Elecsys N-MID Osteocalcin

cobas®

REF		Σ	SYSTEM
07027591190	07027591501	100	**cobas e** 402 **cobas e** 801

English

For use in the USA only

System information

Short name	ACN (application code number)
OSTEOC	10060

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 **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 Da. 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 and its measurement is useful in disorders of bone metabolism, e.g. osteoporosis in particular, but also in primary and secondary hyperparathyroidism.[1,2,3,4,5] 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.[6,7,8] 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.[4,9] 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.[10]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 12 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 II 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 **cobas** link.

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

Reagents - working solutions

The **cobas e** pack is labeled as OSTEOC.

M Streptavidin-coated microparticles, 1 bottle, 6.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-N-MID Osteocalcin-Ab~biotin, 1 bottle, 10.3 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~$Ru(bpy)_3^{2+}$, 1 bottle, 7.2 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: Caution: Federal law restricts this device to sale by or on the order of a physician.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: 1-800-428-2336

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 available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **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

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on the analyzers	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Criterion: Recovery within 90-110 % of serum value or slope 0.90-1.10 + intercept ≤ ± 0.80 ng/mL + coefficient of correlation ≥ 0.95.

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

Serum and heparinized plasma: Stable for 8 hours at 20-25 °C, 3 days at 2-8 °C, 3 months at -20 °C (± 5 °C). Freeze only once.

EDTA plasma: Stable for 2 days at 20-25 °C, 3 days at 2-8 °C, 3 months at -20 °C (± 5 °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 and calibrators are at 20-25 °C prior to measurement.

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

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 11972111122, N-MID Osteocalcin CalSet, for 4 x 1.0 mL
- REF 05618860160, PreciControl Varia, for 4 x 3.0 mL
- REF 07299001190, Diluent Universal, 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500160, ISE Cleaning Solution/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.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

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

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 **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack 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 **cobas e** pack, 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.

If necessary, repeat the measurement of the samples concerned.

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 effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1129 µmol/L or ≤ 66 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 205 nmol/L or ≤ 50 ng/mL
Rheumatoid factors	≤ 2200 IU/mL

Criterion: For concentrations of 0.5-20 ng/mL the deviation is ≤ 2 ng/mL. For concentrations > 20 ng/mL the deviation is ≤ 10 %.

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.

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

Pharmaceutical substances

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

In addition, the following special drugs were tested. No interference with the assay was found.

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Special drugs

Drug	Concentration tested mg/L
Actonel (risedronate)	150
Fosamax (alendronate)	350
β-Estradiol	2.5
β-Estradiol-17-Valerate	2.5
β-Estradiol-3-Sulfate	2.5

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

0.5-300 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.5 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

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.4 ng/mL

Limit of Detection = 0.5 ng/mL

Limit of Quantitation = 4 ng/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

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 analyzers or manually). The concentration of the diluted sample must be ≥ 50 ng/mL.

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

After dilution by the analyzers, the 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	133	18.5	9.7-35.1
Afro-American	160	16.3	7.6-30.7
• Postmenopausal, w/o diagnosed osteoporosis			
Caucasian	141	17.5	7.3-37.8
Afro-American	160	18.1	8.4-38.5
Osteoporosis patients	103	29.1	17.3-48.6
Healthy men			
• 30-50 yrs.			
Caucasian	130	22.9	10.2-36.7
Afro-American	151	18.3	8.4-33.6
• 51-70 yrs.			
Caucasian	117	18.5	10.8-31.1
Afro-American	117	17.6	9.9-35.6
• > 70 yrs.			
Caucasian	25	15.9	Percentile range not reliable for sample size less than 110
Afro-American	13	14.9	

In patients with renal failure the osteocalcin values can be elevated, both directly, due to impaired clearance and indirectly, due to renal osteodystrophy.[11]

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 (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	6.94	0.096	1.4	0.140	2.0
Human serum 2	11.0	0.139	1.3	0.179	1.6
Human serum 3	42.1	0.636	1.5	0.929	2.2
Human serum 4	155	2.24	1.4	3.65	2.4
Human serum 5	250	4.04	1.6	5.43	2.2
PC[b)] Varia1	20.4	0.142	0.7	0.203	1.0
PC Varia2	103	0.840	0.8	1.30	1.3

b) PreciControl

Method comparison

A comparison of the Elecsys N-MID Osteocalcin assay, REF 07027591190 (**cobas e** 801 analyzer; y) with the N-MID Osteocalcin assay, REF 12149133122 (**cobas e** 601 analyzer; x) gave the following correlations (ng/mL):

Number of samples measured: 123

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Passing/Bablok[12]	Linear regression
y = 1.05x - 0.379	y = 1.05x - 0.285
τ = 0.990	r = 1.00

The sample concentrations were between approximately 0.964 and 278 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

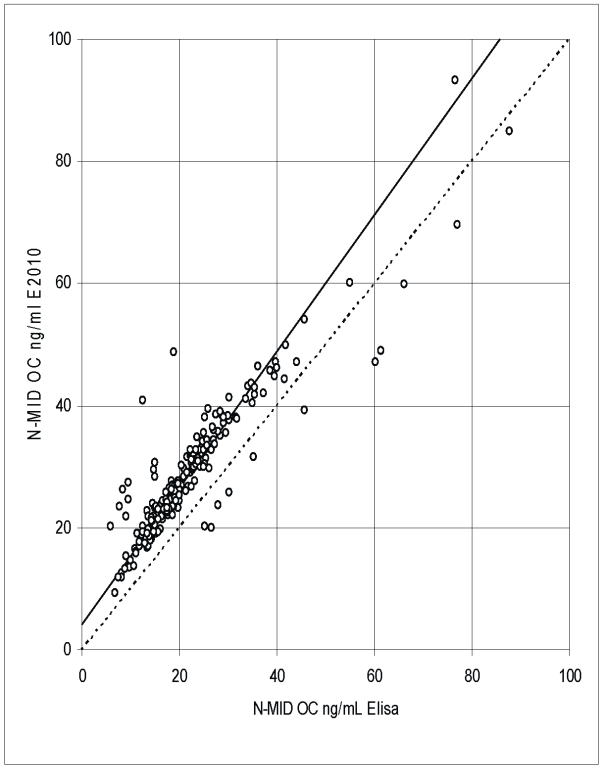
Passing/Bablok[12]

y = 1.12x + 4.4

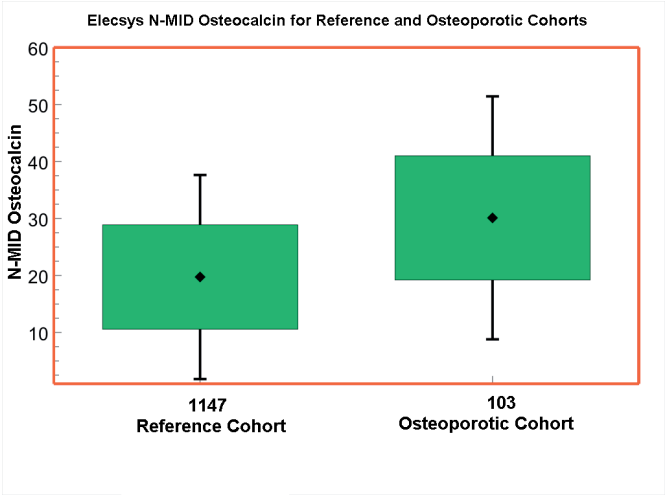
r = 0.922

md(68) = 1.337

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



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.



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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets 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 (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used

Elecsys N-MID Osteocalcin

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REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

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