DRG

DRG® Osteocalcin ELISA (EIA-3375)

CE

RUO in the USA

Revised 24 Feb. 2010 rm (Vers. 5.0)

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

1 INTENDED USE

Immunoenzymetric assay for the *in vitro* quantitative measurement of intact human Osteocalcin (OST) in serum.

2 CLINICAL BACKGROUND

2.1 Biological activities

Osteocalcin or bone Gla protein (B.G.P) is the major non-collagen protein of the bone matrix. It has a molecular weight of 5800 Da and contains 49 amino-acids, including 3 residues of gamma carboxyl glutamic acid. Osteocalcin is synthesized in the bone by the osteoblasts. After production, it is partly incorporated in the bone matrix and the rest is found in the blood circulation. The exact physiological function of osteocalcin is still unclear. A large number of studies show that the circulating levels of osteocalcin reflect the rate of bone formation.

2.2 Clinical application

The determination of the blood levels of osteocalcin is valuable for :

- The identification of women at risk of developing osteoporosis
- Monitoring bone metabolism during the perimenopause and postmenopause
- Monitoring bone metabolism during hormone replacement therapy and treatment of premenopausal women with LH-RH agonists
- Monitoring bone metabolism in patients with growth hormone deficiency, hypothyroidism, hyperthyroidism, chronic renal failure.

3 PRINCIPLES OF THE METHOD

The hOST-EASIA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on breakable microtiterplates. The assay uses monoclonal antibodies (MAbs) directed against distinct epitopes of human osteocalcin. Calibrators and samples react with the capture monoclonal antibody (MAb 1) coated on microtiter well and with a monoclonal antibody (MAb 2) labelled with horseradish peroxidase (HRP). After an incubation period allowing the formation of a sandwich: coated MAb 1 – human osteocalcin – MAb 2 – HRP, the microtiterplate is washed to remove unbound enzyme labelled antibody. Bound enzyme-labelled antibody is measured through a chromogenic reaction. Chromogenic solution (TMB ready for use) is added and incubated. The reaction is stopped with the addition of Stop Solution and the microtiterplate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is proportional to the osteocalcin concentration.

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

4 REAGENTS PROVIDED

	Reagents	96 tests Kit	Color Code	Reconstitution
	Microtiterplate with 96 anti OST (monoclonal antibodies) coated breakable wells	96 wells	blue	**Ready** for use
CONJ BUF	Conjugate buffer: TRIS-HCl buffer with bovine serum albumin, bovine casein, EDTA, gentamycin and thymol	1 vial 12 ml	red	**Ready** for use
Ab HRP CONC	Conjugate: HRP labelled anti-OST (monoclonal antibodies) in Stabilizing Buffer	1 vial 0.4 ml	red	**Dilute** 50 x with conjugate buffer
CAL 0	Zero calibrator in human serum with protease inhibitors and benzamidin	1 vial lyophilized	yellow	**Add** 1.0 ml distilled water
CAL N	Calibrator N = 1 to 5 (see exact values on vial labels) in human serum with protease inhibitors and benzamidin	5 vials lyophilized	yellow	**Add** 0.5 ml distilled water
WASH SOLN CONC	Wash Solution (Tris-HCl)	1 vial 10 ml	brown	**Dilute** 200 x with distilled water (use a magnetic stirrer).
CONTROL N	Controls - N = 1 or 2 in human serum with protease inhibitors, benzamidin and thymol	2 vials lyophilized	silver	**Add** 0.5 ml distilled water
CHROM TMB	Chromogenic TMB Solution (Tetramethylbenzydine)	1 vial 12 ml	black	**Ready** for use
STOP SOLN	Stop Solution: HCl 1N	1 vial 12 ml	white	**Ready** for use

Note: 1. Use the zero calibrator for sample dilutions.
2. The OST calibrator is calibrated on a synthetic peptide (Peninsula 6045).

5 SUPPLIES NOT PROVIDED

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

1. High quality distilled water
2. Trasylol® at 10000IU/ml
3. Pipettes for delivery of: 25 µl, 100 µl, , 500 µl and 1 ml (the use of accurate pipettes with disposable plastic tips is recommended)
4. Vortex mixer
5. Magnetic stirrer
6. Washer for Microtiterplates
7. Microtiterplate reader capable of reading at 450 nm and 650 nm (bichromatic reading)

6 REAGENT PREPARATION

A. Calibrators :

Reconstitute the zero calibrator with 1.0 ml distilled water and other calibrators with 0.5 ml distilled water.

