DRG® IGF-BP3 ELISA (EIA-5049)

CE

Revised 10 Nov. 2010 rm (Vers. 2.1) **USA: RUO**

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

Immunoenzymetric assay for measurement of the human Insulin-like Growth Factor Binding Protein-3 (IGFBP-3) in serum.

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

2 PRINCIPLES OF THE METHOD

The IGFBP3-EASIA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiterplates.

Standards and samples react with the capture monoclonal antibody (MAb 1) coated on microtiter well and with a monoclonal antibody (MAb 2) labelled with horseradish peroxidase (HRP). After an incubation period allowing the formation of a sandwich: coated MAb 1 – human IGFBP3– MAb 2 – HRP, the microtiterplate is washed to remove unbound enzyme labelled antibody. Bound enzyme-labelled antibody is measured through a chromogenic reaction. Chromogenic solution (TMB) is added and incubated. The reaction is stopped with the addition of Stop Solution and the microtiterplate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is proportional to the human IGFBP3 concentration.

A calibration curve is plotted and IGFBP3 concentration in samples is determined by interpolation from the calibration curve.

3 REAGENTS PROVIDED

Reagents	96 tests Kit	Color Code	Reconstitution
Microtiterplate with 96 anti IGFBP3 (monoclonal antibodies) coated breakable wells	96 wells	blue	Ready for use
[Ab HRP CONC] Conjugate: HRP labelled anti-IGFBP3 (monoclonal antibodies) in TRIS buffer with bovine serum albumin and thymol	1 vial 0.5 ml	red	**Dilute** 20 x with conjugate buffer
[CONJ BUF] Conjugate Buffer: Tris buffer with bovine serum albumin and thymol	1 vial 10 ml	red	Ready for use
[CAL N] Calibrators - N = 1 to 5 , in phosphate buffer with bovine serum and thymol. See exact values on vial labels. **Standards are prediluted.** **! Use dilution buffer as zero calibrator.**	5 vials lyophilized	yellow	**Add** 1 ml distilled water

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[DIL BUF] Dilution Buffer: Phosphate buffer with bovine albumin, bovine serum and thymol.	1 vial 100 ml	black	Ready for use
[CONTROL N] Controls - N = 1 or 2 in human serum with thymol. Controls are prediluted.	2 vials lyophilized	silver	**Add** 1 ml distilled water
[WASH SOLN CONC] **Wash Solution** (Tris-HCl)	1 vial 10 ml	brown	**Dilute** 200 x with distilled water (use a magnetic stirrer).
[CHROM TMB] Chromogenic TMB Solution (Tetramethylbenzydine)	1 vial 12 ml	white	Ready for use
[STOP SOLN] Stop Solution: HCl 1.0 N	1 vial 12 ml	black	Ready for use

Note: use the dilution buffer as zero standard.

The calibrators are standardized against the NIBSC/WHO recombinant IGFBP-3, reference reagent coded 93/560.

4 SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. High quality distilled water
2. Pipettes for delivery of: 50 µl, 100 µl and 1 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Plastic tubes for dilution of samples
4. Vortex mixer
5. Magnetic stirrer
6. Washer for microtiterplates
7. Microtiterplate reader capable of reading at 450 nm and 650 nm (monochromatic reading)

5 REAGENT PREPARATION

A. Calibrators:

Reconstitute calibrators with 1 ml distilled water.

! Use dilution buffer as zero calibrator

B. Controls :

Reconstitute the controls with 1 ml distilled water.

C Working IGFBP3-HRP conjugate :

Prepare an adequate volume of conjugate solution by adding for example : 100 µl of the 20 x concentrated IGFBP3-HRP conjugate to 2 ml of conjugate buffer. Use a vortex to homogenize. Extemporaneous preparation is recommended.

D Working Wash solution :

Prepare an adequate volume of Working Wash solution by adding 199 volumes of distilled water to 1 volume of Wash Solution (200x). Use a magnetic stirrer to homogenize.
Discard unused Working Wash solution at the end of the day.

6 STORAGE AND EXPIRATION DATING OF REAGENTS

Before opening or reconstitution, all kit components are stable until the expiry date, indicated on the vial label, if kept at 2°C to 8°C.

Unused strips must be stored, at 2-8°C, in a sealed bag containing a desiccant until expiration date.

After reconstitution, standards and controls are stable for one week at 2°C to 8°C. For longer storage periods, aliquots should be made and kept at -20°C for maximum 3 months. Avoid successive freezing and thawing

The concentrated Wash Solution is stable at room temperature until expiration date.

The Working IGFBP3-HRP conjugate is stable for 4 hours at room temperature, avoid direct sunlight.

Alterations in physical appearance of kit reagents may indicate instability or deterioration.

7 SPECIMEN COLLECTION AND PREPARATION

Serum must be kept at 2°C - 8°C.
If the test is not run within 24 hours, storage in aliquots at -20°C is recommended. Avoid subsequent freeze thaw cycles.
Prior to use, all samples should be at room temperature. It is recommended to vortex the samples before use.
Do not use haemolysed samples.

8 PROCEDURE

8.1 Handling notes

Do not use the kit or components beyond expiry date.

Do not mix materials from different kit lots.

Bring all the reagents to room temperature prior to use.

Thoroughly mix all reagents and samples by gentle agitation or swirling.

Perform standards, controls and samples in duplicate. Vertical alignment is recommended.

Use a clean plastic container to prepare the Wash Solution.

In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.

For the dispensing of the Chromogenic Solution and the Stop Solution avoid pipettes with metal parts.

