

BIOCLONE AUSTRALIA PTY LIMITED

THE DIAGNOSIS OF

GROWTH DISORDERS

ASSOCIATED WITH

GROWTH HORMONE

EXCESS OR DEFICIENCY

TECHNICAL BRIEF

FORM # GHDFLY Ed. 7



Bioclone's Quality Management System certified to ISO 9001, ISO 13485, GMP and CE Mark

Bioclone Australia Pty Limited (a subsidiary of Hitachi Chemical Co., Ltd) ABN 14 002 036 071

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Bioclone also markets a large range of immunoassay kits - IRMAs, RIAs, ELISAs, and allergen products.

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The Diagnostic Application of Serum Growth Hormone, Insulin-like Growth Factor (IGF) and IGF Binding Protein Measurement:

Teale, J.D., JIFCC, Volume 6 Issue 5 November 1994

Summary of recommended diagnostic procedures:

Short Stature:

...”GH Deficiency. Serum GH measurement following or during vigorous exercise or sleep in infants (screening tests). If impaired response, proceed to one of the following provocation tests: glucagon; clonidine or insulin hypoglycemia, if the first options produced impaired responses. Random serum IGF-I and IGFBP-3 measurements can be used.”

...” Receptor deficiencies. Random serum GH, IGF-I (and GH-BP).”

Acromegaly:

...”A random serum IGF-I screening test is recommended. If normal IGF-I, proceed to the GH suppression test (serum GH measurement following glucose load).”

Diabetes:

...”The IGFBP is GH-dependent and inversely related to insulin secretion. Therefore, when diabetic control is poor, IGFBP-1 levels are highest, and IGF-I bioactivity depressed, causing a lack of growth.”

Nutrition:

...”Undernutrition can be detected by the measurement of persistently elevated random serum GH levels in association with low IGF-I concentrations.”

Malignancy:

...”A high IGF-II to IGF-I molar ratio (apparently normal or elevated IGF-II will suppress IGF-I) has been proposed as a sensitive and specific diagnostic indicator of NICTH.”

...”The measurement of IGFBP-1, IGFBP-2 and IGFBP-3 produces a recognizable pattern which in combination with IGF levels can enhance the diagnostic strategy of NICTH.

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**THE DIAGNOSIS
OF
GROWTH DISORDERS**

GH ELISA or IRMA	(Growth Hormone)
UGH ELISA	(Urinary Growth Hormone)
IGF-I RIA	(Insulin-like Growth Factor-I)
IGFBP-3 RIA	(Insulin-like Growth Factor Binding Protein-3)
ALS RIA	(Acid-Labile Subunit)
IGFBP-2 ELISA	(Insulin-like Growth Factor Binding Protein-2)

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Technical Data Sheet:

ELEGANCE Growth Hormone ELISA Kit

Catalogue#: 40 480096 (96 wells)

Description: ELISA kit with Biotin-Streptavidin amplification system designed for *in vitro* quantitative measurement of human Growth Hormone in serum or plasma.

Background: Growth Hormone is a polypeptide of 191 amino acids (molecular weight ~ 22,000 Da) synthesised in the somatotroph cells of the anterior pituitary. GH is the principal regulator of body growth and has a number of direct metabolic and cellular effects in addition to acting indirectly by inducing the release of IGFs (insulin-like growth factors) (1-5). The secretion of GH is pulsatile and is under complex neurogenic, metabolic and hormonal control. Factors influencing GH secretion include stress, exercise, sleep, dopamine, noradrenaline, serotonin, hypoglycaemia, amino acids, rising fatty acid levels and IGF-I. Hyposecretion in children results in short stature or dwarfism. Hypersecretion of GH, which most commonly results from a pituitary tumour, causes gigantism in children and acromegaly in adults.

Calibrators: Calibrators are lyophilised, calibrated and labelled in mIU/L referenced to the International Standard for Growth Hormone, human (1st I.S. 80/505, 1987).

Sensitivity: < 0.1 mIU/L

Precision:

Intraassay CV%			Interassay CV%		
Mean(mIU/L)	CV%		Mean(mIU/L)	CV%	
A 6.5	4.4%		E 5.5	7.2%	
B 17.6	3.5%		F 15.8	6.9%	
C 35.4	3.9%		G 30.4	8.7%	

Specificity: The specificity of the kit was assessed by addition of purified human prolactin (40,000 mIU/L) and human placental lactogen (1.0 mIU/L) to separate samples from which endogenous GH had been removed. The apparent GH concentration was undetectable in each case.

