Leptin-Ria-CT

KIPMR44

DIAsource ImmunoAssays S.A. - Rue de l'Industrie, 8 - B-1400 Nivelles - Belgium

LOT : 110204/1

Human-Leptin-RIA-CT

Radioimmunoassay with Coated Tubes for the Quantitative Detection of human Leptin in serum and plasma

KIPMR44

IN VITRO DIAGNOSTIC USE

en

Rue de l'Industrie 8, B-1400 Nivelles, Belgium - Tel: +32 67 88 99 99 - Fax : +32 67 88 99 96

TECHNICAL FEATURES+APPLICATIONS

- Measures total Leptin concentration in **serum, EDTA/Heparin plasma**
- Calibrated against the **WHO** International Standard: **NIBSC Code 97/594**
- High affinity antibodies, no influence of Leptin Bindingprotein
- ready-to-use calibrators with **0-64 ng/ml** are
- Small serum volumina sufficient, measurement in capillar blood possible
- Easy handling

INTRODUCTION

Leptin, the product of the ob gene (1,2), is a recently discovered single-chain proteohormone with a molecular weight of 16 kD, which is thought to play a key role in the regulation of body weight. Its amino acid sequence exhibits no major homologies with other proteins (1). Leptin is almost exclusively produced by differentiated adipocytes (3-5). It acts on the central nervous system, in particular the hypothalamus, thereby suppressing food intake and stimulating energy expenditure (2,6-9). Leptin receptors - alternatively spliced forms exist that differ in length - belong to the cytokine class I receptor family (10-12). They are found ubiquitously in the body (10, 11, 13, 14) indicating a general role of leptin, which is currently not fully understood. A circulating form of the leptin receptor exists which acts as one of several leptin binding proteins (15). Besides its metabolic effects, leptin was shown to have a strong influence on a number of endocrine axes. In male mice, it blunted the starvation-induced marked decline of LH, testosterone, thyroxine and the increase of ACTH and corticosterone. In female mice, leptin prevented the starvation-induced delay in ovulation (16). Ob/ob mice, which are leptin deficient due to an ob gene mutation, are infertile. This defect could be corrected by administration of leptin, but not through weight loss due to fasting (17), suggesting that leptin is pivotal for reproductive functions.

All these actions may, at least in part, be explained by the suppressive effect of leptin on neuropeptide Y (NPY) expression and secretion by neurons in the arcuate nucleus (6,18,19). NPY is a strong stimulator of appetite (20,21) and is known to be involved in the regulation of various pituitary hormones, e.g. suppression of GH through stimulation of somatostatin (22,23), suppression of gonadotropins (23) or stimulation of the pituitary-adrenal axis (21).

The most important variable that determines circulating leptin levels is body fat mass (24-26). Obviously, under conditions of regular eating cycles, leptin reflects the proportion of adipose tissue (27) showing an exponential relationship (37). This constitutive synthesis of leptin is modulated by a number of non-hormonal and hormonal variables. Stimulators in both rodents and humans are overfeeding (28,29), insulin (3,5,30-33) and glucocorticoids (5,34-36). Suppression has been shown for fasting (27), cAMP and beta-3-adrenoceptor agonists (35). From these findings it becomes clear that leptin is an integral component of various metabolic and endocrine feedback loops (38).

For clinical purposes, it is important to note that serum leptin levels show a moderate circadian variation with a peak during the night at about 2 a.m. (37). The leptin values at this time are about 30 to 100 % higher than the levels measured in the morning or early afternoon. This variation together with the influence of food intake needs to be taken into account, when blood samples are collected.

Under fairly standardized conditions, i.e. normal eating cycles and blood sampling in the morning or early afternoon, a single leptin measurement is informative.

For the appropriate interpretation of measured leptin levels, reference ranges are required. Because body fat mass is the major confounding variable, these ranges should be referred to measures of the percentage body fat such as body mass index (BMI) or percent body fat determined by, e.g., bioelectric impedance assessment (BIA). Leptin levels are higher in females than in males (38,39) and an age dependence was shown in children and adolescents (40). Therefore, reference ranges referring to measures of body fat should be stratified according to gender and pubertal development.

Leptin levels are high in most obese patients suggesting the presence of leptin insensitivity (20,26,37,38,41,42). In a small percentage of patients, however, leptin levels have been found inappropriately low with respect to their fat mass. It remains for future studies to prove that these patients represent a new pathophysiologic entity: leptin deficiency. Since leptin has also been shown to be of great importance for reproductive functions, possible new pathophysiologic mechanisms may be discovered relating infertility to insufficient leptin production.

The discovery of leptin has released an avalanche of research activities seeking to understand the regulation and actions of this new hormone. Most importantly, it has provided a key to better understand the physiology of body weight regulation and to unveil possible pathophysiologic mechanisms in both obesity and eating disorders. Further, it may provide new insights into certain causes of infertility.

INTENDED USE

The widespread importance makes leptin an interesting parameter for physicians dealing with **metabolic syndrome**, **obesity**, **cachexia** and other **metabolic disturbances**, as **diabetologists, endocrinologists**, **gynaecologists**, **andrologists**, and **psychiatrists** treating patients with **eating disorders**.

This radio immunoassay kit is suited for measuring human leptin in serum or plasma, and conditioned adipocyte culture media for scientific and diagnostic purposes.

Measuring leptin in anorectic or cachectic patients, young children or in specimen other than serum, such as urine, cerebrospinal fluid, and certain cell culture media, is also possible with this kit.

Under conditions of normal eating cycles, measurement in a single blood sample collected in the morning or early afternoon is sufficient.

The comparison with BMI-related reference ranges may be useful to detect conditions of relative **leptin deficiency** as a possible cause of obesity or provide an indication for **leptin resistance** respectively.

Due to its high correlation with body fat mass leptin measurements under standardized conditions may be used as a simple and inexpensive test for determination of **body fat**.

PRINCIPLE

For the Radioimmunoassay for the determination of human Leptin a polyclonal rabbit-antibody of high specificity is used. Leptin is measured quantitatively and binding protein does not influence the test results. Tubes were coated with streptavidin and separation is facilitated by biotinylated anti-rabbit-antibody.

Calibrators are prepared of recombinant human leptin in concentrations between **1, 2, 4, 8, 16, 32 and 64 ng/ml.**

Calibration of the Assay

The Assays is calibrated against the International Standard for human leptin. The standard preparation of the WHO with code 97/594 (y) is available from the NIBSC (z). One ampoule of the preparation, reconstituted in 1 ml Assay Buffer, will be quantified with the nominal content of 5000 ng human leptin.

PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical **sensitivity** of the assay yields **0.1 ng/ml** (2x SD of zero calibrators)

Intra-Assay-Variation

	Number of determinations	Mean value [ng/ml]	Standard Deviation [ng/ml]	VC%
Sample 1	6	19.67	0.87	4.4
Sample 2	6	6.95	0.33	4.8

Inter-Assay-Variation

	Mean value (ng/ml)	Standard deviation (ng/ml)	VC% (%)
Sample 1	8.975	0.48	5.3
Sample 2	6.3	0.32	5.1
Sample 3	20.9	1.05	5.0

Linearity

The Leptin RIA Leptin KIPMR44 can be used for diluted samples. The linearity of serum dilutions is excellent (Fig.9).

Recovery

Serum spiking experiments with recombinant human leptin yielded a recovery of 97% (± 2%).

SPECIMEN COLLECTION, PREPARATION, AND STORAGE

Serum as well as EDTA/Heparin **plasma samples** are suitable (significant deviation of hLeptin levels in corresponding Serum or -Plasma samples were not found). Conditioned adipocyte culture medium was found to be suitable. An external sample preparation prior to assay is not required.
Samples should be handled as recommended in general: as fast as possible and chilled as soon as possible. In case there will be a longer period between the sample withdrawal and determination store the undiluted samples frozen at –20°C or below in tightly closable plastic tubes. Avoid on principal repeated freeze-thaw cycles of serum/plasma (if required, please subaliquote) although Leptin levels were found to be unaffected by few cycles (5x) in our experiments.

The high sensitivity of the assay allows measurement of hLeptin in small sample volumes. Because of the wide effective range of this RIA kit a preparative sample dilution is generally not necessary. For most of the determinations (serum or plasma samples, and no extreme values expected) **the use of undiluted samples 25 µl per tube**, **should be appropriate.** In case Leptin levels of more than 64 ng/ml are expected, eg. obese patients (BMI>35), the sample should and can be diluted, e.g. 1:10.

The hLeptin concentrations may be completely different in body fluids of human origin other than serum or cell culture supernatants.

REAGENTS PROVIDED

ASS BUF	Assay buffer (1 bottle, 125 ml; ready for use)
Ab BIOT	Capture-antibody: anti-rabbit IgG, biotin-conjugated. Reconstitute in 7 ml Assay buffer. (1 bottle, 7 ml; lyophilized)
Ab	Specific antibody: rabbit-anti-human leptin antibody. Reconstitute in 7 ml Assay buffer. (1 bottle, 7 ml; lyophilized)
Ag 125I	Tracer: ^{125}I-leptin; < 2.25 µCi or < 83 kBq respectively. Reconstitute in 13 ml Assay buffer. (1 bottle, 13 ml; lyophilized, red coloured)
CAL N	Calibrators 1-7. (7 vials, 750 µl each; ready for use). Contain recombinant Leptin. Use 25 µl / tube. See exact values on the vial label

CONTROL	1

Control 1
(1 vial, 0.75 ml; human serum; lyophilized). Reconstitute with 750 µl Assay buffer.
See exact values on the vial label

Tubes (125 streptavidin-coated tubes)

MATERIALS REQUIRED BUT NOT PROVIDED
Precision pipettes (50, 100 and 500 µl) Micropipettes and multichannel pipettes with disposable plastic tips
Vortex-mixer
Device to aspirate the fluid from the tubes (recommended because of the potential danger of radioactivity and infection by human samples)
Shaking Device : 350 rpm
Gamma Counter

REAGENT PREPARATION
In conducting the assay, follow strictly the test protocol. Room temperature incubation means: Incubation at 20 - 25°C.

Reagents with different lot numbers should not be mixed. All reagents are stable unopened until the expiry date, if stored in the dark at 2° - 8°C (see label).

Control 1, Capture Antibody, Specific Antibody and **Tracer** have to be reconstituted in **Assay Buffer.** It is recommended to keep the reconstituted reagents at room temperature for 30 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer.

The shelf life of the components after opening is not affected, if used appropriately. Reconstituted Components (**Control 1, Capture Antibody, Specific Antibody** and **Tracer**) should be stored at -20°C (or below). Repeated freeze-thaw cycles have to be avoided.

Before use, all kit components should be brought to room temperature. **Precipitates, possible in buffers, should be dissolved before use through mixing and warming**.

WARNINGS AND PRECAUTIONS

For in-vitro diagnostic use only. For professional use only.
Possession and use of the kit is subject to the regulations of the national nuclear regulatory authorities.

Reagents with different lot numbers should not be mixed.

Reagents contain Sodium-Azide as preservative, however, highly diluted (0.02%). Avoid any skin contact.

Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.

Before use, all kit components should be brought **to room temperature at 20 - 25°C**. Precipitates in buffers should be dissolved before use by thorough mixing and warming. **Temperature WILL affect the** the assay. However, Values for the patient samples will not be affected.

Caution: This kit contains material of human and/or animal origin. Source human serum for the Control 1 provided in this kit was tested by FDA recommended methods and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV) antibody. No known test methods can offer total assurance of the absence of infectious agents; therefore all components and patient's specimens should be treated as potentially infectious.

Radioactivity - Before ordering or using radioactive materials, it is necessary to take the appropriate actions to ensure compliance with national regulations governing their use. Local rules in each establishment, which define actions and behaviour in the radioactivity working areas, should also be adhered to. The advice given here does not replace any local rules, instructions or training in the establishment, or advice from the radiation protection advisers. It is important to follow the code of good laboratory practice in addition to the specific precautions relating to the radionuclide I-125 used.

Iodine-125 has a radioactive half-life T1/2 of 60 days and emits 35.5 keV gamma radiation, 27 – 32 keV x-rays and no beta radiation. Shielding is effective done by lead, first half value layer is 0.02 mm lead, reduction to 10 % is made by 0.2 mm.

To reduce the radiation dose time spent handling radioactivity should be minimized (plan ahead), and distance from source of radiation should be maximized (doubling the distance from the source quarters the radiation dose).

Formation of aerosols, e.g. by improper opening and mixing of vials or pipetting of solutions which may cause minute droplets of radioactivity become airborne, is a hazard and should be avoided.

