PROCEDURAL NOTES

1. It is recommended that all calibrators, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused streptavidin-coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
7. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.
8. Prepare a calibration curve for each run. Do not use data from previous runs.
9. To avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.

INTERPRETION OF RESULTS

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the zero calibrator from the average absorbance of all other readings to obtain corrected absorbance.
3. The calibration curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs (e.g. Point-to-Point, 4-Parameter) may also be used for the calculation of results.
4. The sample human osteocalcin concentrations for the controls and samples are read directly from the calibration curve using their respective corrected absorbance.

LIMITATIONS OF THE PROCEDURE

1. An abnormally high osteocalcin value is likely to indicate a more significant bone turnover condition of a sample. For sample values reading greater than the highest calibrator, it is recommend to re-assay sample with dilution.
2. Different age group and gender may show a different normal range of osteocalcin.
3. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

QUALITY CONTROL

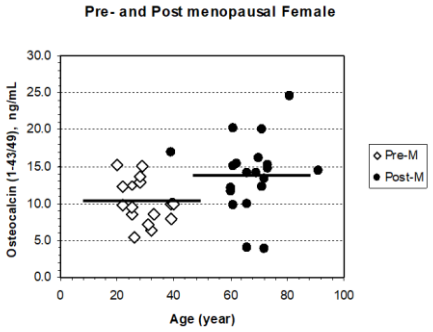
To assure the validity of the results each assay should include adequate controls.

EXPECTED VALUES

Forty serum samples from normal healthy adults with age of 26 – 58 were collected and measured with this ELISA. The normal osteocalcin range was found to be 3.8 – 25.3 ng/mL and the mean osteocalcin level of this group was 11.7 ng/mL (median: 11.4 ng/mL) and a Standard Deviation of 3.8 ng/mL. The ninety-five percentile normal high cut-off is 17 ng/mL based on this study group.

A validation study of pre- and post-menopausal women, as well as a group of male subjects, indicated a well-differentiated serum osteocalcin level of post-menopausal women from other two groups with this ELISA. The data is summarized in the following table and figure.

	Premenopausal Women (n = 16)	Postmenopausal Women (n = 19)	Male (n = 15)
Mean	29.0	68.7	50.3
Standard Deviation	6.3	7.9	9.9
Range	21 – 40	60 – 91	37 – 76
Osteocalcin (1-43/49) (ng/mL)			
Mean	10.3	13.8	10.8
Standard Deviation	3.0	5.0	3.6
Range	5.4 – 15.2	3.9 – 21.6	5.4 – 15.1



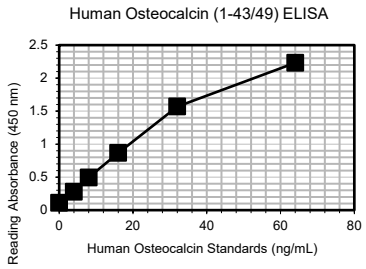
Forty serum samples from patients with end-stage renal diseases on hemodialysis were also measured with this ELISA. Except for one patient, all other 39 patients showed their osteocalcin values above the normal high cut-off ranging from 21 ng/mL to 119 ng/mL with a mean value of 60.6 ng/mL (median: 59.6 ng/mL, SD: 26.2 ng/mL).

EXAMPLE DATA

A typical absorbance data and the resulting calibration curve from human osteocalcin ELISA are represented.

Note: This curve should not be used in lieu of calibration curve run with each assay.

Well ID	Reading Absorbance (450 nm)		Concentration (ng/mL)
	Average	Corrected	
Calibrator Level 1: 0 ng/mL	0.112	0.000	
Calibrator Level 2: 4 ng/mL	0.279	0.167	
Calibrator Level 3: 8 ng/mL	0.494	0.382	
Calibrator Level 4: 16 ng/mL	0.866	0.754	
Calibrator Level 5: 32 ng/mL	1.570	1.458	
Calibrator Level 6: 64 ng/mL	2.232	2.120	
Control 1	0.363	0.251	5.26
Control 2	0.663	0.551	11.69



PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of this human osteocalcin ELISA as determined by the 95% confidence limit on 8 replicate determinations of both zero and level 2 calibrators is approximately 0.31 ng/mL.

Hook Effect

This assay has showed that it did not have any high dose "hook" for sample osteocalcin level up to 1,250 ng/mL.

Specificity

This assay shows less than 15% cross reactivity to uncarboxylated osteocalcin.