Reference Ranges: It is recommended that each laboratory establish its own ranges. Samples from healthy individuals were analysed and gave results between 0 and 15.4 mIU/L.
95% of these values fall in the range 0 - 3.5 mIU/L.
n = 179 GH Mean = 0.812 mIU/L GH SD = 2.127 mIU/L

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References:

1. Underwood, LE and Van Wyk, JJ Normal and Aberrant growth. In Williams Textbook of Endocrinology, Wilson, JD and Foster DW (eds), WB Saunders Co, Philadelphia, 180-195. (1985).
2. Cockram, CS, Sonksen, PH and West, TET " Growth Hormone", In Hormones in Blood, 3rd Ed., Vol 4, CH Gray and VHT James (Eds), Academic Press, N.Y., 334. (1979). pp 65-86.
3. Lazarus, L, "Growth Hormone", In: Endocrine Disorders, A guide to Diagnosis, 1st Ed., RA Donal (Ed), (1984), pp 273-302.
4. Baxter, RC "The Somatomedins: Insulin-like growth factors." Adv. Clin. Chem., 25, (1986). 49-115.
5. Reutens, AT, Hoffman, DM, Leung, KC and Ho, KKY. Evaluation and Application of a Highly Sensitive Assay for Serum Growth Hormone (GH) in the study of Adult GH Deficiency. J Clin Endocrin and Metab, 80, pp480-485, (1995).

Precautions:

For clinical laboratory and research use. Not for human or therapeutic use.
Sodium azide yields highly toxic acid under acidic conditions. When disposing of reagents use copious amounts of water to prevent build-up of potentially explosive azides.
Some kit components contain thiomersal. This reagent should not be drained or disposed of without proper treatment according to local regulations.
Avoid any skin contact with the substrate OPD. The substrate solution of OPD should be disposed of according to local regulations.

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Technical Data Sheet:

Growth Hormone IRMA Kit

Catalogue#: 20 280125 (125 tubes)

Description: Immunoradioimmunoassay kit designed for *in vitro* quantitative measurement of human Growth Hormone in serum or plasma.

Background: Growth Hormone is a polypeptide of 191 amino acids (molecular weight ~ 22,000 Da) synthesised in the somatotroph cells of the anterior pituitary. GH is the principal regulator of body growth and has a number of direct metabolic and cellular effects in addition to acting indirectly by inducing the release of IGFs (insulin-like growth factors) (1-4). The secretion of GH is pulsatile and is under complex neurogenic, metabolic and hormonal control. Factors influencing GH secretion include stress, exercise, sleep, dopamine, noradrenaline, serotonin, hypoglycaemia, amino acids, rising fatty acid levels and IGF-I. Hyposecretion in children results in short stature or dwarfism. Hypersecretion of GH, which most commonly results from a pituitary tumour, causes gigantism in children and acromegaly in adults.

Calibrators: Calibrators are calibrated and labelled in mIU/L referenced to the International Standard for Growth Hormone, human (1st I.S. 80/505, 1987).

Sensitivity: < 0.2 mIU/L

Precision:	Intraassay CV%		Interassay CV%		
	Mean(mIU/L)	CV%	Mean(mIU/L)	CV%	
A	5.2	1.4%	E	5.17	3.2%
B	12.1	1.1%	F	12.0	3.9%
C	21.6	1.0%	G	20.8	4.1%

Specificity: The specificity of the kit was assessed by measuring the apparent response of the assay to various potentially crossreactive analytes.

PL At a concentration of 10 mIU/L, the apparent GH was 1.5 mIU/L

Prl At a concentration of 8000 mIU/L, the apparent GH was 0.2 mIU/L

Reference Ranges: The reference range should be used as a guide only. It is recommended that each laboratory establish its own ranges. Because GH is secreted in a pulsatile manner, the reference range given is for a basal level.
GH < 5 mIU/L.

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1. Sonksen, PH and West, TET " Growth Hormone", In Hormones in Blood, 3rd Ed., Vol 1, CH Gray and VHT James (Eds), Academic Press, N.Y., 334. (1979).
2. Cockram, CS, Sonksen, PH and West, TET " Growth Hormone", In Hormones in Blood, 3rd Ed., Vol 4, CH Gray and VHT James (Eds), Academic Press, N.Y., 334. (1979). pp 65-86.
3. Lazarus, L, "Growth Hormone", In: Endocrine Disorders, A guide to Diagnosis, 1st Ed., RA Donal (Ed), (1984), pp 273-302.
4. Baxter, RC "The Somatomedins: Insulin-like growth factors." Adv. Clin. Chem., 25, (1986). 49-115.

Precautions:

For clinical laboratory and research use. Not for human or therapeutic use.
Sodium azide yields highly toxic acid under acidic conditions. When disposing of reagents use copious amounts of water to prevent build-up of potentially explosive azides.

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Technical Data Sheet:

ELEGANCE Urinary Growth Hormone ELISA Kit

Catalogue#: 40 GHU96 (96 wells)

Description: The Bioclone Urinary Growth Hormone ELISA kit, which is a two-step enzyme immunoassay utilizing a colorimetric reaction in its detection system, has been developed to enable a highly sensitive, simple, and rapid *in vitro* measurement of urinary GH (without dialysis).