Solutions containing iodine should not be made acidic, because this might lead to the formation of volatile elemental iodine.
As some iodo-compounds can penetrate rubber gloves, it is advisable to wear two pairs, or polyethylene gloves over rubber.

For cleaning of contaminated areas or equipment, the Iodine-125 should be rendered chemically stable by using alkaline sodium thiosulphate solution together with paper or cellulose tissue.

General first aid procedures:
Skin contact: Wash affected area thoroughly with water. Discard contaminated cloths and shoes.
Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids.
Ingestion: If swallowed, wash out mouth thoroughly with water. Immediately see a physician.
Do not eat, drink or smoke in these areas.
Never pipette the materials with the mouth.
Spilled material must be wiped off immediately and should become disinfected. Clean contaminated areas and equipment with a suitable detergent.

The handling of radioactive and potentially infectious material must comply with the following guidelines:

The material should be stored and used in a special designated area.

Do not eat, drink or smoke in these areas.

Never pipette the materials with the mouth.

Avoid direct contact with these materials by wearing laboratory coats and disposable gloves.

Spilled material must be wiped off immediately. Clean contaminated areas and equipment with a suitable detergent.

Unused radioactive material and radioactive waste should be disposed according to the recommendations of the national regulatory authorities.

ASSAY PROCEDURE

NOTES: All determinations (Calibrators, Control 1 and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.
When performing the assay, the Calibrators, Control 1 and the samples should be pipette as fast as possible.

Flow Chart of Assay Protocol

#	Tube	Assay Buffer, Calibrators, Control 1, Samples µl	Capture-antibody µl	Specific antibody µl	Tracer µl
1,2	TC	---	---	---	100
3,4	B_0	Assay Buffer :25	50	50	100
5-18	Calibrators	Cal 1-7: 25	50	50	100
19,20	Control 1	Control 1: 25	50	50	100
21,22	Sample 1	25	50	50	100
23,24	Sample 2	25	50	50	100
etc.					

1) Labelling of the assay tubes should be done in the following order:

1, 2 total counts (**TC**),
3, 4 zero calibrator (**B_0**),
5 - 18 duplicates of calibrators (**1-7**),
19, 20 duplicates of control (**1**),
21, 22 etc. duplicates of samples.

2) Add **25** µl of **Assay Buffer** to tubes 3 and 4.
Add **25** µl of **Calibrators** to tubes 5 - 18:
5, 6 calibrator **1**
7, 8 calibrator **2**, etc.

3) Add **25** µl of **control 1** to tubes 19 and 20.

4) Add **25** µl of the first **sample** to tubes 21 and 22,etc.

5) Add **50** µl **capture-antibody** beginning with tube 3.

6) Add **50** µl **specific antibody** beginning with tube 3.

7) Add **100** µl **tracer** to all tubes.

8) Remove tubes 1 and 2 (total counts) or seal with a stopper.

9) Shake the tubes on a shaking device (350 rpm) **overnight** (at least 15 h) **at room temperature**. (steps 9 to 12 may not be performed with tubes 1 & 2)

10) Aspirate the liquid (except tubes 1 and 2 !) completely.

Take care that the coating of the tubes remains intact. Depending on laboratory equipment and common laboratory practice, aspiration of the liquid can be replaced by careful decantation.

11) Add **500** µl of **assay buffer** to the tubes (except tubes 1 and 2 !).

12) Aspirate the liquid (see step 10).

13) Count the radioactivity of **all** tubes.

CALCULATION OF RESULTS

Establishing the Calibration Curve

Calibrator	**B_0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
ng/ml	0	1	2	4	8	16	32	64

1. Calculate the average counts of each pair of tubes. This equals to the binding value B.
2. The mean of the zero standard (tubes 3 and 4) equals B_0.
3. Calculate the percent bound (% B/B_0) by dividing the B values by B_0:
   $\%B/B_0 = B/B_0 \times 100\%$.

4. Plot % B/B_0 versus the calibrator concentrations on a semi-logarithmic or logit-log paper respectively. For convenience, it is recommended to use computer assisted data reduction programs.
5. Calculate the 'percentage bound of the zero calibrator':
   Average of tubes 3, 4 (B_0) divided by average of tubes 1, 2 (total counts) multiplied by 100%.
   It should be $\%B_0/TC > 20\%$.

Evaluation of sample concentrations:
Read the concentration value (abscissa) corresponding to the $\%B/B_0$ of the sample as in the example given below:

average counts of zero calibrator (B_0): 8648 cpm
average counts of sample tube: 4283 cpm

$\%B/B_0$ = sample counts / B_0 x 100%
= 4283 / 8648 x 100%
= 0.495 x 100% = 49.5%

For a 49.5% value on the y-axis (ordinate) a value of 7.28 ng/ml on the x-axis (abscissa) may be obtained.
In case of dilution of the samples, multiplication of this value determined graphically or by the aid of a computer program with the dilution factor gives the leptin concentration of the sample.

Concentration of the control:
The control 1 should fit with the labelled concentration range. The measured Leptin concentration are only valid, if the measured value of the control is in the labelled concentration range. Otherwise process analysis is required and depending on the results the measured values are accepted or not.

EXPECTED NORMAL VALUES

Serum leptin levels are mainly determined by body fat mass with low levels in lean individuals and high levels in obese subjects. In addition, there is a clear gender difference with higher levels in females at a given percentage body fat. Further, leptin levels are influenced by pubertal development. Any attempt, therefore, to give ranges of expected leptin levels must account for these relationships.
Various methods for the estimation of body fat are available such as calculation of body mass index (weight (kg) divided by the square of height (m)) (BMI), bioelectric impedance assessment (BIA) or total body dual energy x-ray absorptiometry (DXA). Although the accuracy of BMI with respect to reflecting true fat mass is inferior to other more sophisticated methods such as BIA or DXA, BMI provides a number of advantages:

1) It is independent of the regression models applied.
2) It is easy to determine, only weight and height measurements are required.
3) It is retrospectively mostly available.
4) It is the most precise measure during short-term changes of fat mass, e.g. during fasting.

