



Read entire protocol before use.

# IGF-I-D-RIA-CT

Bio-Line S.A. - Rue André Fauchille.17 - B-1150 Bruxelles – Belgium

## I. INTENDED USE

Radioimmunoassay for the in vitro quantitative measurement of human Insulin-like Growth Factor-I (IGF-I) in serum.

## II. GENERAL INFORMATION

A. Name: Bio-Line **IGF-I-D-RIA-CT** Kit  
B. Catalogue number: **BL-46-CT**: 100 tests  
C. Manufactured by: Bio-Line S.A.  
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## III. CLINICAL BACKGROUND

### a. Structure and function (clinical background)

Insulin-like growth factor I (IGF-I) or Somatomedin-C (SM-C) is a basic 70 amino acid single chain polypeptide (MW: 7649 Da) similar to proinsulin (50% sequence homology), and to the other well-characterized member of the somatomedin family: IGF II (67AA, 70 % sequence homology with IGF-I).

IFG-I is the most important factor, which mediates the growth promoting actions of growth hormone, a pituitary hormone with highly fluctuating blood levels due to pulsatile release. The blood concentration of IFG-I is more stable due to the binding to carrier proteins. The concentration of the predominant binding protein (MW 53000) as well as the production of IFG-I, are regulated by growth hormone.

IFG-I is produced by the liver, and other tissues, and it has endocrine, paracrine and autocrine activities. It stimulates growth and regulates differentiation of various tissues, displays insulin-like activities and promotes cartilage growth.

Although GH is the most important factor controlling IFG-I secretion and concentration, other factors are also determinant: the age (with a peak at adolescence), the sex, the nutritional status, and other hormones (oestrogen, thyroxin, prolactin,...). Specific trophic stimuli mainly control IFG-I secretion in the local microenvironment of a particular organ (paracrine activities), while blood IFG-I concentration is the most important variable for balanced systemic growth (endocrine activities).








### b. Intended use (clinical applications)

- **Growth retardation:** Growth retardation may be due to several causes, among which deficient GH production (hypopituitarism), which is associated with low IFG-I blood levels. Because of the difficulties to get interpretable results from GH measurements (by dynamic multiple or stimulation tests), the determination of the stable IFG-I concentration in plasma is often considered as a simple screening test to evaluation "GH impregnation" of the patient before deciding more extensive investigations. In several clinical situations with impaired growth, low IFG-I levels may be observed despite normal or high GH production (i.e. malnutrition, chronic diseases states, some genetic dwarfs like Pygmies,...). Interestingly, children with discrete GH neuro-secretery dysfunction may display low IFG-I values despite normal GH levels by conventional testing. The results of IFG-I assay must be interpreted cautiously by considering the normal variations of IFG-I during childhood and adolescence (see Rosenfeld et al).
- **Acromegaly:** IFG-I levels are elevated in acromegaly (excess production of GH) and may serve as an indicator of disease severity. Results are more readily interpreted because the normal values are more easily defined in adults. IFG-I measurements are also useful to monitor treatment.
- **Research:** The IFG-I RIA kit is an invaluable tool to study the modifications of this growth factor during physiologic (i.e. pregnancy) or pathologic (i.e. diabetes) situations, and the local regulation of IFG-I production in relation to its paracrine and autocrine activities (wound healing, organ regeneration, neoplastic growth, foetal development, gonadal regulation, etc).

#### IV. PRINCIPLES OF THE METHOD

In the present kit, Bio-Line has introduced a pre-treatment step in order to improve the clinical performance of the assay. It is well established that the binding proteins interfere with the radioimmunoassay for IGF-I. A fixed amount of <sup>125</sup>I labelled IGF-I competes with the IGF-I to be measured present in the sample or in the calibrator for a fixed amount of antibody sites immobilized on the wall of a polystyrene tube. After 2 hours with shaking incubation at room temperature, an aspiration step terminates the competition reaction. The tubes are then washed with 2 ml of washing solution and aspirated. A calibration curve is plotted and the IGF-I concentrations of the samples are determined by dose interpolation from the calibration curve.