Reproducibility and Precision

The intra-assay precision is validated by measuring two samples in a single assay with 16 replicate determinations. The inter-assay precision is validated by measuring two control samples in duplicate in 6 individual assays. The results are as follows:

	Intra-Assay		Inter-Assay	
Sample	**1**	**2**	**1**	**2**
Mean (ng/mL)	11.9	40.2	5.6	11.9
CV (%)	4.7	5.0	8.3	5.7

Linearity

Two human serum samples from dialysis patients were diluted with a BSA based 0.01M phosphate, 0.15M sodium chloride buffer matrix and assayed. The results are as follows:

Samples	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Sample A	69.6	-	-
50%	34.5	34.8	99
25%	15.1	17.4	87
Sample B	42.1	-	-
50%	21.4	21.1	101
25%	10.4	10.5	99

Spike Recovery

Two serum samples are spiked with three assay calibrators in equal volume (1 vol. + 1 vol. mixture) and assayed. The results are as follows:

Samples	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Sample A	33.4	-	-
+ Level 3	18.5	20.7	89
+ Level 4	23.8	24.7	96
+ Level 5	30.4	32.7	93
Sample B	15.7	-	-
+ Level 3	11.4	11.9	96
+ Level 4	15.3	15.9	96
+ Level 5	24.4	23.9	102

Interfering Substances

Lipids 3000 mg/mL	**Test Osteocalcin (ng/mL)**	**Control Osteocalcin (ng/mL)**	**Dcut (ng/mL)**	**Bias (ng/mL)**	**Bias (%) (dobs)**
	42.6	42.6	3.2	0.0	0.0
	13.1	12.1	1.0	1.0	8.2
	8.8	8.2	0.6	0.6	7.3
Hemoglobin 200 mg/mL	**Test Osteocalcin (ng/mL)**	**Control Osteocalcin (ng/mL)**	**Dcut (ng/mL)**	**Bias (ng/mL)**	**Bias (%) (dobs)**
	42.0	41.3	3.1	0.7	1.7
	13.6	13.4	1.0	0.2	1.5
	9.7	9.1	0.7	0.6	6.6
Hemoglobin 66.6 mg/mL	**Test Osteocalcin (ng/mL)**	**Control Osteocalcin (ng/mL)**	**Dcut (ng/mL)**	**Bias (ng/mL)**	**Bias (%) (dobs)**
	42.3	41.3	3.1	1.0	0.2
	13.5	13.4	1.0	0.1	0.7
	9.4	9.1	0.7	0.3	3.3
Bilirubin 20 mg/mL	**Test Osteocalcin (ng/mL)**	**Control Osteocalcin (ng/mL)**	**Dcut (ng/mL)**	**Bias (ng/mL)**	**Bias (%) (dobs)**
	22.2	22.9	1.7	0.7	3.1
	7.4	7.5	0.6	0.1	1.3
	4.7	4.6	0.3	0.1	2.2

WARRANTY

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REFERENCES

1. Rosenquist C, Qvist P, Bjarnason N, Christiansen C. Measurement of a more stable region of osteocalcin in serum by ELISA with two monoclonal antibodies. Clin Chem. 1995 Oct;41(10):1439-45.

2. Takahashi M, Kushida K, Nagano A, Inoue T. Comparison of the analytical and clinical performance characteristics of an N-MID versus an intact osteocalcin immunoradiometric assay.
3. Clin Chim Acta. 2000 Apr;294(1-2):67-76.

4. Nagasue K, Inaba M, Okuno S, Kitatani K, Imanishi Y, Ishimura E, Miki T, Kim M, Nishizawa Y. Serum N-terminal midfragment vs. intact osteocalcin immunoradiometric assay as markers for bone turnover and bone loss in hemodialysis patients. Biomed Pharmacother. 2003 Mar;57(2):98-104.

5. Garnero P, Grimaux M, Seguin P, Delmas PD. Characterization of immunoreactive forms of human osteocalcin generated in vivo and in vitro. J Bone Miner Res. 1994 Feb;9(2):255-64

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

IVD In Vitro Diagnostic Device	RUO For Research Use Only	LOT Lot Number
REF Catalog Number	 Read instructions before use	 Number of Tests
 Store at	 Use by	 Keep away from heat and direct sun light
 Manufacturer	EC REP Authorized Representative in Europe	CE European Conformity

SHORT ASSAY PROCEDURE

1. Add **25 µL** of the calibrators, controls, and samples into the designated microwells.
2. Add **200 µL** of the working antibody solution to each well.
3. Mix, cover, and incubate at **room temperature (20-25 ºC)** with **shaking 350 rpm – 450 rpm** for **60 minutes**.
4. Wash each well five times.
5. Add **200 µL** of substrate to each well.
6. Cover and incubate at **room temperature (20-25 ºC)** for **20 minutes**.
7. Add **50 µL** of the stop solution to each well.
8. Read the absorbance at **450nm.**