Background: Human growth hormone (GH) is a single-chain polypeptide hormone comprised of 191 amino acids (1), and physiologically known to promote the growth of humans. In diagnosis of short children and other diseases related to pituitary gland, the status of GH secretion has been examined. At a drug load test, the decrease of GH reactivity is considered as an important indicator of pituitary dwarfism. For diagnosis of GH Neurosecretory Dysfunction (GHND), the capability of secreting GH into blood is usually assessed (2). However, due to the significant fluctuation of GH content in blood, repeated blood draws are required for the assessment (3), and inflict pain on the patient. In recent years, urinary GH has been measured to indirectly determine the concentration of GH in blood. Research results are suggesting the clinical significance of this method (4, 5, 6).

Calibrators: Calibrators are lyophilised, calibrated and labelled in pg/mL referenced to the International Standard for Growth Hormone, human (1st I.S. 80/505, 1987).

Sensitivity: Measurement was carried out five times or more using 0pg/mL and 5pg/mL urinary GH calibrators. Mean of OD values and SD were calculated. "Mean for 0pg/mL calibrator + 2SD" was smaller than "Mean for 5pg/mL calibrator - 2SD". Thus sensitivity <5pg/mL.

Precision:

No.	Urine A	Urine B	Urine C
1	6.0	13.9	22.7
2	6.3	14.4	29.1
3	5.8	14.7	21.7
4	5.7	12.9	23.1
5	5.7	14.4	22.4
Mean	5.90	14.06	22.60
SD	0.25	0.71	0.58
CV(%)	4.3	5.0	2.6

Specificity: The OD for controls were in the range of 80 - 120% of its known concentration.

Reference Ranges: Using the Bioclone Urinary Growth Hormone ELISA kit, the measurable range of human GH is 5 - 200pg/mL. It is recommended that each laboratory establish its own reference range.

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References:

- 1) Nail, H.D. et al. Recent Prog. Horm. Res. 28:887, 1973
- 2) Spiliotis, B.C., et al: JAMA. 251:2228, 1984
- 3) Sizume, K. et al: Growth Hormone and Its Related Peptides, Asakura Shoten, 1992
- 4) Bala, R.M. & Beck, J.C.: J. Clin. Endocr, 33:799, 1971
- 5) Kato, Y. et al: Hormone & Clinical Vol. 36:53-56, 1988
- 6) Tanaka, T., et al: Hormone & Clinical Vol. 38:73-77, 1988

Precautions:

For clinical laboratory and research use. Not for human or therapeutic use.

Avoid ingestion or skin contact. If it happens, wash with copious amounts of water and consult a doctor when necessary.

Some kit components contain sodium azide. Sodium azide may react with lead and copper plumbing to form potentially explosive azides. When disposing of this reagent, dilute with copious amounts of water to prevent build-up of azide.

Some kit components contain thiomersal. This reagent should not be drained or disposed of without proper treatment according to local regulations.

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Technical Data Sheet:

IGF-I RIA Kit

Catalogue#: 10 IGF50 (50 tubes), 10 IGF100 (100 tubes)

Description: Radioimmunoassay kit designed for *in vitro* quantitative measurement of IGF-I in human serum or plasma. Contains an acid-ethanol extraction step to ensure accurate determinations.

Background: IGF-I is useful in the diagnosis of acromegaly and other growth disorders associated with lack of growth hormone (GH). Whereas GH is secreted in sharp pulses, IGF-I levels in serum are much more stable (1). Recent studies also show that measurement of IGF-I may be useful in determining nutritional status and in monitoring the remission of breast cancer under treatment with an oestrogen receptor antagonist (2, 3). The radioligand used in the assay, des(1-3)IGF-I, is an analogue of IGF-I with reduced IGF binding protein (IGFBP) affinity. Des(1-3)IGF-I provides true assay results when there are residual (post-extraction) IGFBPs in samples (4).

Protocol: The following is a brief protocol description only, samples and controls are extracted prior to assaying. Add 100µl of standard / extracted sample into tubes. Add 100µl IGF-I tracer followed by 100µl IGF-I antiserum. Incubate for 2 hours at room temperature. Add 250µl Separation Reagent to all tubes except TC. Incubate at room temperature for 15 minutes. Separate antibody from unbound label by magnetic separation for 2 minutes, then decant the tubes. Add 500µl wash then repeat the separation and decant the tubes. Count tubes and calculate results.

Calibrators: Lyophilised calibrators are calibrated against the International Reference Reagent for IGF-I for Immunoassay (IRR IGF-I, 87/518). The calibrators are pre-adjusted to allow for the extraction step (effective dilution factor).

Controls: A serum control is included in the kit, for easy Quality Control.

Samples: Samples may be either serum or plasma, and are treated prior to immunoassay with a quick and easy acid-ethanol extraction. This is viewed by many researchers as essential to obtaining accurate determinations. (5,6). Recovery of IGF-I in the extraction procedure is 98.5%.

Sensitivity: Typically less than 1.0 ng/ml.

Precision: Intraassay precision typically less than 5% CV. Interassay precision typically less than 7% CV.

