



# IGFBP-3 RIA KIT

**REF** 10 095100

**Σ** 100



## 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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Manufactured by Bioclone Australia Pty Limited  
(a subsidiary of Hitachi Chemical Co., Ltd) ABN 14 002 036 071  
71-73 Railway Parade Marrickville NSW AUSTRALIA 2204  
Tel +61 (0) 2 9517 1966 Fax +61 (0) 2 9517 2990 Freecall 1800 251 138  
Email sales@bioclone.com.au Web www.bioclone.com.au



Hitachi Chemical Diagnostics Inc.

Hitachi Europe Limited, Whitebrook Park, Lower Cookham Road  
Maidenhead, Berkshire, SL6 8YA, UK. Phone: +44 1628 585 591

## INTENDED USE

The IGFBP-3 RIA has been designed for the quantitative *in vitro* diagnostic measurement of IGFBP-3 (Insulin-like growth factor binding protein-3) in serum or plasma.

## PRINCIPLES OF THE RIA

The RIA is a double antibody radioimmunoassay system. The analyte competes with <sup>125</sup>I labelled tracer antibody for binding to a constant amount of antibody.

A second antibody/PEG complex is used to separate antibody-bound from free <sup>125</sup>I labelled tracer antibody. Following centrifugation, the supernatant is discarded and the pellet containing the bound radioactivity is counted using a gamma counter. The concentration of the analyte is inversely proportional to the bound radioactivity in the pellet. Counts from the calibrators are plotted and samples are read from the constructed calibrator curve.

## REAGENTS PROVIDED, STABILITY AND STORAGE

Kit size - 100 tests. The kit and all its components, unopened or opened, should be stored at 2-8°C until the listed expiry dates.

## IGFBP-3: Tracer

**1 vial REF # BP11**  
11 mL <sup>125</sup>I labelled IGF-I covalently linked to IGFBP-3 (1μCi) in BSA PBS buffer containing a red dye. Contains sodium azide, 0.1% w/v. Ready to use.

## IGFBP-3: Antiserum

**1 vial REF # BPA1**  
11 mL containing rabbit IGFBP-3 antiserum diluted in BSA PBS buffer and a blue dye. Contains sodium azide, 0.1% w/v. Ready to use.

## Precipitating Reagent

**1 vial REF # SME1**  
53 mL containing animal serum and polyethylene glycol in BSA PBS buffer. Contains Bronidox L 0.25% v/v. Mix thoroughly before use.

## IGFBP-3: Calibrators

**1 vial REF # BPS1**  
50 mL each of Calibrator A (0 μg/mL concentrate), containing a 4 x concentrated solution of BSA PBS buffer. Contains sodium azide 0.4% w/v.

To be diluted before use.

## 5 vials REF # BPS2-6

1.0 mL each of Calibrator B-F in BSA PBS buffer. Contains sodium azide, 0.1% w/v. Lyophilized.

## IGFBP-3: Controls

**2 vials REF # BPC1-2**  
1.0 mL each in BSA PBS buffer. Contains sodium azide, 0.1% w/v. Lyophilized.

**Do not dilute.**

## 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, Calibrators and Controls

The source material of the calibrators and controls 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 sodium azide and Bronidox L as a preservative. As reagents contain a potentially toxic preservative, care should be taken in handling, to avoid ingestion or skin contact. Sodium azide may react with lead and copper plumbing to form potentially explosive azides.

## Radioactive Material

The tracer contains radioactive material.

## SPECIMEN COLLECTION AND HANDLING

No special patient preparation is required. Specimens can be either serum or plasma collected in a manner appropriate for laboratory testing. Serum is preferred, however the anticoagulants heparin or EDTA can be employed without sacrificing accuracy.

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
- \* Disposable plastic test tubes with caps 12 x 75 mm
- \* Precision pipettes
- \* Repeating pipettes
- \* 100 mL measuring cylinder
- \* Vortex mixer
- \* Timer
- \* Refrigerated centrifuge capable of 2000 x g
- \* Absorbent paper
- \* Gamma counter

## 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.

All assay steps should be performed without interruption.

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

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

If a centrifuge does not attain at least 2000 x g, an unstable pellet may result. Therefore the centrifuge time must be increased.

## 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

#### Precipitating Reagent

Mix thoroughly before use

#### Calibrators and Controls

To reconstitute the lyophilized calibrators and controls, add the volume of deionized 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 controls should be stored at -20°C.

#### Dilution Procedure

##### Calibrator A

##### 0 μg/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 1:199. Low samples may be diluted 1:99 and these values should be divided by 2 when calculating serum values.

1. Label dilution tubes (1 per sample)
- 2a. For 1:99 - Pipette 10µL sample, add 0.99 mL of 0 µg/mL diluent.  
b. For 1:199 - Pipette 10µL sample, add 1.99 mL of 0 µg/mL diluent.
3. Vortex.

