polyclonal anti-IGFBP-2 (antibody reagent) and biotinylated IGFBP-2 (conjugate reagent). An anti-rabbit polyclonal is bound to microwells as a capture antibody. Samples are prediluted 1:25 with Calibrator A. The kit is a competitive assay between calibrator or sample IGFBP-2 and biotin-labelled IGFBP-2. After an overnight incubation with the antibody, the microwells are washed to remove any unbound material. Streptavidin-peroxidase (Amplification Reagent) is added and binds to the biotinylated antibody at many sites. After washing, the Substrate Solution reacts with any bound peroxidase to produce colour in direct proportion with the amount of sample antigen, which can be calculated from the calibrator curve.

ELEGANCE REAGENTS PROVIDED, STABILITY AND STORAGE

Kit size - 96 tests. The kit and all its components, unopened or opened, should be stored at 2-8°C until the listed expiry dates.

IGFBP-2:

Coated Microwells

96 wells REF # BP2A96

Frame containing microwells coated with anti-IGFBP-2 antibody. Ready to use.

IGFBP-2:

Antibody Reagent

1 vial REF # BP2B96

2.5 mL of IGFBP-2 antibody in a buffered solution containing BSA, and a blue dye.

Contains Bronidox L, 0.2% v/v and thiomersal, 0.02% w/v. Ready to use.

IGFBP-2:

Amplification Reagent

1 vial REF # BP2P96

10 mL streptavidin-peroxidase (streptavidin from *S. avidinii*) in a buffered solution containing BSA and a violet dye.

Contains Bronidox L, 0.2% v/v and thiomersal, 0.02% w/v. Ready to use.

Wash Concentrate

1 vial REF # EWC96

50 mL of a 15 x concentrated wash solution. Contains thiomersal, 0.09% w/v. To be diluted before use.

IGFBP-2:

Conjugate Reagent

1 vial REF # BP2C96

2.5 mL biotinylated anti-IGFBP-2 antibody in a buffered solution containing BSA and a red dye. Contains Bronidox L, 0.2% w/v and thiomersal, 0.02% w/v. Ready to use.

Substrate Solution TMB N

1 vial REF # TMBB96

10 mL 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxidase in a stabilising solution.

Ready to use.

IGFBP-2:

Calibrators/ Control

1 vial REF # EBP2S1

5 vials REF # EBP2S2-6

1 vial REF # EBP2C1

20 mL of Calibrator A

(0 ng/mL concentrate)

containing a 4 x concentrated solution of BSA PBS buffer.

Contains thiomersal

0.01% w/v and Bronidox L, 0.2% w/v. To be diluted before use.

0.5 mL in Calibrators B-F and

Control 1 each in 1% BSA

PBS. Contains thiomersal

0.01% w/v and Bronidox L,

0.2% w/v. Lyophilized.

PRECAUTIONS AND WARNINGS TO USERS

Handling of specimens and kit components, their use, storage and disposal should be in accordance with any local or national laboratory safety procedures or regulations.

Specimens and Calibrators

The source material of the calibrators has been tested by an approved accredited method for the presence of hepatitis B surface antigen, antibody to hepatitis C and antibody to HIV - 1/2 (AIDS) and has been found to be non-reactive for all. However, it is recommended that all samples be handled as if capable of transmitting infectious disease.

Preservatives

The kit contains thiomersal and Bronidox L as preservatives. As reagents contain potentially toxic preservatives, care should be taken in handling to avoid ingestion or skin contact.

Substrate Solution

Avoid any skin contact.

SPECIMEN COLLECTION AND HANDLING

No special patient preparation is required. Specimens are serum collected in a manner appropriate for laboratory testing.

Avoid grossly haemolytic, lipaemic and turbid specimens. Specimens can be stored at 2-8°C for up to 48 hours. Specimens held for longer should be stored at or below -20°C.

Specimens should not be frozen and thawed repeatedly. Thawed specimens should be checked for flocculent matter and mixed by inversion just prior to testing.