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Reference Ranges

Approximately 600 samples were assayed with the Bioclone IGF-I RIA kit. The results obtained are listed below. Units are in ng/mL.

DEFINITIONS – *Median* – When values are ranked in ascending order, half of the results obtained fall below this value.

- *nth %tile* – When values are ranked in ascending order, n% of the results obtained fall below this value.

All (Male and Female)

AGE	n	MEAN	MEDIAN	SD	25th %tile	75th %tile	5th %tile	95th %tile	MIN	MAX
0 yrs	28	68.5	58.5	62.4	38.8	76.4	19.8	108	18.0	362
1 - 3 yrs	40	98.6	88.1	58.1	64.4	109	39.7	229	10.0	270
4 - 6 yrs	29	117	97.9	67.1	63.6	160	45.3	248	19.7	274
7 - 9 yrs	39	203	184	107	130	236	71.7	450	55.8	484
10 -12 yrs	38	327	324	150	204	450	123	521	29.7	701
13-15 yrs	52	388	388	140	297	499	158	605	70.0	664
16-18 yrs	27	461	454	109	422	513	282	641	215	705
19-30 yrs	106	294	282	96.7	224	374	152	424	103	581
31-40 yrs	55	220	215	75.1	183	246	118	326	98.9	556
41-50 yrs	68	193	196	65.2	137	238	108	297	68.7	384
51-60 yrs	61	170	163	58.9	128	210	82.2	268	54.3	339
> 60 yrs	25	165	157	54.6	134	198	86.8	241	60.2	319

Male

AGE	n	MEAN	MEDIAN	SD	25th %tile	75th %tile	5th %tile	95th %tile	MIN	MAX
0 yrs	18	70.0	57.2	75.8	38.5	72.7	19.5	132	18.0	362
1 - 3 yrs	19	97.2	87.6	49.6	73.0	107	44.6	204	41.5	227
4 - 6 yrs	10	121	80.4	84.8	64.4	184	31.5	250	19.7	274
7 - 9 yrs	19	193	170	100	125	228	71.4	375	55.8	457
10 -12 yrs	20	298	308	128	195	383	123	497	120	499
13-15 yrs	21	398	397	101	307	430	289	556	254	645
16-18 yrs	7	477	470	123	418	537	320	641	294	663
19-30 yrs	70	296	298	98.3	221	385	151	423	103	572
31-40 yrs	30	214	201	86.5	153	245	118	311	98.9	556
41-50 yrs	28	184	189	71.7	115	239	101	282	92.9	384
51-60 yrs	31	183	187	64.6	134	222	91.6	284	72.0	339
> 60 yrs	17	169	174	64.8	129	210	78.0	258	60.2	319

Female

AGE	n	MEAN	MEDIAN	SD	25th %tile	75th %tile	5th %tile	95th %tile	MIN	MAX
0 yrs	10	65.6	71.8	28.6	43.9	81.5	26.8	103	19.8	117
1 - 3 yrs	21	99.8	88.6	66.1	51.2	108	38.7	256	10.0	270
4 - 6 yrs	19	115	99.0	58.2	71.0	137	56.0	225	44.8	265
7 - 9 yrs	20	213	185	115	144	239	101	451	59.0	484
10 -12 yrs	18	360	388	168	225	467	136	583	29.7	701
13-15 yrs	31	382	376	163	278	516	113	607	70.0	664
16-18 yrs	20	455	443	107	425	508	274	588	215	705
19-30 yrs	36	289	265	94.6	232	347	184	438	131	581
31-40 yrs	25	228	222	59.3	203	247	133	322	104	382
41-50 yrs	40	200	196	61.4	172	234	125	308	68.7	382
51-60 yrs	30	157	153	50.2	125	179	80.1	248	54.3	268
> 60 yrs	8	157	151	22.3	142	166	133	191	130	198

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Specificity: The rabbit anti-IGF-I antiserum is highly specific. No detectable crossreactivity/interference was found in the assay with the following compounds at specified concentrations:

Analyte	Concentration Assayed
Human GH	1000 nmol/L
Prolactin	25000 mIU/L
IGFBP-1	1000 ng/mL
IGFBP-2	1000 ng/mL
IGF-II	4000 ng/mL

Species Specificity

Samples of various animal sera were tested in the BIOCLONE IGF-I KIT to determine whether there is any immunoreactive response for IGF-I. Immunoreactive and non-immunoreactive sera are listed below.

