N-MID Osteocalcin

cobas®

Osteocalcin (OCN)

REF	Σ	SYSTEM
12149133 160	100	Elecsys 2010 MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

For USA: Elecsys N-Mid Osteocalcin Immunoassay

English

For use in the USA only

Intended use

Immunoassay for the in vitro quantitative determination of N-MID osteocalcin in human serum and plasma. The determination of osteocalcin, an indication of human bone formation and osteoblastic activity, may be useful as an aid in the management of postmenopausal osteoporosis.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Osteocalcin, the most important non-collagen protein in bone matrix, is a bone-specific, calcium-binding protein which is dependent on vitamin K. It contains 49 amino acids and has a molecular weight of approximately 5800 daltons. It contains up to three γ-carboxyglutamic acid residues (bone-GLA-protein, BGP).

During bone synthesis osteocalcin is produced by the osteoblasts. Its production is dependent upon vitamin K (formation of γ-carboxyglutamic acid residues) and is stimulated by vitamin D3. After release from the osteoblasts, osteocalcin is not only assimilated into the bone matrix but also secreted into the blood stream. Accordingly, the serum (plasma) osteocalcin level is related to the rate of bone turnover in various disorders of bone metabolism, e.g. osteoporosis in particular, but also in primary and secondary hyperparathyroidism or Paget's disease.

Osteocalcin is therefore termed a bone turnover marker and is used for this purpose. By means of osteocalcin measurements it is possible to monitor therapy with antiresorptive agents (bisphosphonates or hormone replacement therapy, HRT) in, for example, patients with osteoporosis or hypercalcemia.

Both intact osteocalcin (amino acids 1-49) and the large N-MID fragment (amino acids 1-43) occur in blood. Intact osteocalcin is unstable due to protease cleavage between amino acids 43 and 44. The N-MID-fragment resulting from cleavage is considerably more stable.

The Elecsys N-MID Osteocalcin assay uses two monoclonal antibodies specifically directed against epitopes on the N-MID-fragment and the N-terminal-fragment. The assay hence detects the stable N-MID-fragment as well as the (still) intact osteocalcin. The test is non-dependent on the unstable C-terminal-fragment (amino acids 43-49) of the osteocalcin molecule and thus ensures constant measurement results under routine conditions in the laboratory.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample, a biotinylated monoclonal N-MID osteocalcin-specific antibody, and a monoclonal N-MID osteocalcin-specific antibody labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack is labeled as OSTEOC.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-N-MID Osteocalcin-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-N-MID Osteocalcin antibody (mouse) 1.5 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

R2 Anti-N-MID Osteocalcin-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 mL: Monoclonal anti-N-MID Osteocalcin antibody (mouse) labeled with ruthenium complex 1.3 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

For USA: For prescription use only.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	8 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes.

Li-heparin and K_3-EDTA plasma.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Note: Avoid hemolysis! Erythrocytes contain proteases which degrade osteocalcin. It is recommended that blood be centrifuged immediately.

Stability of serum and heparinized plasma: 8 hours at 15-25 °C, 3 days at 2-8 °C, 3 months at -20 °C. Freeze once only.

Stability of EDTA-plasma: 2 days at 15-25 °C, 3 days at 2-8 °C, 3 months at -20 °C. Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary

tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 11972111122, N-MID Osteocalcin CalSet, for 4 x 1 mL
- REF 05618860160, PreciControl Varia, for 2 x 3 mL each of PreciControl Varia 0, 1 and 2
- REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500160, Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against in-house reference standards: osteocalcin in analyte-free human serum matrix.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Varia.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample either in ng/mL or µg/L.

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 205 nmol/L or < 50 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

Hemolysis interferes. Erythrocytes contain proteases which degrade osteocalcin.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 2200 U/mL.

There is no high-dose hook effect at N-MID osteocalcin concentrations up to 4200 ng/mL.

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.500-300 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.500 ng/mL. Values above the measuring range are reported as > 300 ng/mL (or up to 1500 ng/mL for 5-fold diluted samples).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: < 0.500 ng/mL.

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Dilution

Samples with N-MID osteocalcin concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:5 (either automatically by the MODULAR ANALYTICS E170, Elecsys 2010 or **cobas e** analyzers or manually). The concentration of the diluted sample must be > 60 ng/mL.

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After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** software automatically takes the dilution into account when calculating the sample concentration.

Expected values

The reference ranges are test-dependent. Completed studies with the Elecsys N-MID Osteocalcin assay have revealed the following ranges in ng/mL:

	Number	N-MID Osteocalcin	
		Median (ng/mL)	5-95th Percentile
Healthy women			
• Premenopausal, > 21 yrs. Caucasian Afro-American	 133 160	 18.5 16.3	 9.7-35.1 7.6-30.7
• Postmenopausal, w/o diagnosed osteoporosis Caucasian Afro-American	 141 160	 17.5 18.1	 7.3-37.8 8.4-38.5
Osteoporosis patients	103	29.1	17.3-48.6
Healthy men			
• 30-50 yrs. Caucasian Afro-American	 130 151	 22.9 18.3	 10.2-36.7 8.4-33.6
• 51-70 yrs. Caucasian Afro-American	 117 117	 18.5 17.6	 10.8-31.1 9.9-35.6
• > 70 yrs. Caucasian Afro-American	 25 13	 15.9 14.9	 Percentile range not reliable for sample size less than 110

