Product information

User's Manual

hOST-IRMA

Immunoradiometric assay kit for the in vitro quantitative measurement of human intact osteocalcin (OST) in serum and plasma.

Catalog #: **IB79151**
Tests: **96**

IVD	**Europe:**	**For in-vitro diagnostic use only**
RUO	**USA:**	**For research use only**

CE

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Product information

1. INTENDED USE

Immunoradiometric assay kit for the in vitro quantitative measurement of human intact osteocalcin (OST) in serum and plasma.

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 BL-America hOST-IRMA is an immunoradiometric assay based on coated-tube separation. Mabs1, the capture antibodies, are attached to the lower and inner surface of the plastic tube. Calibrators or samples added to the tubes will at first show low affinity for Mabs1. Addition of Mab2, the signal antibody labelled with ^{125}I, will complete the system and trigger the immunological reaction. After washing, the remaining radioactivity bound to the tube reflects the antigen concentration. The use of several distinct Mabs avoids hyperspecificity, common to two-site IRMA.

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4. REAGENTS PROVIDED

Reagents	96 tests Kit	Colour Code	Reconstitution
Tubes coated with anti OST (monoclonal antibodies)	2 x 48	brown	**Ready** for use
Ab | ^{125}I TRACER: ^{125}Iodine labelled anti-OST (monoclonal antibodies) in TRIS buffer with bovine serum albumin, azide (<0.1%), EDTA, protease inhibitors and an inert red dye	1 vial 5.5 ml 440 kBq	red	**Ready** for use
CAL | 0 Zero calibrator in osteocalcin free human serum with benzamidine and protease inhibitors	1 vial lyophil.	yellow	**Add** 1.0 ml distilled water
CAL | N Calibrator N = 1 to 5 (see exact values on vial labels) in osteocalcin free human serum with benzamidine and protease inhibitors	5 vials lyophil.	yellow	**Add** 0.5 ml distilled water
WASH | SOLN | CONC Wash solution (TRIS-HCl)	1 vial 10 ml	brown	**Dilute** 70x with distilled water (use a magnetic stirrer).
CONTROL | N Controls 1 and 2 in human serum with thymol, benzamidine and protease inhibitors	2 vials lyophil.	silver	**Add** 0.5 ml distilled water

Note: 1. Use the zero calibrator for sample dilutions.
2. The origin of the osteocalcin for the preparation of the calibrators is human.

5. SUPPLIES NOT PROVIDED

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

1. Distilled water
2. Trasylol® at 10000IU/ml
3. Pipettes for delivery of: 50 µl, 500 µl and 1 ml (the use of accurate pipettes with disposable plastic tips is recommended)
4. Vortex mixer
5. Tube shaker (400 rpm)
6. Magnetic stirrer
7. Refrigerated centrifuge
8. 5 ml automatic syringe (Cornwall type) for washing
9. Aspiration system (optional)
10. Any gamma counter capable of measuring ^{125}I may be used (minimal yield 70%).

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6. REAGENT PREPARATION

- **Calibrators** : Reconstitute the zero calibrator with 1.0 ml distilled water and other calibrators with 0.5 ml distilled water.
- **Controls** : Reconstitute the controls with 0.5 ml distilled water.
- **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.

7. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kit components are stable until the expiry date, indicated on the vial label, if kept at 2 to 8°C.
- 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°. Freezing should be performed immediately after use, do not wait for freezing until all the samples are pipetted.
  Avoid subsequent freeze-thaw cycles.
- 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.

8. SPECIMEN COLLECTION AND PREPARATION

- Serum or heparin and EDTA plasma provide similar results.
- Collect blood by venipuncture, taking care to avoid hemolysis, the samples must be kept in an ice bath. Separate the plasma or serum from the cells within 3 hours, the use of a refrigerated centrifuge is recommended. Add 100 µl Trasylol® (10000IU/ml) to the plasma or 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 citrate plasma, hemolyzed 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. 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.

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9.2. Procedure

1. Label coated tubes in duplicate for each calibrator, sample and control. For determination of total counts, label 2 normal tubes.
2. Briefly vortex calibrators, controls, samples and dispense 50 µl of each into the respective tubes.
3. Dispense 50 µl of anti-OST-^{125}I tracer into each tube, including the uncoated tubes for total counts.
4. Shake the rack containing the tubes gently by hand to liberate any trapped air bubbles.
5. Incubate for 2 hours 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). Avoid foaming during the addition of the Working Wash solution.
8. Aspirate (or decant) the content of each tube (except total counts).
9. Wash tubes again with 2 ml Wash solution (except total counts) and aspirate (or decant).
10. After the last washing, let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
11. Count tubes in a gamma counter for 60 seconds.

10. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. On semi logarithmic or linear graph paper plot the c.p.m. (ordinate) for each calibrator against the corresponding concentration of OST (abscissa) and draw a calibration curve through the calibrator points, reject the obvious outliers.
3. Read the concentration for each control and sample by interpolation on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is to be used, a 4-parameter logistic function curve fitting is recommended.
5. 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.

hOST-IRMA		cpm	B/T (%)
Total count		160142	100
Calibrator	0.0 ng/ml	197	0.12
	1.9 ng/ml	2050	1.27
	4.5 ng/ml	5467	3.40
	19.5 ng/ml	26254	16.31
	46.0 ng/ml	60957	37.86
	69.0 ng/ml	86764	53.89

12. PERFORMANCE AND LIMITATIONS

12.1. Detection limit

Twenty zero calibrators were assayed along with a set of the other calibrators. The detection limit, defined as the apparent concentration of the average count at zero binding plus two standard deviations, was 0.22 ng/ml.

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12.2. Specificity

This method detects intact human osteocalcin. N-terminal and C-terminal fragments, 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.

added Hormone	OST CAL 1 ng/ml	OST CAL 2 ng/ml
-	20	100
N-terminal fragment 1-18 at 28 mM	18.5	125
C-terminal fragment at 5.5 mM	19.2	97

12.3. Precision

INTRA ASSAY				INTER ASSAY			
Serum	**Replicate**	**<X> ± SD (ng/ml)**	**CV (%)**	**Serum**	**Replicate**	**<X> ± SD (ng/ml)**	**CV (%)**
A	10	2.99 ± 0.06	2.0	A	10	9.03 ± 0.54	5.9
B	10	8.60 ± 0.08	1.0	B	10	20.1 ± 0.4	4.2
C	10	20.0 ± 0.2	1.0				

SD : Standard Deviation; CV: Coefficient of variation

12.4. Accuracy

RECOVERY TEST

Added OST (ng/ml)	Recovered OST (ng/ml)	Recovery (%)
7.5	7.6	101
15.0	14.6	97
30.0	32.9	109
60.0	73.3	122

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DILUTION TEST			
Sample	**Dilution**	**Theoretical Concent. (ng/ml)**	**Measured Concent. (ng/ml)**
A	1/1	-	60.5
	1/2	30.3	29.0
	1/4	15.1	12.7
	1/8	7.6	6.7
	1/16	3.8	3.6
	1/32	1.9	2.0

Samples were diluted with zero calibrator.

To the best of our knowledge, no international reference material exists for this parameter.

12.5. Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 30 minutes after the calibrator has been added to the coated tubes.

TIME DELAY				
Sample	**0'**	**10'**	**20'**	**30'**
X	3.4	3.3	3.3	3.4
Y	8.8	9.1	8.8	8.7
Z	20.5	20.8	20.4	20.8

12.6. Hook-effect

A sample spiked with OST up to 1000 ng/ml gives higher counts 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.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises

14. REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.
Healthy subjects obtained values ranging from 5 to 25 ng/ml (2.5 to 97.5 percentiles).

Pathology	nr. of subjects	X ± SD ng/ml
Healthy subjects	61	13.7 ± 5.5
Premenopausal women	19	10.6 ± 3.1
Postmenopausal women	25	15.6 ± 5.9
patients with tumor-induced	29	13.0 ± 12.0
hypercalcemia	14	31.6 ± 14.7
patients with hyperparathyroidism	18	5.1 ± 3.2
patients with hypoparathyroidism		

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15. PRECAUTIONS AND WARNINGS

Safety

For *in vitro* diagnostic use only.
This kit contains 125I, emitting ionizing X and γ radiations.
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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17. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS ml	CALIBRATORS ml	SAMPLE(S) CONTROLS ml
Calibrators (0-5) Samples, Controls Tracer	- - 0.05	0.05 - 0.05	- 0.05 0.05
Incubation	2 hours at room temperature with shaking (400 rpm)		
Separation Working Wash solution Separation Working Wash solution Separation	- - - - -	aspirate (or decant) 2.0 aspirate (or decant) 2.0 aspirate (or decant)	
Counting	Count tubes for 60 seconds		

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