Immunoreactive	Non-immunoreactive
Primate (Rhesus Monkey)	Rabbit
Rat	Bovine (F Donor)
Bovine (NBC)	Ovine
Porcine	Murine

References:

1. Minuto, F, Underwood, LE, Grimaldi, P, et al, Decreased somatomedin C concentrations during sleep. Temporal relationship to the nocturnal surges of growth hormone and prolactin. *J.Clin. Endocrinol. Metab.* 52, 508-512 (1981).
2. Burgess, EJ, Insulin-like growth factor 1: a valid nutritional indicator during parenteral feeding of patients suffering an acute phase response. *Ann Clin Biochem* 29, 137-144 (1992).
3. Pollack, M, et al, Effect of tamoxifen on serum insulin like growth factor 1 levels in stage 1 breast cancer patients. *J. of Natl. Can. Inst.* 82 (21), 1693-1696 (1990).
4. Bang, P, Eriksson, U, Sara, V, Wivell, I-L, Hall, K. Comparison of acid ethanol extraction and acid gel filtration prior to IGF-I and IGF-II radioimmunoassays: Improvement of determinations in acid ethanol extracts by the use of truncated IGF-I as radioligand. *Acta Endocrinol.* (Copenh.) 124, 620-629 (1991).
5. Daughaday, WH, Mariz, IK, and Blethan, SL. Inhibition of access to bound somatomedin to membrane receptor and immuno-binding sites: a comparison of radioreceptor and radioimmunoassay of somatomedin in native and acid-ethanol extracted serum. *J.Clin. Endocrinol. Metab.*, 51, 781-788 (1980).
6. Baxter, RC. The somatomedins: insulin-like growth factors. *Adv. Clin. Chem.* 25, 49-115 (1986).

Precautions: For clinical laboratory and research use. Not for human or therapeutic use.
Sodium azide yields highly toxic acid under acidic conditions. When disposing of reagents use copious amounts of water to prevent build-up of potentially explosive azides.

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BIOCLONE

Technical Data Sheet:

IGFBP-3 RIA Kit

Catalogue#: 10 095100 (100 tubes)
Description: Radioimmunoassay kit designed for *in vitro* quantitative measurement of IGFBP-3 in human serum or plasma.

Background#: IGFBP-3 is useful in the diagnosis of acromegaly, and other growth disorders associated with lack of Growth Hormone. A total of six IGF binding proteins (IGFBPs) have been identified in human serum and other body fluids and tissues (1). It is a growth hormone dependant binding protein which carries most of the IGFs in serum. IGFBP-3 in human serum is the ~50kDa acid-labile subunit of a 150 kDa IGF-carrying complex. The functions of IGFBP-3 include the maintenance of a reservoir of IGFs in the circulation and regulation of IGF action. Like IGF-I, IGFBP-3 can be used to assess growth hormone status (2). It has been suggested that IGFBP-3 may be particularly useful in the diagnosis of growth hormone deficiency in children (3).

Calibrators: Calibrators are calibrated against a highly purified preparation of IGFBP-3. The calibrators are pre-adjusted to allow for sample dilution factor.

Controls: Two Controls are included in the kit, for easy Quality Control.

Samples: Samples may be either serum or plasma. Recovery of IGFBP-3 is 98.5 +/- 9%.

Sensitivity: 0.7 +/- 0.5 µg/mL

Precision:

Intraassay CV%			Interassay CV%		
Mean(µg/mL)	CV%		Mean(µg/mL)	CV%	
A 1.6	6.0%		E 1.4	6.9%	
B 3.9	3.7%		F 3.9	8.5%	
C 7.3	2.6%		G 6.8	4.9%	

Specificity:
IGF-I (SMC) <0.001%
Human GH: <0.001%

Species Specificity

Samples of various animal sera were tested in the BIOCLONE IGFBP-3 KIT to determine whether there is any immunoreactive response for IGFBP-3. Immunoreactive and non-immunoreactive sera are listed below.

Immunoreactive
Primate (Rhesus Monkey)

Non-immunoreactive
Rabbit
Bovine (F Donor)
Ovine
Murine
Goat
Equine
Rat
Porcine
Bovine (NBC)

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Reference Ranges:

Approximately 600 samples were assayed with the Bioclone IGFBP-3 RIA kit. The results obtained are listed below.

Units are in $\mu\text{g/mL}$.

DEFINITIONS – *Median* – When values are ranked in ascending order, half of the results obtained fall below this value.

- *nth %tile* – When values are ranked in ascending order, n% of the results obtained fall below this value.

All (Male and Female)

AGE	n	MEAN	MEDIAN	SD	25 th %tile	75 th %tile	5 th %tile	95 th %tile	MIN	MAX
0 - 1 yrs	39	1.46	1.34	.55	1.10	1.68	.78	2.67	.69	2.91
2 - 3 yrs	21	1.42	1.28	.49	1.20	1.70	.70	2.34	.57	2.39
4 - 5 yrs	22	1.40	1.36	.64	1.05	1.86	.36	2.51	.27	2.75
6 - 7 yrs	22	1.93	1.86	.60	1.56	2.14	1.15	2.81	.95	3.48
8 -10 yrs	38	2.19	2.09	.70	1.62	2.62	1.27	3.70	1.10	3.95
11-12 yrs	29	2.52	2.50	.66	2.05	3.07	1.61	3.51	.97	3.75
13-15 yrs	42	3.12	3.19	.71	2.70	3.70	1.72	3.91	1.56	4.26
16-18 yrs	36	3.25	3.23	.60	2.95	3.60	2.12	4.32	1.53	4.44
19-30 yrs	99	2.78	2.80	.65	2.38	3.22	1.52	3.79	1.12	4.47
31-40 yrs	56	2.52	2.48	.67	2.13	2.95	1.46	3.57	1.21	5.13
41-50 yrs	75	2.31	2.38	.60	1.87	2.75	1.23	3.18	.81	3.51
51-60 yrs	54	2.26	2.28	.61	1.84	2.63	1.15	3.34	.99	3.66
> 60 yrs	27	2.10	2.07	.61	1.83	2.43	1.19	3.09	.68	3.22