B. Controls :

Reconstitute the controls with 0.5 ml distilled water.

C. Working anti-OST-HRP conjugate :

Prepare an adequate volume of conjugate solution by adding 40 µl of the concentrated anti-OST-HRP conjugate to 2 ml of conjugate buffer. Use a vortex to homogenize. Extemporaneous preparation is recommended.

D. Working Wash solution :

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

7 STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the vial label, if kept at 2 to 8°C.
- Unused wells must be stored, at 2-8°C, in a sealed bag containing a desiccant until expiration date.
- After reconstitution, calibrators and controls are very unstable, use them immediately after reconstitution. For longer storage periods, aliquots should be made and kept at –20°C for maximally 6 weeks. Freezing should be performed immediately after use, do not wait for freezing until all the samples are pipetted. Avoid subsequent freeze-thaw cycles.
- The concentrated Wash Solution is stable at room temperature until expiration date.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, the concentrated conjugate 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.

8 SPECIMEN COLLECTION AND PREPARATION

Collect blood by venipuncture, taking care to avoid haemolysis, the samples must be kept in an ice bath.
Separate the serum from the cells within 3 hours, the use of a refrigerated centrifuge is recommended.
Add 100 µl Trasylol® (10000IU/ml) to the serum immediately after centrifugation (to obtain 1000 IU Trasylol® per ml sample).

With this treatment the samples are stable for 3 days at 2-8°C.
For a longer delay the samples have to be frozen (- 20°C), however the samples can only be thawn once! For repeat testing freeze the samples in aliquots and discard each sample after the first thawing.

Do not use haemolysed samples or lipemic samples.

9 PROCEDURE

9.1 Handling notes

- Do not use the kit or components beyond expiry date.
- Do not mix materials from different kit lots.
- Bring all the reagents to room temperature prior to use.
- Thoroughly mix all reagents and samples by gentle agitation or swirling.
- Perform calibrators, controls and samples in duplicate. Vertical alignment is recommended.
- Use a clean plastic container to prepare the Wash Solution.
- In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.
- For the dispensing of the Chromogenic Solution and the Stop Solution avoid pipettes with metal parts.
- High precision pipettes or automated pipetting equipment will improve the precision.
- Respect the incubation times.
- To avoid drift, the time between pipetting of the first calibrator and the last sample must be no longer than 30 minutes.
- Prepare a calibration curve for each run, do not use data from previous runs.
- Dispense the Chromogenic Solution within 15 minutes following the washing of the microtiterplate.

– During incubation with Chromogenic Solution, avoid direct sunlight on the microtiterplate.

9.2 Procedure

1. Select the required number of wells for the run. The unused wells should be resealed in the bag with a desiccant and stored at 2-8°C.
2. Secure the wells into the holding frame.
3. Pipette 25 µl of each Calibrator, Control and Sample into the appropriate wells.
4. Pipette 100 µl of working anti-OST-HRP conjugate into all the wells.
5. Incubate for 2 hours at room temperature .
6. Aspirate the liquid from each well.
7. Wash the plate 3 times by:
   Dispensing 0.4 ml of Wash Solution into each well
   Aspirating the content of each well
8. Pipette 100 µl of the chromogenic solution into each well within 15 minutes following the washing step.
9. Incubate the microtiterplate for 30 minutes at room temperature horizontal and avoid direct sunlight.
10. Pipette 100 µl of Stop Solution into each well.
11. Read the absorbancies at 450 nm (reference filter 630 nm or 650 nm) within 1 hour and calculate the results as described in section 10.

10 CALCULATION OF RESULTS

1. Read the plate at 450 nm against a reference filter set at 650 nm (or 630 nm).
2. Calculate the mean of duplicate determinations.
3. On semi-logarithmic or linear graph paper plot the OD values (ordinate) for each calibrator against the corresponding concentration of OST (abscissa) and draw a calibration curve through the calibrator points by connecting the plotted points with straight lines.
4. Read the concentration for each control and sample by interpolation on the calibration curve.
5. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4 parameter logistic function curve fitting is recommended.

If Trasylol® is added to the samples (100 µl/ml), sample values have to be multiplied by 1.1.