High precision pipettes or automated pipetting equipment will improve the precision.

Respect the incubation times.

To avoid drift, the time between pipetting of the first standard and the last sample must be limited to the time mentioned in section 12 paragraph 5 (Time delay).

Prepare a calibration curve for each run, do not use data from previous runs.

Dispense the Chromogenic Solution within 15 minutes following the washing of the microtiter plate.

During incubation with Chromogenic Solution, avoid direct sunlight on the microtiter plate.

8.2 Procedure

1. Label one plain plastic tube for each sample.
2. Dispense 1 ml of Dilution Buffer into each tube.
3. Add 10 µl of sample into these tubes.
4. Vortex pre-diluted samples, reconstituted standards and controls.
5. Select the required number of wells for the run. The unused wells should be resealed in the bag with a desiccant and stored at 2-8°C.
6. Secure the wells into the holding frame.
7. Pipette 100 µl of dilution buffer as zero calibrator.
   Pipette 100 µl of each calibrator, control and diluted sample into the appropriate wells.
8. Pipette 50 µl of IGFBP3-HRP conjugate solution into all the wells.
9. Incubate for 2 hours at room temperature.
10. Aspirate the liquid from each well.
11. Wash the plate 3 times
12. Pipette 100 µl of the Chromogenic solution into each well within 15 minutes following the washing step.
13. Incubate the microtiter plate for 30 minutes at room temperature, avoid direct sunlight
14. Pipette 100 µl of Stop Solution into each well.
15. Read at 450 nm (reference filter 630 nm or 650 nm) within 1 hour and calculate the results as described in section 10

9 CALCULATION OF RESULTS

1. Read the plate at 450 nm against a reference filter set at 650 nm (or 630 nm).
2. Calculate the mean of duplicate determinations.
3. On semi-logarithmic or linear graph paper plot the OD values (ordinate) for each calibrator against the corresponding concentration of Hu IGFBP3 (abscissa) and draw a calibration curve through the calibrator points by connecting the plotted points with straight lines.

4. Read the concentration for each control and sample by interpolation on the calibration curve.
5. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

10 TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

IGFBP-3-EASIA			OD units
Calibrator	0	ng/ml	0.028
	460	ng/ml	0.114
	1270	ng/ml	0.311
	3020	ng/ml	0.778
	6710	ng/ml	1.403
	16070	ng/ml	2.588

11 INTERNAL QUALITY CONTROL

If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.

If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots. Controls which contain azide will interfere with the enzymatic reaction and cannot be used.

Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises

It is recommended that Controls be routinely assayed as unknown samples to measure assay variability. The performance of the assay should be monitored with quality control charts of the controls.

It is good practise to check visually the curve fit selected by the computer.

12 REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

12.1 Age Group	12.2 MALES (ng/ml)			12.3 FEMALES (ng/ml)		
	12.4 Mean	12.5 Range	12.6 N	12.7 Mean	12.8 Range	12.9 N
12.10 0 - 2 years	12.22 2638	12.34 1481-4481	12.46 15	12.58 2348	12.70 1398-3485	12.82 12
12.11 3 - 5 years	12.23 2405	12.35 1478-3052	12.47 12	12.59 2752	12.71 2059-3325	12.83 13
12.12 6 - 8 years	12.24 3186	12.36 2506-4428	12.48 17	12.60 3282	12.72 2469-4495	12.84 13
12.13 9 - 11 years	12.25 3263	12.37 2020-4705	12.49 21	12.61 3298	12.73 2342-4640	12.85 11
12.14 12 - 14 years	12.26 3672	12.38 2238-5971	12.50 19	12.62 4241	12.74 3000-7022	12.86 14
12.15 15 - 17 years	12.27 4031	12.39 2710-5235	12.51 21	12.63 4181	12.75 2539-6607	12.87 20
12.16 18 - 20 years	12.28 3826	12.40 2303-5537	12.52 10	12.64 3709	12.76 2272-6102	12.88 9
12.17 21 - 30 years	12.29 3372	12.41 2092-4552	12.53 11	12.65 3766	12.77 2704-5594	12.89 10
12.18 31 - 40 years	12.30 2704	12.42 1190-4140	12.54 14	12.66 3372	12.78 2659-4533	12.90 12
12.19 41 -50 years	12.31 3885	12.43 2318-6896	12.55 18	12.67 3240	12.79 2322-4046	12.91 16
12.20 51 - 60 years	12.32 3175	12.44 2112-4625	12.56 16	12.68 3830	12.80 1602-5997	12.92 15
12.21 > 60 years	12.33 2826	12.45 1155-3876	12.57 23	12.69 3621	12.81 1995-6505	12.93 21

13 PRECAUTIONS AND WARNINGS

Safety

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.

All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with all reagents, Stop Solution contains HCl. In case of contact, wash thoroughly with water.

Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

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14 SUMMARY OF THE PROTOCOL

	CALIBRATORS CONTROLS µl	SAMPLE(S) µl
DILUTION OF SAMPLES		
Dilution buffer	-	1000
Sample	-	10
Shaking	Vortex	
INCUBATION		
Standards (0 to 5), controls	100	-
Diluted Samples,	-	100
Diluted Conjugate	50	50
Incubate for 2 hours at room temperature. Aspirate the contents of each well. Wash 3 times with 400 µl of Wash Solution and aspirate.		
Chromogenic Solution	100	100
Incubate for 30 min at room temperature		
Stop Solution	100	100
Read on a microtiterplate reader and record the absorbance of each well at 450 nm (versus 630 or 650 nm)		