Male

AGE	n	MEAN	MEDIAN	SD	25 th %tile	75 th %tile	5 th %tile	95 th %tile	MIN	MAX
0 - 7 yrs	50	1.60	1.53	.63	1.15	1.96	.78	2.75	.69	3.48
8 -10 yrs	18	2.23	2.18	.80	1.49	2.62	1.10	3.95	1.10	3.95
11-12 yrs	14	2.20	2.06	.62	1.85	2.71	.97	3.25	.97	3.25
13-15 yrs	14	3.06	3.01	.46	2.72	3.44	2.34	3.80	2.34	3.80
16-18 yrs	13	3.19	3.25	.46	2.77	3.47	2.32	3.99	2.32	3.99
19-30 yrs	64	2.75	2.91	.68	2.41	3.25	1.40	3.55	1.12	4.24
31-40 yrs	28	2.52	2.48	.80	2.03	2.83	1.47	3.63	1.21	5.13
41-50 yrs	31	2.17	2.36	.65	1.65	2.70	.89	3.01	.81	3.03
51-60 yrs	26	2.16	2.28	.63	1.80	2.57	1.00	2.96	.99	3.40
> 60 yrs	13	2.16	2.22	.69	1.96	2.71	.68	3.09	.68	3.09

Female

AGE	n	MEAN	MEDIAN	SD	25 th %tile	75 th %tile	5 th %tile	95 th %tile	MIN	MAX
0 - 7 yrs	54	1.48	1.42	.56	1.20	1.87	.37	2.57	.27	2.81
8 -10 yrs	20	2.17	2.06	.61	1.70	2.56	1.37	3.40	1.35	3.70
11-12 yrs	15	2.81	2.83	.56	2.28	3.33	2.02	3.75	2.02	3.75
13-15 yrs	28	3.15	3.41	.81	2.66	3.77	1.63	3.93	1.56	4.26
16-18 yrs	23	3.28	3.21	.67	2.96	3.72	2.12	4.32	1.53	4.44
19-30 yrs	35	2.83	2.76	.60	2.33	3.19	2.09	4.13	1.87	4.47
31-40 yrs	28	2.53	2.52	.54	2.15	3.01	1.46	3.17	1.42	3.51
41-50 yrs	44	2.41	2.47	.54	1.94	2.75	1.53	3.26	1.25	3.51
51-60 yrs	28	2.35	2.30	.60	1.94	2.71	1.43	3.34	1.26	3.66
> 60 yrs	14	2.04	2.04	.53	1.83	2.27	1.19	3.22	1.19	3.22

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References:

1. Martin, JL and Baxter, RC, (1992). Insulin-like Growth Factor Binding Protein-3: Biochemistry and Physiology. Growth Regulation, 2, 88-99.
2. Baxter, RC and Martin, JL (1986). Radioimmunoassay of Growth Hormone Dependent Insulin-like Growth Factor Binding protein in Human Plasma. J Clin Invest, 78, 1504-1512.
3. Blum, WF, Ranke, MB, Kietzman, K, Gauggel, E, Zeisel, HJ and Bierich, JR (1989). A Specific Radioimmunoassay for the Growth Hormone (GH)-Dependent Somatomedin-Binding Protein: Its Use for Diagnosis of GH Deficiency. J Clin Endocrinol and Metab, 70, 1292-1298.

Precautions:

For clinical laboratory and research use. Not for human or therapeutic use.
Sodium azide yields highly toxic acid under acidic conditions. When disposing of reagents use copious amounts of water to prevent build-up of potentially explosive azides.

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BIOCLONE

Technical Data Sheet:

ALS RIA Kit

Catalogue#: 10 ALS100 (100 tubes)

Description: Radioimmunoassay kit designed for *in vitro* quantitative measurement of ALS in human serum or plasma.

Background: Measurement of serum ALS levels are valuable in the diagnosis of GH deficiency in children, monitoring therapeutic GH replacement (1,2) and as a marker of nutritional status. ALS is synthesised in the liver under GH control, and its serum concentration reflects GH secretory status, being elevated in acromegaly and decreased in GH deficiency (1). Most of the insulin-like growth factor I and II (IGF-I and IGF-II) in the circulation are found as part of a 140kDa ternary complex which also contains the acid stable 40-45kDa IGF binding protein-3 (IGFBP-3) and ALS (Acid-Labile Subunit) an 85kDa glycoprotein (3). ALS has a long circulating half life and, when present in the ternary complex, appears to prevent the passage IGFs from the circulation to the tissues (4). ALS is also not subject to proteolysis in pregnancy serum (5).