Therefore, the following expectation ranges of serum leptin levels were referred to BMI as the major confounding independent variable and were stratified according to gender and pubertal development (45; see figures 1-8 and tables 4 - 11). After the age of 20 years, no significant age dependence was observed. These gender and age adjusted expectation ranges may be used to compare a measured leptin level at a given BMI with normal subjects to detect pathologic deviations.
The best-fit regression lines for the various subgroups are exponential curves of the form $leptin = a \cdot e^{(b \cdot BMI)}$.
The 5th and 95th percentiles are given by the following equations:

$leptin = a \cdot e^{(b \cdot BMI - c)}$ and $leptin = a \cdot e^{(b \cdot BMI + c)}$

In a semi-logarithmic plot (y-axis = log leptin), these curves give straight lines. The values for a, b and c are given in table 3 according to gender and pubertal stage and also for adults. Using these values, the expectation ranges of leptin levels can be easily extended to lower or higher BMI ranges if required.

Example:
The 50th percentile for boys at Tanner stages 3 and 4 is given by the following curve:
$leptin = 0.0181 \cdot e^{(0{,}2067 \,.\, BMI)}$
The 5th percentile is given by: $leptin = 0.0181 \cdot e^{(0{,}2067 \,.\, BMI - 1{,}1919)}$
and the 95th percentile is given by: $leptin = 0.0181 \cdot e^{(0{,}2067 \,.\, BMI + 1{,}1919)}$

In a semi-logarithmic plot, these lines are parallel with an equal distance to the 50th percentile.

Calculation of standard deviation scores (SDS; Z-scores)

A convenient method to detect any deviation of a measured leptin level from the corresponding reference range is to calculate its standard deviation score by relating the leptin level at the patient's BMI to the average leptin value of the corresponding sex and age group and expressing its deviation by the x-fold standard deviation. This method may be considered as normalization to the normal reference cohort. Thus, the leptin values can be adjusted for BMI, gender and pubertal stage/age (i.e., the influence of gender, age and BMI are removed) and may be pooled for further analysis.

Accounting for the logarithmic distribution of leptin levels, the leptin SDS can be calculated by the following equation:

$leptin\ SDS = (\ln(leptin) - \ln(a) - b \cdot BMI) / d$

In this equation, ln represents the natural logarithm (referring to the basis e). The constants a, b and d are given in table 3 according to gender and pubertal stage/age.

Example:

A boy at Tanner stage 3, BMI = 25 kg/m^2, measured leptin concentration = 5 ng/ml.

leptin SDS = (ln(5) - ln (0.0181) - 0.2067·25) / 0.6850 =
(1.6094 - (-4.0118) - 5.1675) / 0.6850 = 0.66

Estimation of optimal dilution of samples

Because serum leptin levels vary widely over several orders of magnitude, depending mainly on the body fat mass, adequate dilution might be a prerequisite for precise measurement. Therefore, samples should be diluted such, that the leptin concentration is near this value in order to take maximum advantage of the precision of the kit. The reference ranges provide a useful tool for a good estimation of the expected leptin value according to BMI, gender and age.

Example:

Adult woman, BMI = 45 kg/m^2 (130 kgs,1.70 m height). From the reference range for adult women, the average leptin level at a BMI of 45 kg/m^2 is approximately 224 ng/ml. The optimal dilution would be 1:30.

APPENDIX

Table 3: Constants a, b, c and d for calculation of leptin reference ranges and leptin SDS based on BMI. Groups of normal healthy individuals were stratified according to gender and pubertal stage/age. TS= Tanner stage, n= number of subjects, a,b,c and d = constants as defined in the text.

Groups	n	a	b	c	d
Males					
TS 1&2	136	0,0146	0,2706	0,8821	0,5379
TS 3&4	50	0,0181	0,2067	1,1919	0,6850
TS 5	112	0,0316	0,1462	1,0821	0,6558
Adults	380	0,0130	0,2200	1,1053	0,6740
Females					
TS 1&2	136	0,0422	0,2499	0,7849	0,4786
TS 3&4	43	0,0543	0,2357	0,5745	0,3379
TS 5	157	0,2550	0,1508	0,7053	0,4301
Adults	587	0,3042	0,1467	0,8548	0,5212

Table 4: Girls Tanner stages 1 and 2

Percentile (µg/L)					
BMI (kg/m²)	1	5	50	95	99
11	0.22	0.30	0.66	1.45	1.99
12	0.28	0.39	0.85	1.86	2.56
13	0.36	0.50	1.09	2.38	3.29
14	0.46	0.64	1.40	3.06	4.22
15	0.60	0.82	1.79	3.93	5.42
16	0.76	1.05	2.30	5.04	6.96
17	0.98	1.35	2.95	6.47	8.93
18	1.25	1.73	3.79	8.31	11.5
19	1.61	2.22	4.87	10.7	14.7
20	2.07	2.85	6.25	13.7	18.9
21	2.65	3.66	8.03	17.6	24.3
22	3.41	4.70	10.3	22.6	31.2
23	4.37	6.03	13.2	29.0	40.0
24	5.62	7.75	17.0	37.2	51.4
25	7.21	9.95	21.8	47.8	65.9
26	9.26	12.8	28.0	61.4	84.7
27	11.9	16.4	35.9	78.8	109.0
28	15.3	21.1	46.1	101.0	140.0
29	19.6	27.0	59.2	130.0	
30	15.2	34.7	76.1		
31	32.3	44.6	97.7		
32	41.5	57.2	125.0		
33	53.2	73.4			
34	68.4	94.3			
35	87.8	121.0			
36	113				
37	145				

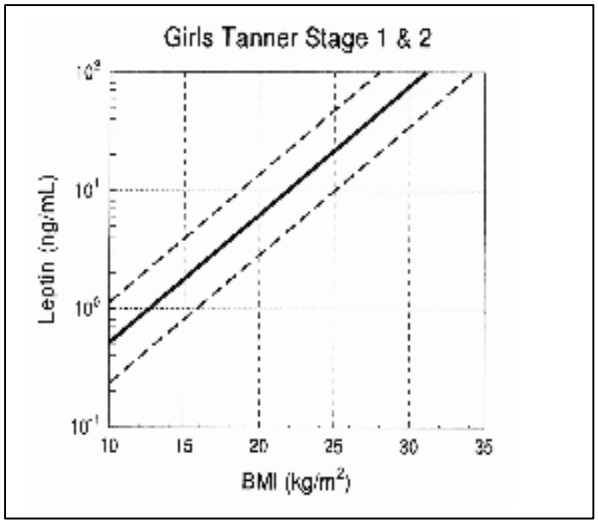

Figure 1: Reference ranges of human serum levels referring to BMI: GirlsTanner stage 1 & 2 (see text for details)