11 TYPICAL DATA

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

1. hOST-EASIA		2. OD units
3. Calibrator	4. 0.0 ng/ml	10. 0.033
	5. 1.56 ng/ml	11. 0.118
	6. 4.1 ng/ml	12. 0.229
	7. 12.7 ng/ml	13. 0.641
	8. 31.5 ng/ml	14. 1.420
	9. 75 ng/ml	15. 2.415

12 PERFORMANCE AND LIMITATIONS

12.1 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 above the average OD at zero binding, was 0.08 ng/ml.

12.2 Specificity

This method detects intact human osteocalcin. N-terminal and C-terminal fragments have been tested at their maximum levels found in normal and pathological samples, were added to a low and a high value calibrator. No cross reactivity was observed at these concentrations.

12.3 Precision

16. INTRA ASSAY				17. INTER ASSAY			
18. Serum	19. N	20. <X> ± SD 21. (ng/ml)	22. CV 23. (%)	24. Serum	25. N	26. <X> ± SD 27. (ng/ml)	28. CV 29. (%)
30. A 31. B	32. 20 33. 20	34. 11.4 ± 0.5 35. 28.2 ± 0.28	36. 4.7 37. 3.1	38. A 39. B	40. 20 41. 20	42. 11.8 ± 0.4 43. 27.7 ± 1.55	44. 3.5 45. 5.6

SD : Standard Deviation; CV: Coefficient of variation

12.4 Accuracy

RECOVERY TEST

46. Sample	47. Added OST 48. (ng/ml)	49. Recovered OST 50. (ng/ml)	51. Recovery 52. (%)
53. 54. Serum 55. 56. 57. 58. 59.	60. 61. 1.4 62. 4.04 63. 8.4 64. 15 65. 31 66. 64.6	67. 68. 1.55 69. 4 70. 8.3 71. 14.5 72. 31 73. 64.4	74. 75. 111 76. 99 77. 99 78. 97 79. 100 80. 99

DILUTION TEST

81. Sample	82. Dilution	83. Theoretical Concent. 84. (ng/ml)	85. Measured Concent. 86. (ng/ml)
87.	99.	111.	123.
88. 1	100.1/1	112.-	124.28.6
89.	101.1/2	113.14.3	125.14.2
90.	102.1/4	114.7.1	126.7.1
91.	103.1/8	115.3.6	127.3.4
92.	104.1/16	116.1.8	128.1.4
93.	105.	117.	129.
94.	106.	118.	130.
95.	107.1/1	119.-	131.30.8
96. 2	108.1/2	120.15.4	132.15
97.	109.1/4	121.7.7	133.7.7
98.	110.1/8	122.3.8	134.3.7

Samples were diluted with zero calibrator.

12.5 Hook effect

A sample spiked with OST up to 625 ng/ml gives higher OD's than the last calibrator point.

13 INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots. Controls which contain azide will interfere with the enzymatic reaction and cannot be used.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises
- It is recommended that Controls be routinely assayed as unknown samples to measure assay variability. The performance of the assay should be monitored with quality control charts of the controls.
- It is good practise to check visually the curve fit selected by the computer.

14 REFERENCE INTERVALS

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

Normal values are expected between 5 to 25 ng/ml.

15 PRECAUTIONS AND WARNINGS

Safety

For *in vitro* use only.

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

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

Avoid any skin contact with all reagents, Stop Solution contains HCl, the chromogenic solution contains TMB and H_2O_2. In case of contact, wash thoroughly with water.

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

16 SUMMARY OF THE PROTOCOL

135.	136.CALIBRATORS 137.(µl)	138.SAMPLE(S) / CONTROLS 139.(µl)
140. 141.Calibrators (0-5) 142.Samples, Controls 143.Working Anti-OST-HRP conjugate	144. 145.25 146.- 147.100	148. 149.- 150.25 151.100
152.Incubate for 2 hours at room temperature. 153.Aspirate the contents of each well. 154.Wash 3 times with 400 µl of Wash Solution and aspirate.		
155. 156.Chromogenic Solution	157. 158.100	159. 160.100
161. 162.Incubate for 30 min at room temperature . 163.		
164. 165.Stop Solution	166. 167.100	168. 169.100
170.Read on a microtiterplate reader and record the absorbance of each well at 450 nm (versus 630 or 650 nm)		

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SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..