Turbid specimens or specimens containing particulate matter should be centrifuged prior to use.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- * Distilled or deionised water
- * 2M HCl
- * Precision pipettes
- * Repeating pipette
- * 1L measuring cylinder
- * Absorbent tissue (lint-free)
- * Timer
- * Vortex mixer
- * Microtitre plate shaker
- * Microtitre plate washer
- * Microplate reader system.

PROCEDURAL NOTES

Bring all reagents and specimens to room temperature (20-25°C) and mix by gentle inversion prior to use. Duplicates are recommended. Contamination of reagents will lead to poor performance. A calibrator curve should be run with each assay. Specimens suspected of having concentrations above top calibrator should be diluted in zero calibrator before assay. All assay steps should be performed without interruption, but if the wells cannot be filled with Amplification Reagent or Substrate Solution immediately after washing, then the microwells may be left upside down on absorbent lint-free tissue for a maximum of 15 minutes.

Reagents are matched in each kit and therefore reagents from different lot numbers should not be mixed.

The photometer and all pipettes used should be calibrated appropriately before use.

Washing

The efficiency of the wash step is vital for good precision. Microwells are washed using an automatic plate washer. Avoid overflows from one well to another.

Quality Control

Control specimens should be run in every assay to ensure correct procedure. Control values should lie within laboratory ranges before assay is approved.

ASSAY PROCEDURE

Preparation of Reagents

Wash Solution

Dilute the wash concentrate 1 in 15 with deionised water. The wash solution can be stored at room temperature (20-25°C) for 12 weeks.

Calibrators and Control

To reconstitute the lyophilized calibrators and control, add the volume of deionised water indicated on each vial label. Allow the vials to sit undisturbed until completely dissolved (at least 30 minutes) and then mix by gentle inversion. Exact concentrations determined lot-to-lot are stated on a separate label inside the kit. After reconstitution the calibrators and control should be stored at -20°C for up to 4 weeks.

Dilution Procedure

Calibrator A

0 ng/mL Concentrate

Dilute Calibrator A, 1 in 4 with deionised water. If the Calibrator A has crystallised, warm to 37°C. The Calibrator A solution is then also used as the sample diluent and can be stored at 2-8°C until the listed expiry date.

Sample Preparation

Samples (not calibrators/controls) should be diluted 1:25.

- Label dilution tubes. (1 per sample)
- Pipette 10 µL sample, add 250 µL of Calibrator A diluent. Vortex.

Protocol

- Assemble the microwells in the frame according to the number of tests required. Bag and return unused wells to 2-8°C.
- Pipette 50 µL of sample (calibrator, control, specimen) in duplicate into the appropriate wells. Time taken to dispense the samples should not exceed 20 minutes.
- Pipette 25 µL IGF2P-2 Conjugate Reagent (red) into all wells.
- Pipette 25 µL of IGF2P-2 Antibody Reagent (blue) into all wells.
- Cover microwells with lid and incubate overnight (16-24 hours) stationary at room temperature (20-25°C).
- After incubation, wash the microwells. Aspirate the liquid and rinse each well 4 times with 250 µL wash solution. After the final wash, invert the microwells and tap firmly on absorbent tissue to remove any remaining wash solution. Ensure that no air bubbles remain in the wells.
- Pipette 100 µL of IGF2P-2 Amplification Reagent (violet) into all wells.
- Cover microwells with lid and incubate for 10 minutes on a plate shaker at room temperature (20-25°C).
- After incubation, repeat wash step.
- Pipette 100 µL of Substrate Solution into all wells. Timing of the incubation step is measured from the addition of substrate solution to the first well.

- Cover microwells with lid and incubate for 5 minutes on a plate shaker at room temperature (20-25°C).
- Pipette 50 µL of 2M HCl into all wells in the same timed sequence as for Substrate Solution addition.
- Carry out an end-point reading at 450 nm and process data as described in the microplate reader user's manual. This reading step should be carried out within 30 minutes of stopping the reaction.

CALCULATION OF RESULTS

Calculation of results can be carried out manually if there is no automatic data reduction. Determine the OD for each well. Plot the calibrator curve using log-log graph paper with concentration of calibrators on the x-axis and OD on the y-axis. The curve may be drawn point-to-point or a curve-fitting routine, such as spline interpolation, may be used. Interpolate the sample values from OD measured from this calibrator curve. Record the value of each sample in ng/mL IGF2P-2. Calibrators are established to allow for a sample dilution of 1 in 26.