### Protocol

1. Assemble and label test tubes in duplicate according to the number of tests required. Include Total Counts (TC), Non-Specific Binding (NSB), calibrators, controls and diluted specimens.
2. Pipette 200 µL of 0 µg/mL calibrator diluent in duplicate into the NSB tubes.
3. Pipette 100 µL of sample (calibrator, control, diluted specimen) in duplicate into the appropriate tubes.
4. Pipette 100 µL of IGFBP-3 Antiserum (blue) to all tubes except NSB and TC.
5. Pipette 100 µL of IGFBP-3 Tracer (red) into all tubes.
6. Vortex tubes gently and incubate overnight at room temperature (20-25°C, 16-24 hours). All tubes should be purple except NSB and TC tubes.
7. At the end of the incubation period, pipette 500 µL of the thoroughly mixed Precipitating Reagent into all tubes except TC and vortex. Set TC tubes aside, and incubate for 15 minutes at room temperature (20-25°C).
8. Centrifuge all tubes for 20 minutes at 2000 x g in a refrigerated centrifuge (4°C).
9. Immediately after centrifugation, decant the supernatant completely. Tap the tubes gently onto absorbent paper and blot the rims to remove all residual supernatant.
10. Count the tubes for one minute using a gamma counter. Counting longer will reduce statistical counting error. Record the cpm of each tube.
11. Calculate results.

### CALCULATION OF RESULTS

Calculation of results can be carried out manually if there is no automatic data reduction. Calibrators are established to allow for a sample dilution of 1 in 200.

1. Determine the average cpm for duplicate tubes.
2. Plot the calibrator curve on a semi-log or log-linear graph paper using the method below:

Use the following formula to calculate %B/T:

$$\%B/Bo = \frac{\text{cpm (Sample)} - \text{cpm (NSB)}}{\text{cpm (Calibrator A)} - \text{cpm (NSB)}} \times 100$$

3. Plot %B/Bo on the y axis versus the stated concentrations of the calibrators.
4. Read samples directly off the calibrator curve as µg/mL.

### MODEL CALCULATIONS

ID	Ave cpm	%B/Bo	IGBP-3 (µg/mL)
TC	7019		
NSB	113		
0	3311	100.0	
1	2798	84.0	
4	1798	52.7	
10	1123	31.6	
20	710	18.7	
60	413	9.4	
Sample 1	951	26.1	12.9
Sample 2	2087	61.7	2.9

### CALIBRATION

The calibrators supplied in this kit are calibrated to highly purified IGFBP-3.

### LIMITATIONS

Serum specimens showing gross haemolysis, gross lipaemia, or turbidity may give false results. Samples that contain appreciable background radioactivity should not be used. Any suspect samples should be screened for radioactivity before performing the assay and should be held until the radioactivity has decayed, or a new sample requested.

### 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:

Normal Adults	1.7 - 4.0 µg/mL
Mean	2.7 µg/mL
(n=103)	

### PERFORMANCE CHARACTERISTICS

#### Intra-assay Precision

Sample	n	Mean ± 2SD (µg/mL)	%CV
1	10	4.0 ± 0.4	4.7
2	10	1.6 ± 0.2	6.0
3	10	3.9 ± 0.3	3.7
4	10	7.3 ± 0.4	2.6

n=diluted aliquots

#### Inter-assay Precision

Sample	n *	Mean ± 2SD (µg/mL)	%CV
1	9	3.9 ± 0.2	6.1
2	9	1.4 ± 0.1	6.9
3	9	3.9 ± 0.3	8.5
4	9	6.8 ± 0.3	4.9

n=diluted aliquots, \* duplicate

#### Specificity

Analyte	Concentration Assayed	Apparent IGFBP-3 Result
Human GH	50 ng/mL	undetectable
IGF-1	22 ng/mL	undetectable

#### Accuracy

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

Sample	IGFBP-3 (µg/mL) Observed	IGFBP-3 (µg/mL) Expected	% Recovery
1	6.52	6.84	95.3
2	5.66	5.68	99.6
3	6.86	6.92	99.1

#### Dilution

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

Sample	IGFBP-3 (µg/mL) Observed	IGFBP-3 (µg/mL) Expected	% Recovery
1/50	24.73	24.73	100.0
1/100	12.75	12.36	103.2
1/200	5.77	6.18	93.4
1/400	2.81	3.09	90.9
1/800	1.52	1.55	98.1

#### Sensitivity

The sensitivity, defined as that concentration of analyte corresponding to two standard deviations from the mean of the dose response variable of the zero calibrator (measured in 9 assays), is 0.7 µg/mL. In terms of the actual concentration of IGFBP-3, the sensitivity is 3.5 ng/mL.

#### 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 IGFBP-3 RIA is manufactured by:  
Bioclone Australia Pty Limited,  
71-73 Railway Parade, Marrickville  
NSW 2204, AUSTRALIA.  
Telephone +61 (0) 2 9517 1966 Freecall 1800 251 138  
Fax +61 (0) 2 9517 2990  
Email sales@bioclone.com.au Web: www.bioclone.com.au

### TECHNICAL SERVICE

Full technical service is available by calling Bioclone on +61 (0) 2 9517 1966 or Freecall 1800 251 138

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