#### V. REAGENTS PROVIDED

Reagents	100 Test Kit	Colour Code	Reconstitution
 Tubes coated with anti IGF-I	2 x50	green	Ready for use
 <sup>125</sup> I TRACER: <sup>125</sup> Iodine labelled IGF-I (HPLC grade) in phosphate buffer with bovine casein and azide (<0.1%)	1 vial 21 ml 210 kBq	red	Ready for use
 CAL N Calibrators - N = 0 to 5 (see exact values on vial labels) in phosphate buffer with ovalbumin and azide (<0.1%) Calibrator are prediluted	6 vials lyoph.	yellow	Add 1 ml dilution buffer
 PRE SOL Pre-treatment Solution containing HCl 0.1N	1 vial 5 ml	black	Ready for use
 DIL BUF Dilution buffer containing Tris-HCl buffer with bovine casein and azide (<0.1%)	1 vial 50 ml	green	Ready for use
 WASH SOLN CONC Wash solution (TRIS-HCl)	1 vial 10 ml	brown	Dilute 70 x with distilled water (use a magnetic stirrer).
 CONTROL N Controls - N = 1 or 2 in human serum with thymol	2 vials lyoph.	silver	Add 0.5 ml distilled water

**Note** :Use the zero dilution buffer for sample dilutions.  
1 ng of the calibrator preparation is equivalent to 1 ng of the 1<sup>st</sup> IRR 87/518.

#### VI. SUPPLIES NOT PROVIDED

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

1. Distilled water
2. Pipettes for delivery of: 50 µl, 100 µl, 500 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. 5 ml automatic syringe (Cornwall type) for washing
7. Aspiration system (optional)
8. Tube shaker (400rpm)
9. Any gamma counter capable of measuring <sup>125</sup>I may be used (minimal yield 70%).

#### VII. REAGENT PREPARATION

- A. Calibrators:** Reconstitute the calibrators with 1ml dilution buffer.
- B. Controls:** Reconstitute the controls with 0.5 ml distilled water.
- C. Working Wash solution:** Prepare an adequate volume of Working Wash solution by adding 69 volumes of distilled water to 1 volume of Wash Solution (70x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

#### VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kit components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, calibrators and controls are stable for one week at 2 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.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, tracer is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

#### IX. SPECIMEN COLLECTION AND PREPARATION

- Serum samples must be kept at 2-8°C.
- If the test is not run within 48 hrs, storage at -20°C is recommended.
- Avoid subsequent freeze-thaw cycles.

#### X. PROCEDURE

##### A. 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. In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample. High precision pipettes or automated pipetting equipment will improve the precision. Respect the incubation times. Prepare a calibration curve for each run, do not use data from previous runs.

##### B. Pre-treatment step

1. Label one plastic tube for each sample and control.
2. Dispense 50 µl of each sample and control into the tube.
3. Add 50 µl of pre-treatment solution into this tube.
4. Vortex each tube during 5 seconds.
5. Incubate 30 minutes at room temperature.
6. Add 1 ml of the dilution buffer to the tube.
7. Vortex each tube.

##### C. Procedure

1. Label coated tubes in duplicate for each calibrator, control and sample. For the determination of total counts, label 2 normal tubes.
2. Briefly vortex calibrators, controls and samples and dispense 100µl of each into respective tubes.
3. Dispense 200 µl of <sup>125</sup>Iodine labelled IGF-I into each tube, including the uncoated tubes for total counts.
4. Shake the tube rack gently by hand to liberate any trapped air bubbles.
5. Incubate for 120 minutes at room temperature on a tube shaker (400 rpm).
6. Aspirate (or decant) the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
7. Wash tubes with 2 ml Working Wash solution (except total counts) and aspirate (or decant). Avoid foaming during the addition of the Working Wash solution.
8. Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
9. Count tubes in a gamma counter for 60 seconds.

## XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. Calculate the bound radioactivity as a percentage of the binding determined at the zero calibrator point (0) according to the following formula :

$$B/B_0(\%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \times 100$$

3. Using a 3 cycle semi-logarithmic or logit-log graph paper, plot the (B/B<sub>0</sub>(%)) values for each calibrator point as a function of the IGF-I concentration of each calibrator point. Reject obvious outliers.
4. Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
5. By interpolation of the sample (B/B<sub>0</sub> (%)) values, determine the IGF-I concentrations of the samples from the calibration curve.
6. For each assay, the percentage of total tracer bound in the absence of unlabelled IGF-I (B<sub>0</sub>/T) must be checked.