The range of the *ELEGANCE* IGF2P-2 ELISA is from 0 to approx. 3000 ng/mL, but the maximum concentration that can be reported is limited by the linear performance characteristics of the photometer used. If the OD value of the highest calibrator is above the range of the photometer, then this calibrator must be omitted from the plot of the calibrator curve.

MODEL CALCULATIONS

Endpoint Data

ID	Ave OD	IGFBP-2 (ng/mL)
0	2.171	
82.7	1.786	
262	1.287	
529	0.839	
1226	0.531	
2766	0.321	
Control 1	0.779	642.0

CALIBRATION

The calibrators supplied in this kit are calibrated and labelled in ng/mL, referenced to primary material, quantitated by amino acid analysis.

To convert to nmol/L use:

$$\text{nmol/L} = \frac{\text{ng/mL}}{0.0321}$$

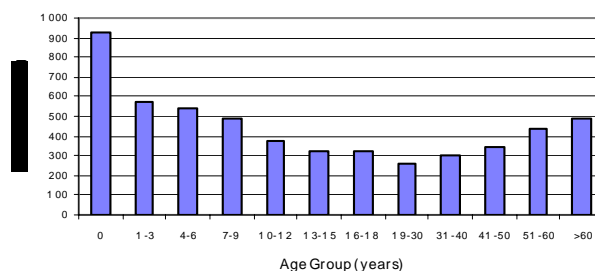
LIMITATIONS

Serum specimens showing gross haemolysis, gross lipaemia, or turbidity may give false results.

EXPECTED VALUES

It is recommended that each laboratory establish its own reference range based on a representative sample population. The following reference range was obtained by assaying serum samples from healthy individuals and is given as a guide only:

Combined Normal Range (IGFBP-2) (n=537)



PERFORMANCE CHARACTERISTICS

Intra-assay Precision

Sample	n	Mean ± 2SD (ng/mL)	%CV
1	19	156 ± 32	10.2
2	19	585 ± 65	5.5
3	19	1567 ± 221	7.1

Inter-assay Precision

Sample	n *	Mean ± 2SD (ng/mL)	%CV
1	18	113 ± 22	9.7
2	18	684 ± 118	8.6
3	18	1313 ± 182	6.9

* duplicate

Specificity

Analyte	Concentration Assayed	Xreactivity
IGFBP-1	130 µg/mL	Not Detectable
IGFBP-3	130 µg/mL	Not Detectable
IGFBP-4	130 µg/mL	Not Detectable
IGFBP-5	130 µg/mL	Not Detectable
IGFBP-6	130 µg/mL	Not Detectable
IGF-I	130 µg/mL	Not Detectable
IGF-II	130 µg/mL	Not Detectable
GH	11 µg/mL	Not Detectable

Accuracy

Recovery was calculated by assaying before and after addition of exogenous analyte.

Sample	IGFBP-2 (ng/mL)		% Recovery
	Observed	Expected	
1	125	121	103.0
2	270	282	96.3
3	934	910	103.0
4	1457	1520	95.7

Dilution

A sample was diluted in zero, assayed and recovery calculated.

Sample	IGFBP-2 (ng/mL)		% Recovery
	Observed	Expected	
Neat	1866		
1/2	1039	933	111.4
1/4	467	467	100.0
1/8	221	233	94.9

Sensitivity

The sensitivity of the assay is typically 10 ng/mL. In terms of actual concentration of IGF2P-2, the sensitivity is typically less than 0.4 ng/mL. The sensitivity is defined as that concentration of analyte which corresponds to the dose response variable (OD) that is two standard deviations from the mean dose response variable of at least 8 replicate determinations of the zero calibrator run in three different assays.

Interference

No interference with analyte recovery was observed for concentrations of haemoglobin up to 250 mg/dL, bilirubin up to 10 mg/dL and triglycerides up to 970 mg/dL.

ORDERING INFORMATION

The *ELEGANCE* IGF2P-2 ELISA is manufactured by:
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