In patients with renal failure the osteocalcin values can be elevated, both directly, due to impaired clearance and indirectly, due to renal osteodystrophy.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	6.01	0.085	1.4	0.186	3.1
Human serum 2	12.2	0.135	1.1	0.373	3.1
Human serum 3	35.6	0.601	1.7	1.06	3.0
Human serum 4	169	3.12	1.8	5.56	3.3
Human serum 5	8.11	0.091	1.1	0.159	2.0

Elecsys 2010 and **cobas e** 411 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
PreciControl Varia 1	19.3	0.164	0.8	0.267	1.4
PreciControl Varia 2	93.2	1.01	1.1	1.65	1.8

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	6.11	0.056	0.9	0.120	2.0
Human serum 2	12.0	0.126	1.1	0.240	2.0
Human serum 3	34.5	0.361	1.0	0.677	2.0
Human serum 4	160	2.03	1.3	3.65	2.3
Human serum 5	7.49	0.066	0.9	0.107	1.4
PreciControl Varia 1	17.9	0.166	0.9	0.207	1.2
PreciControl Varia 2	85.9	0.755	0.9	1.12	1.3

Method comparison

A comparison of the Elecsys N-MID Osteocalcin assay (y) with a commercially available N-MID osteocalcin test (x) using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 48

Passing/Bablok

$y = 1.31x + 2.0$

$r = 0.966$

$md(68) = 0.816$

The sample concentrations were between approximately 2.58 and 49.8 ng/mL.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found:

No cross-reactivity detectable for β-CrossLaps, parathyroid hormone, and bone-specific alkaline phosphatase.

Clinical Data

Elecsys N-MID Osteocalcin assay and a commercially available N-MID osteocalcin test were used to measure retrospective samples from 57 subjects participating in a clinical study involving two Calcitonin treatments over six months.

Samples were taken from patients at weeks 0, 4, 12 and 24. The method comparison is presented to describe the linear regression. Number of samples measured: 228

Passing/Bablok

$y = 1.12x + 4.4$

$r = 0.922$

$md(68) = 1.337$

The sample concentrations were between approximately 9.19 and 93.4 ng/mL.

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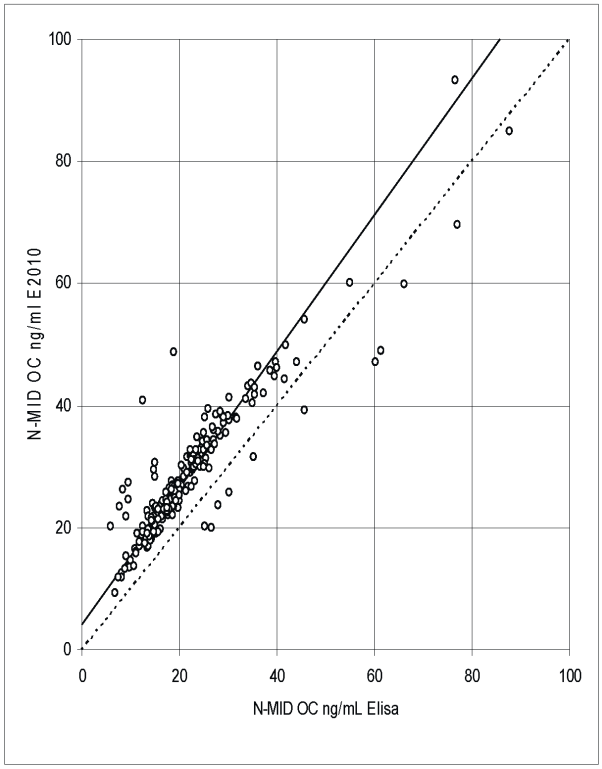
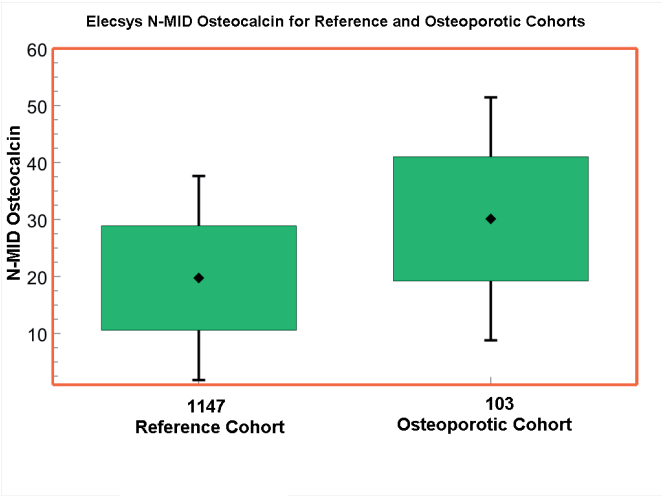


A box and whiskers plot for the clinical study population is also presented below. A clinical population of 1147 reference cohort individuals and 103 osteoporotic cohort individuals (pretreatment) is depicted.



References

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

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