Table 5: Boys Tanner stages 1 and 2

Percentile / Perzentile (µg/L)					
BMI (kg/m²)	1	5	50	95	99
11	0.08	0.12	0.29	0.69	0.99
12	0.01	0.16	0.38	0.91	1.30
13	0.14	0.20	0.49	1.19	1.71
14	0.19	0.26	0.65	1.56	2.24
15	0.24	0.35	0.85	2.04	2.93
16	0.32	0.46	1.11	2.68	3.84
17	0.41	0.60	1.45	3.51	5.04
18	0.55	0.79	1.90	4.60	6.60
19	0.72	1.03	2.50	6.03	8.66
20	0.94	1.35	3.27	7.90	11.3
21	1.24	1.77	4.29	10.4	14.9
22	1.62	2.33	5.62	13.6	19.5
23	2.12	3.05	7.37	17.8	25.5
24	2.78	3.99	9.66	23.3	33.5
25	3.65	5.24	12.7	30.6	43.9
26	7.78	6.87	16.9	40.1	57.5
27	6.27	9.0	21.7	52.5	75.4
28	8.22	11.8	28.5	68.9	98.8
29	10.7	15.5	37.4	90.3	129.0
30	14.1	20.3	48.9	118.0	
31	18.5	26.6	64.2		
32	24.3	34.8	84.1		
33	31.8	45.6	110.0		
34	41.7	59.8	144.0		
35	54.6	78.4			
36	71.6	102.0			
37	93.9	134.0			
38	123.0				

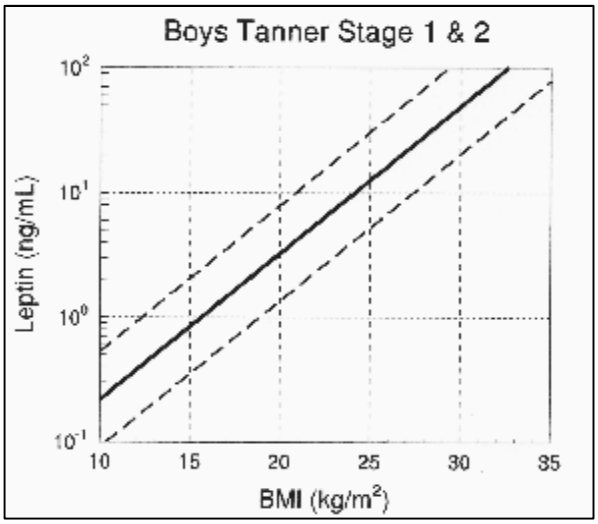

Figure 2: Reference ranges of human serum levels referring to BMI: Boys Tanner stage 1 & 2 (see text for details)

Table 6: Girls Tanner stages 3 and 4.

Percentile (µg/L)					
BMI (kg/m²)	1	5	50	95	99
11	0.32	0.41	0.73	1.29	1.63
12	0.41	0.52	0.92	1.63	2.06
13	0.52	0.66	1.16	2.07	2.61
14	0.65	0.83	1.47	2.61	3.31
15	0.83	1.05	1.87	3.31	4.19
16	1.05	1.33	2.36	4.19	5.30
17	1.33	1.68	2.99	5.30	6.71
18	1.68	2.13	3.78	6.71	8.49
19	2.13	2.69	4.79	8.5	10.8
20	2.69	3.41	6.06	10.7	13.6
21	3.41	4.31	7.67	13.61	17.2
22	4.32	5.46	9.71	17.2	21.8
23	5.46	6.91	12.3	21.8	27.6
24	6.91	8.75	15.6	27.6	34.9
25	8.75	11.1	19.7	34.9	44.2
26	11.1	14.0	24.9	44.2	56.0
27	14.0	17.7	31.6	56.0	70.9
28	17.8	22.5	39.9	70.9	89.7
29	22.5	28.4	50.5	89.7	114.0
30	28.4	36.0	63.9	114.0	144.0
31	36.0	45.6	80.9	144.0	
32	45.6	57.7	80.2	144.0	
33	57.7	73.0	102.0		
34	73.0	92.4	130.0		
35	92.4	117.0			
36	117.0	148.0			
37	148.0				

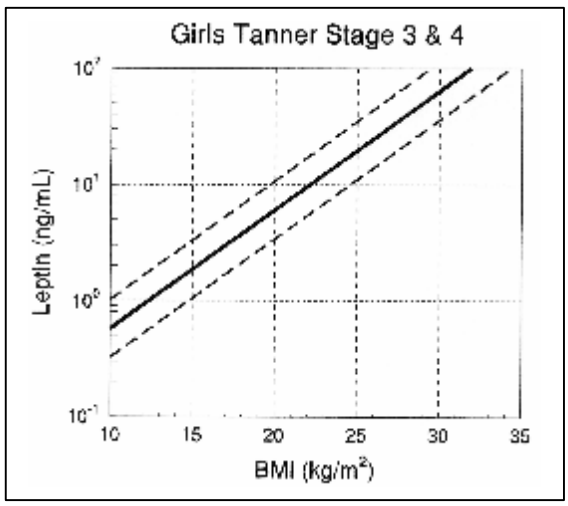

Figure 3: Reference ranges of human serum levels referring to BMI: Girls Tanner stage 3 & 4 (see text for details).

Table 7 : Boys Tanner stage 3 & 4

Percentile (µg/L)					
BMI (kg/m²)	1	5	50	95	99
11	0.03	0.05	0.18	0.58	0.94
12	0.04	0.07	0.22	0.71	1.16
13	0.49	0.08	0.27	0.88	1.43
14	0.06	0.10	0.33	1.08	1.75
15	0.07	0.12	0.40	1.32	2.16
16	0.09	0.15	0.49	1.63	2.65
17	0.11	0.18	0.61	2.00	3.26
18	0.14	0.23	0.75	2.46	4.01
19	0.17	0.28	0.92	3.03	4.93
20	0.21	0.34	1.13	3.72	6.06
21	0.26	0.42	1.39	4.58	7.46
22	0.32	0.52	1.71	5.63	9.17
23	0.39	0.64	2.10	6.92	11.3
24	0.48	0.78	2.58	8.51	13.9
25	0.59	0.96	3.18	10.5	17.0
26	0.73	1.19	3.91	12.9	21.0
27	0.89	1.46	4.80	15.8	25.8
28	1.10	1.79	5.90	19.4	31.7
29	1.35	2.20	7.26	23.9	39.0
30	1.66	2.71	8.93	29.4	48.0
31	2.05	3.33	11.0	36.2	58.9
32	2.51	4.09	13.5	44.5	72.4
33	3.09	5.04	16.6	54.7	89.1
34	3.80	6.20	20.4	67.2	109.0
35	4.68	7.62	25.1	82.6	134.0
36	5.75	9.37	30.9	101.0	
37	7.07	11.5	37.9	124.0	
38	8.7	14.2	46.7		
39	10.7	17.4	57.4		
40	13.1	21.4	70.5		