Calibrators: Calibrators are calibrated to highly purified ALS (Mwt: 63,3000 subjected to amino acid analysis). The calibrators are pre-adjusted to allow for sample dilution factor.

Controls: Two Controls are included in the kit, for easy Quality Control.

Samples: Samples may be either serum or plasma. Recovery of ALS is 102 +/- 10%.

Sensitivity: 0.02 nmol/L

Precision:

Intraassay CV%			Interassay CV%		
	Mean(nmol/L)	CV%		Mean(nmol/L)	CV%
A	26.1	5.0%	E	50.6	4.2%
B	48.5	5.4%	F	104	3.5%
C	207	2.8%	G	176	3.9%

Specificity: The rabbit anti-ALS antiserum is highly specific. No detectable crossreaction was found in the assay with the following compounds:

IGFBP-1
IGFBP-2
IGFBP-3
IGF-I
IGF-II

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Reference Ranges:

Approximately 600 samples were assayed with the Bioclone ALS RIA kit. The results obtained are listed below. Units are in nmol/L.

Males:				Females:		
Age (years):	Mean:	n:	Range:	Mean:	n:	Range:
0	80.7	18	4.94 - 286	79.5	12	17.7 - 152
1 - 3	105	22	37.9 - 182	101	23	11.2 - 212
4 - 6	123	12	7.17 - 261	123	22	4.27 - 235
7 - 9	164	22	63.6 - 300	183	21	75.9 - 338
10 - 12	217	21	60.3 - 321	227	19	44.8 - 323
13 - 15	261	20	170 - 367	260	33	92.9 - 400
16 - 18	286	7	230 - 348	293	20	137 - 393
19 - 30	217	69	88.8 - 497	243	36	182 - 361
31 - 40	201	26	97.7 - 313	220	29	144 - 295
41 - 50	184	29	77.3 - 295	215	47	122 - 292
51 - 60	181	28	60 - 273	183	29	79.5 - 251
>60	164	9	60.2 - 222	176	8	91.8 - 244

Note:

The above ranges are actual lowest to highest values found.

References:

1. Baxter, RC. Circulating levels and molecular distribution of the acid-labile (alpha) subunit of the high molecular weight insulin-like growth factor-binding protein complex. *J. Clin. Endocrinol. Metab.* 70, 1347-1353(1990).
2. De Boer, H, Blok, GJ, Popp-Snijders, C, Stuurman, L, Baxter, RC, Van der Veen, E. Monitoring of growth hormone replacement therapy in adults, based on measurement of serum markers. *J. Clin. Endocrinol. Metab.* 81, 1371 -1377 (1996).
3. Baxter, RC and Martin, JL. Structure of the Mr 140 000 growth hormone-dependent insulin-like growth factor binding protein complex: Determination by reconstitution and affinity-labelling. *Proc. Natl. Acad. Sci. USA.* 86, 6898-6902 (1989).
4. Baxter, RC. Circulating binding proteins for the insulin-like growth factors. *Trends Endocrinol. Metab.* 4, 91-96 (1993).
5. Suikkari, A-M, Baxter, RC. Insulin-like growth factor-binding protein-3 is functionally normal in pregnancy serum. *J. Clin. Endocrinol. Metab.* 74, 177-183 (1992).

Precautions:

For research use only. Not for human or therapeutic use.

Sodium azide yields highly toxic acid under acidic conditions. When disposing of reagents use copious amounts of water to prevent build-up of potentially explosive azides.

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Technical Data Sheet:

IGFBP-2 ELISA Kit

Catalogue#: 40 BP296 (96 wells)

Description: Enzyme immunoassay kit designed for the quantitative *in vitro* measurement of IGFBP-2 in serum.

Background: 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. Patient samples are pre-diluted 1:25 with zero standard.

The test is a competitive assay between standard or sample IGFBP-2 and biotin-labelled IGFBP-2. After an overnight incubation with the antibody, unbound IGFBP-2 is removed by a wash step. Amplification of signal is achieved with the addition of streptavidin-peroxidase, which binds to the biotin on the IGFBP-2. After another wash step to remove unbound streptavidin-peroxidase, a substrate solution, containing TMB and peroxide, is added. These react with the enzyme to produce colour in inverse proportion to the sample IGFBP-2 concentration. From the photometric optical density readings a standard curve is produced and IGFBP-2 in patient samples can be quantitated.

Clinical Significance: IGFBP-2 is a 31.2 kDa protein encoded by a single gene (1), it is part of a family of six IGFBPs in humans. It has a relatively higher affinity for IGF-II than IGF-I (1,2). Levels are highest in newborns then decrease and remain relatively stable in adults (3,4). IGFBP-2 may be useful in the diagnosis of growth hormone deficient (GHD) children. IGFBP-2 has been found to be elevated in these patients which generally also have low IGFBP-3 and IGF-I levels (3). There is a negligible variation in IGFBP-2 during the day although it is elevated by fasting over a period of days (3). In NICTH, IGFBP-2 is negatively correlated with blood glucose. IGFBP-2 is elevated in prostate cancer but not prostatic hyperplasia and maybe useful in conjunction with PSA testing (5).