## XII. TYPICAL DATA

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

IGF-I	cpm	B/Bo (%)
Total count	71038	
Calibrator 0.0 ng/ml	38117	100.0
33.0 ng/ml	35753	93.8
81.4 ng/ml	30816	80.8
228.8 ng/ml	19914	52.2
640.2 ng/ml	10525	27.6
1529.0 ng/ml	5331	14.0

## XIII. PERFORMANCE AND LIMITATIONS

### A. Detection limit

Twenty zero calibrators were assayed along with a set of other calibrators.

The detection limit, defined as the apparent concentration two standard deviations below the average counts at zero binding, was 3.41 ng/ml

### B. Specificity

The percentages of cross-reaction estimated by comparison of the concentration yielding a 50% inhibition are respectively:

Compound	Cross-Reactivity (%)
IFG-I	100.0
IGF-II	0.7
Insuline	ND
GH	ND

**Note:** this table shows the cross-reactivity for the anti IFG-I.

### C. Precision

#### INTRA-ASSAY PRECISION

#### INTER-ASSAY PRECISION

Serum	N	<X> ± SD (ng/ml)	CV (%)	Serum	N	<X> ± SD (ng/ml)	CV (%)
A	20	36.1 ± 3.3	9.1	A	17	129.1 ± 11.6	9.0
B	20	81.4 ± 1.9	1.9	B	17	362.5 ± 14.9	4.1
C	20	402.8 ± 6.8	1.7				

SD: Standard Deviation; CV: Coefficient of variation

## D. Accuracy

### DILUTION TEST

Sample	Dilution	Theoretical Concent. (ng/ml)	Measured Concent. (ng/ml)
A	1/1	-	818
	1/2	409	418
	1/4	205	216
	1/8	102	110
	1/16	51	54
B	1/1	-	501
	1/2	251	250
	1/4	125	130
	1/8	63	62
	1/16	31	26

Samples were diluted with Dilution Buffer.

### RECOVERY TEST

Sample	added IGF-I (ng/ml)	Recovered IGF-I (ng/ml)	Recovered (%)
C1	17	18	108
C2	53	55	104
C3	160	164	103
C4	382	411	107

### Conversion factor:

From ng/ml to nmol/L: x 0.1307

From nmol/L to ng/ml: x 7.649

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

## XV. REFERENCE INTERVALS

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

Normal subjects - Mean and range based on 2.5% & 97.5% percentiles

Age Group	MALES (ng/ml)			FEMALES (ng/ml)		
	Mean	Range	N	Mean	Range	N
0 - 2 years	102	73-184	14	98	59-143	3
3 - 5 years	124	103-189	7	159	84-447	9
6 - 8 years	177	115-249	9	276	79-432	10
9 - 11 years	362	181-656	9	272	175-445	8
12 - 14 years	315	168-557	10	418	202-1101	19
15 - 17 years	409	224-592	9	414	138-658	25
18 - 20 years	330	190-390	4	355	144-519	10
21 - 30 years	313	235-408	6	310	191-478	52
31 - 40 years	225	154-270	10	279	180-437	53
41 - 50 years	222	160-318	15	233	123-406	39
51 - 60 years	195	144-286	26	217	122-327	36
> 60 years	171	94-245	23	186	91-320	27

## XVI. PRECAUTIONS AND WARNINGS

### Safety

For *in vitro* diagnostic use only.

This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals.

All radioactive handling should be executed in a designated area, away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.

Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

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 reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up.

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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## XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS µl	CALIBRATORS µl	DILUTED SAMPLE (21x) µl
Pre-treatment Samples, controls Pre-treatment sol.	- -	- -	50 50
Incubation	Vortex 5 secondes and Incubate 30 min RT		
Dilution Buffer	-	-	1000
Shaking	Vortex		
Calibrators (0 to 5)	-	100	-
Diluted Samples	-	-	100
Tracer	200	200	200
Incubation	120 min at room temperature (400 rpm)		
Separation Working Wash solution Separation	-	Aspirate (or decant) 2.0 ml Aspirate (or decant)	
Counting	Count tubes for 60 seconds		

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