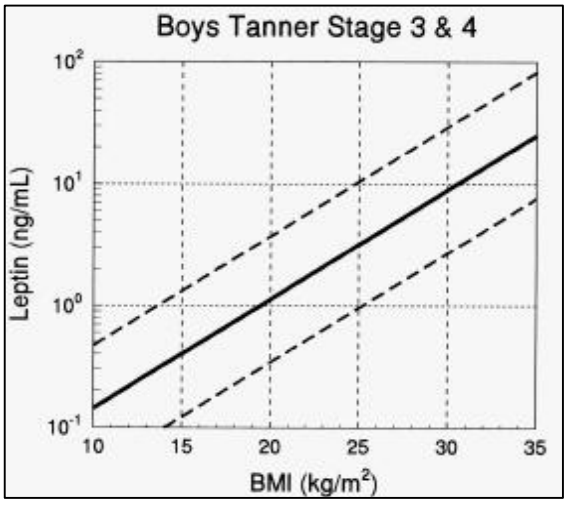

Figure 4: Reference ranges of human serum levels referring to BMI: Boys Tanner stage 3 & 4 (see text for details).

Table 8 : Girls Tanner stage 5.

Percentile (µg/L)					
BMI (kg/m²)	1	5	50	95	99
11	0.50	0.66	1.34	2.71	3.62
12	0.58	0.77	1.56	3.15	4.21
13	0.67	0.89	1.81	3.67	4.89
14	0.78	1.04	2.11	4.26	5.69
15	0.91	1.21	2.45	4.96	6.62
16	1.05	1.41	2.85	5.76	7.70
17	1.22	1.64	3.31	6.70	8.95
18	1.42	1.90	3.85	7.79	10.4
19	1.66	2.21	4.48	9.06	12.1
20	1.93	2.57	5.20	10.5	14.1
21	2.24	2.99	6.05	12.3	16.4
22	2.60	3.48	7.03	14.2	19.0
23	3.03	4.04	8.18	16.6	22.1
24	3.52	4.70	9.51	19.3	25.7
25	4.09	5.46	11.0	22.4	29.9
26	4.76	6.35	12.9	26.0	34.8
27	5.53	7.39	15.0	30.3	40.4
28	6.43	8.59	17.39	35.2	47.0
29	7.48	9.99	20.2	40.9	54.7
30	8.70	11.6	23.5	47.6	63.5
31	10.1	13.5	27.3	55.3	73.9
32	11.8	15.7	31.8	64.4	85.9
33	13.7	18.3	37.0	74.9	99.9
34	15.9	21.2	43.0	87.0	116.0
35	18.5	24.7	50.0	101.0	135.0
36	21.5	28.7	58.1	118.0	
37	25.0	33.4	67.6	137.0	
38	29.1	38.8	78.6		
39	33.8	45.1	91.4		
40	39.4	52.5	106.0		

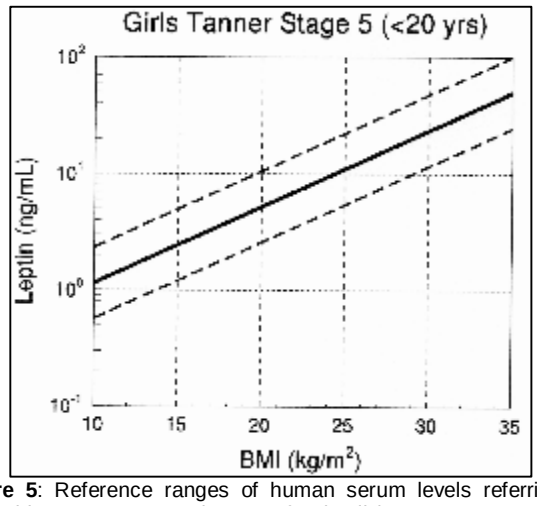

Figure 5: Reference ranges of human serum levels referring to BMI: Girls Tanner stage 5 (see text for details)

Table 9 : Boys Tanner stage 5

Percentile (µg/L)					
BMI (kg/m²)	1	5	50	95	99
11	0.03	0.05	0.16	0.47	0.73
12	0.04	0.06	0.18	0.54	0.84
13	0.05	0.07	0.21	0.62	0.97
14	0.05	0.08	0.24	0.72	1.12
15	0.06	0.10	0.28	0.84	1.30
16	0.07	0.11	0.33	0.97	1.51
17	0.08	0.13	0.38	1.12	1.74
18	0.1	0.15	0.44	1.3	2.02
19	0.11	0.17	0.51	1.50	2.34
20	0.13	0.2	0.59	1.74	2.7
21	0.15	0.23	0.68	2.01	3.13
22	0.17	0.27	0.79	2.33	3.62
23	0.20	0.31	0.91	2.69	4.19
24	0.23	0.36	1.05	3.12	4.85
25	0.27	0.41	1.22	3.61	5.62
26	0.31	0.48	1.41	4.17	6.5
27	0.36	0.55	1.63	4.83	7.52
28	0.41	0.64	1.89	5.59	8.71
29	0.48	0.74	2.19	6.47	10.1
30	0.55	0.86	2.54	7.49	11.7
31	0.64	1.00	2.94	8.67	13.5
32	0.74	1.15	3.4	10.0	15.6
33	0.86	1.33	3.94	11.6	18.1
34	0.99	1.54	4.55	13.4	20.9
35	1.15	1.79	5.27	15.6	24.2
36	1.33	2.07	6.10	18.0	28.1
37	1.54	2.39	7.06	20.8	32.5
38	1.78	2.77	8.17	24.1	37.6
39	2.06	3.21	9.46	27.9	43.5
40	2.38	3.71	10.9	32.3	50.3

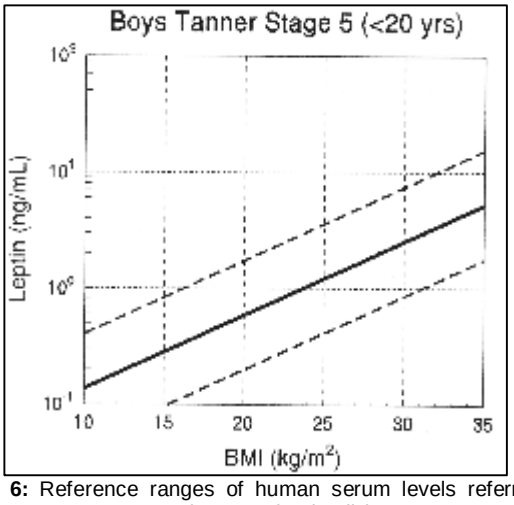

Figure 6: Reference ranges of human serum levels referring to BMI: Boys Tanner stage 5 (see text for details).