Calibrators: Calibrators are calibrated against a highly purified preparation of rIGFBP-2. The calibrators are pre-adjusted to allow for sample dilution factor.

Controls: One Control is included in the kit, for easy Quality Control.

Samples: Samples are serum only.

Sensitivity: Typically less than 13ng/ml.

Intraassay Coefficient of Variation (CV)

Sample	n	Mean (ng/ml)	Standard Deviation	%CV
1	22	6.01	0.61	10.0%
2	22	22.5	1.25	5.56%
3	22	60.3	4.25	7.05%

Interassay Coefficient of Variation (CV)

Sample	n	Mean (ng/ml)	Standard Deviation	%CV
4	18	4.34	0.42	9.68%
5	19	26.3	2.27	8.63%
6	18	50.5	3.51	6.95%

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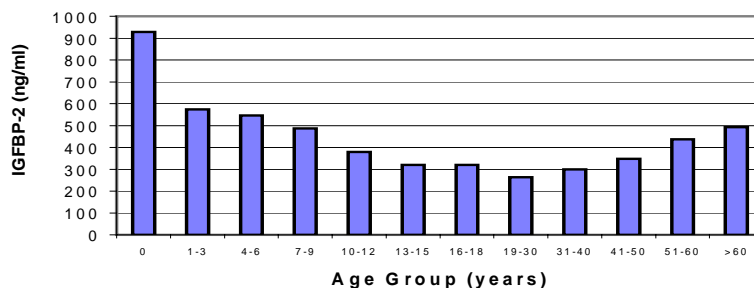


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Reference Ranges:

It is recommended that each laboratory establish its own ranges. The following data is given by way of example:

Combined (M & F) Normal Range (IGFBP-2) (n=537)



Specificity / Interference:

The rabbit anti-IGFBP-2 antiserum is highly specific. No detectable crossreactivity was found in the assay with the following compounds at specified concentrations (prior to samples dilution). Interference by IGFs and GH was also tested, with no detectable result at specified concentrations.

Compound	Concentration tested
IGFBP-1	130 µg/ml
IGFBP-3	130 µg/ml
IGFBP-4	130 µg/ml
IGFBP-5	130 µg/ml

Compound	Concentration tested
IGFBP-6	130 µg/ml
IGF-I	130 µg/ml
IGF-II	130 µg/ml
GH	11 µg/ml

Species Specificity:

Samples of various animal sera were tested in the BIOCLONE IGFBP-2 KIT to determine whether there is any immunoreactive response for IGFBP-2. Immunoreactive and non-immunoreactive sera are listed below.

Immunoreactive

Primate (Rhesus Monkey)
Bovine (F Donor)
Bovine (NBC)
Murine
Porcine
Equine
Ovine
Rat
Goat

Non-immunoreactive

Rabbit

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References:

1. Clemmons RD; Insulinlike Growth Factor Binding Proteins. *Trends Endocrinol. Metab.*, Vol. 1: 412-417, 1990.
2. Baxter RC; Circulating Binding Proteins for the Insulinlike Growth Factors. *Trends Endocrinol. Metab.*, Vol. 4, No. 3: 91-96, 1993.
3. Clemmons DR, *et al*; Variables Controlling the Secretion of Insulin-Like Growth Factor Binding Protein-2 in Normal Human Subjects. *J. Clin. Endocrinol. Metab.*, Vol. 73, No. 4: 727-733, 1991.
4. Juul A, *et. al.*; Serum Levels of Insulin-Like Growth Factor (IGF)-Binding Protein-3 (IGFBP-3) in Healthy Infants, Children, and Adolescents: The relation to IGF-I, IGF-II, IGFBP-1, IGFBP-2, Age, Sex, Body Mass Index, and Pubertal Maturation. *J. Clin. Endocrinol. Metab.*, Vol. 80, No. 8: 2534-2542, 1995.
5. Ho PJ & Baxter RC; Insulin-Like Growth Factor-Binding Protein-2 in Patients with Prostate Carcinoma and Benign Prostatic Hypertrophy. *XIIIth Scientific Research Meeting, RNS Hospital, Sydney, UTS Sydney, 15th-16th November, 1995.*

Precautions:

For clinical laboratory and research use. Not for human or therapeutic use.

Avoid ingestion or skin contact. If it happens, wash with copious amounts of water and consult a doctor when necessary.

Some kit components contain sodium azide. Sodium azide may react with lead and copper plumbing to form potentially explosive azides. When disposing of this reagent, dilute with copious amounts of water to prevent build-up of azide.

Some kit components contain thiomersal. This reagent should not be drained or disposed of without proper treatment according to local regulations.

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