Table 10: Adult women

Percentile (µg/L)					
BMI (kg/m²)	1	5	50	95	99
11	0.46	0.65	1.53	3.59	5.10
12	0.53	0.75	1.77	4.16	5.90
13	0.61	0.87	2.05	4.82	6.83
14	0.71	1.01	2.37	5.58	7.91
15	7.82	1.17	2.75	6.46	9.17
16	0.95	1.35	3.18	7.48	10.61
17	1.10	1.57	3.68	8.66	12.3
18	1.28	1.81	4.27	10.0	14.2
19	1.48	2.10	4.94	11.6	16.5
20	1.71	2.43	5.72	13.4	19.1
21	1.99	2.82	6.62	15.6	22.1
22	2.30	3.26	7.67	18.0	25.6
23	2.66	3.78	8.88	20.9	29.3
24	3.08	4.38	10.3	24.2	34.3
25	3.57	5.07	11.9	28.0	39.7
26	4.13	5.87	13.8	32.4	46.0
27	4.79	6.79	16.0	37.5	53.3
28	5.54	7.87	18.5	43.5	61.7
29	6.42	9.11	21.4	50.4	71.5
30	7.43	10.6	24.8	58.3	82.8
31	8.61	12.2	28.7	67.5	95.8
32	9.97	14.1	33.3	78.2	111.0
33	11.5	16.4	38.5	90.5	129.0
34	13.4	19.0	44.6	105.0	149.0
35	15.5	22.0	51.6	121.0	
36	17.9	25.4	59.8	141.0	
37	20.8	29.5	69.3		
38	24.0	34.1	80.2		
39	27.8	39.5	92.9		
40	32.2	45.7	108.0		

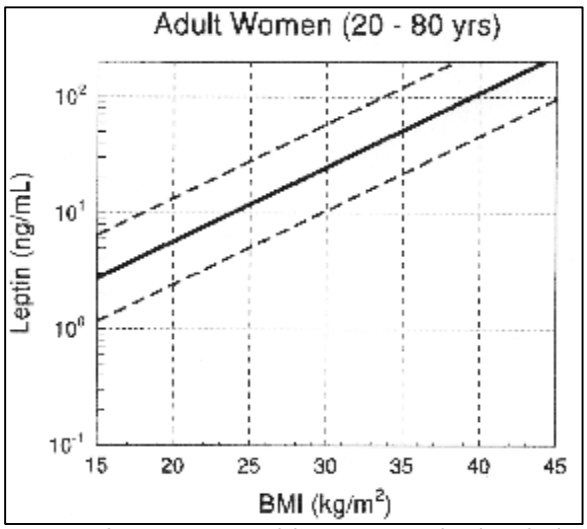

Figure 7: Reference ranges of human serum levels referring to BMI: Adult women (see text for details)

Table 11 Adult men

Percentile (µg/L)					
BMI (kg/m²)	1	5	50	95	99
11	0.03	0.05	0.15	0.44	0.69
12	0.04	0.06	0.18	0.55	0.87
13	0.05	0.08	0.23	0.69	1.08
14	0.06	0.09	0.28	0.85	1.34
15	0.07	0.12	0.35	1.06	1.67
16	0.09	0.15	0.44	1.33	2.09
17	0.12	0.18	0.55	1.65	2.60
18	0.14	0.23	0.68	2.06	3.24
19	0.18	0.28	0.85	2.57	4.04
20	0.22	0.35	1.06	3.20	5.03
21	0.23	0.44	1.32	3.98	6.27
22	0.35	0.54	1.64	4.97	7.81
23	0.43	0.78	2.05	6.19	9.73
24	0.54	0.85	2.55	7.71	12.1
25	0.67	1.05	3.18	9.61	15.1
26	0.83	1.31	3.96	12.0	18.8
27	1.04	1.64	4.94	14.9	23.5
28	1.30	2.04	6.15	18.6	29.2
29	1.61	2.54	7.67	23.2	36.4
30	2.01	3.16	9.56	28.9	45.4
31	2.51	3.94	11.9	36.0	56.6
32	3.12	4.91	14.8	44.9	70.5
33	3.89	6.12	18.5	55.8	87.8
34	4.85	7.63	23.0	69.6	109.0
35	6.04	9.51	28.7	86.7	136.0
36	7.53	11.8	35.8	108.0	
37	9.38	14.8	44.6	135.0	
38	11.7	18.4	55.5		
39	14.6	22.9	69.2		
40	18.2	28.6	86.2		

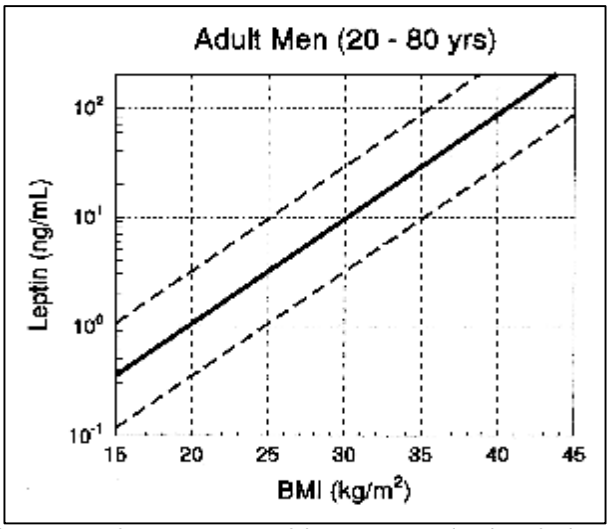

Figure 8: Reference ranges of human serum levels referring to BMI: Adult men (see text for details).

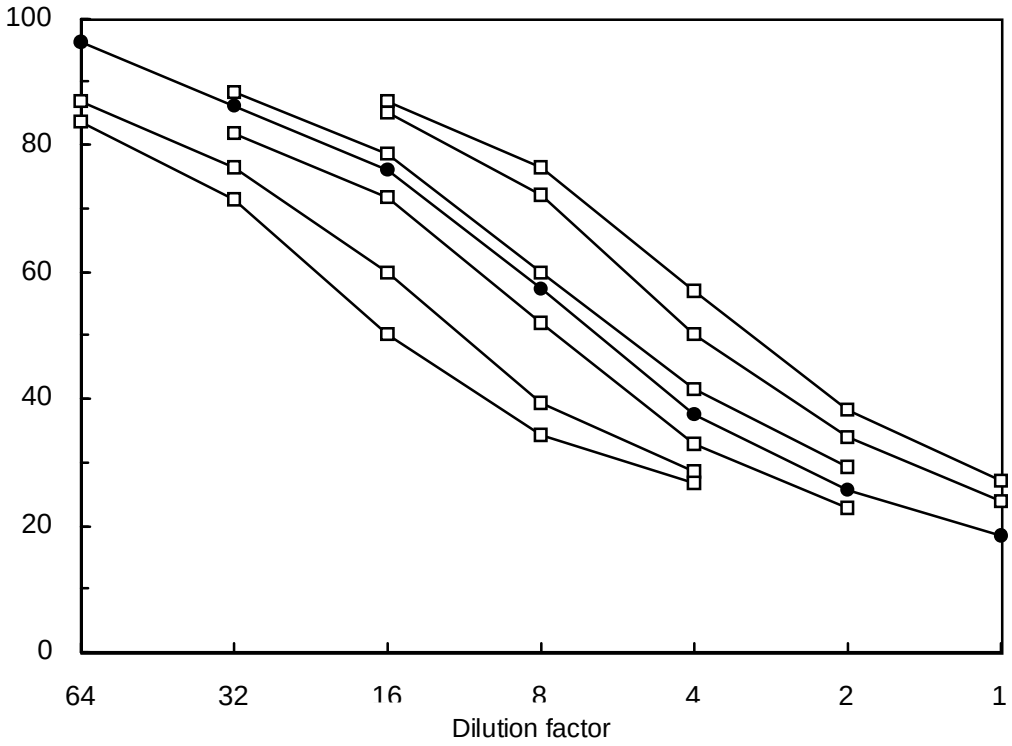

Figure 9: Serial dilution of leptin calibrator (filled circle) and different sera with varying concentrations of leptin. The leptin level of the undiluted calibrator was 16 ng/ml. Means of duplicate measurements are shown

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SUMMARY – DIAsource Hu LEPTIN RIA

Reagent Preparation:	**Reconstitution:**
Capture Antibody	in **7 ml Assay Buffer**
Specific Antibody	in **7 ml Assay Buffer**
Tracer	in **13 ml Assay Buffer**
Control 1	in **750 µl Assay Buffer**
Plasma/Serum Samples can be used undiluted.	

Proposed Assay Procedure for Double Detemination

Addition of Reagent [µl]					
Nr. of Tubes	**Contents of Tubes**	**Assay Buffer Calibrators Control 1 Samples**	**Capture Antibody**	**Specific Antibody**	**Tracer**
1,2	TC	–	–	–	100
3,4	B_0	**Ass Buf**: 25	50	50	100
5-18	Calibrators	**CAL 1-7**: 25	50	50	100
19,20	Control 1	25	50	50	100
21,22	Sample 1	25	50	50	100
23,24 (etc)	Sample 2 (etc)	25	50	50	100

Tubes Nr.:1,2 remove until counting the activity
Incubate, over night, at least. **15 hours** at **RT, 350 rpm**
Aspirate the liquid completely. Take care that the coating of the tubes remains intact.
Add **500 µl** of **Assay Buffer** to the tubes.
Aspirate the liquid completely (see above).
Count the radioactivity of **all** tubes.

Revision Date : 2011-02-04

	Used symbols
	Consult instructions for use
	Storage temperature
	Use by
LOT	Batch code
REF	Catalogue number
CONTROL	Control
I V D	In vitro diagnostic medical device
	Manufacturer
	Contains sufficient for <n> tests
WASH SOLN CONC	Wash solution concentrated
CAL 0	Zero calibrator
CAL N	Calibrator #
CONTROL N	Control #
Ag 125I	Tracer
Ab 125I	Tracer
Ag 125I CONC	Tracer concentrated
Ab 125I CONC	Tracer concentrated
	Tubes
INC BUF	Incubation buffer
ACETONITRILE	Acetonitrile
SERUM	Serum
DIL SPE	Specimen diluent
DIL BUF	Dilution buffer
ANTISERUM	Antiserum
IMMUNOADSORBENT	Immunoadsorbent
DIL CAL	Calibrator diluent
REC SOLN	Reconstitution solution
PEG	Polyethylene glycol
EXTR SOLN	Extraction solution
ELU SOLN	Elution solution
GEL	Bond Elut Silica cartridges
PRE SOLN	Pre-treatment solution
NEUTR SOLN	Neutralization solution
TRACEUR BUF	Tracer buffer
	Microtiterplate
Ab HRP	HRP Conjugate
Ag HRP	HRP Conjugate
Ab HRP CONC	HRP Conjugate concentrate
Ag HRP CONC	HRP Conjugate concentrate
CONJ BUF	Conjugate buffer
CHROM TMB CONC	Chromogenic TMB concentrate
CHROM TMB	Chromogenic TMB solution
SUB BUF	Substrate buffer
STOP SOLN	Stop solution
INC SER	Incubation serum
BUF	Buffer
Ab AP	AP Conjugate
SUB PNPP	Substrate PNPP
BIOT CONJ CONC	Biotin conjugate concentrate
AVID HRP CONC	Avidine HRP concentrate
ASS BUF	Assay buffer
Ab BIOT	Biotin conjugate
Ab	Specific Antibody
SAV HRP CONC	Streptavidin HRP concentrate
NSB	Non-specific binding
2nd Ab	2nd Antibody
ACID BUF	Acidification Buffer
DIST	Distributor