

# Elecsys N-MID Osteocalcin

REF			SYSTEM
12149133122	12149133500	100	<b>cobas e 411</b> <b>cobas e 601</b> <b>cobas e 602</b>

## English

### System information

For **cobas e 411** analyzer: test number 660

For **cobas e 601** and **cobas e 602** analyzers: Application Code Number 122

### Intended use

Immunoassay for the in vitro quantitative determination of N-MID osteocalcin in human serum and plasma. The determination is used for the control of antiresorptives therapeutic efficiency, e.g. for patients with osteoporosis or hypercalcemia.

The electrochemiluminescence immunoassay "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 Da. It contains up to three  $\gamma$ -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  $\gamma$ -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.<sup>1,2,3,4,5</sup> 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.<sup>6,7,8</sup> 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.<sup>4,9</sup> 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.<sup>10</sup>

### Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20  $\mu$ L of sample, a biotinylated monoclonal N-MID osteocalcin-specific antibody, and a monoclonal N-MID osteocalcin-specific antibody labeled with a ruthenium complex<sup>a)</sup> 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 or e-barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)<sub>3</sub><sup>2+</sup>)

### 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)<sub>3</sub><sup>2+</sup> (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 for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:

Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:

Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

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: all countries: +49-621-7590

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.

# Elecsys N-MID Osteocalcin



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, K<sub>2</sub>-EDTA and K<sub>3</sub>-EDTA plasma.

Criterion: Method comparison serum versus plasma, slope 0.9-1.10 + intercept within  $\leq \pm 1$  ng/mL + coefficient of correlation  $\geq 0.95$ .

**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 ( $\pm 5$  °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 ( $\pm 5$  °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, 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 1.0 mL
- REF 05618860190, PreciControl Varia, for 4 x 3.0 mL
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- cobas e** analyzer

Additional materials for the **cobas e 411** analyzer:

- 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, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for **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, 48 magazines x 84 reaction cups or pipette tips, waste bags

- REF 03023150001, WasteLiner, waste bags

- REF 03027651001, SysClean Adapter M

Additional materials for all analyzers:

- REF 11298500316, 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. Read in the test-specific parameters via the reagent barcode. In exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

**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).

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 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.

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	$\leq 1112$ µmol/L or $\leq 65$ mg/dL
Intralipid	$\leq 1500$ mg/dL
Biotin	$\leq 205$ nmol/L or $\leq 50$ ng/mL

# Elecsys N-MID Osteocalcin



Compound	Concentration tested
Rheumatoid factors	≤ 2200 IU/mL

Criterion: Recovery within ± 2 ng/mL of initial value for samples ≤ 20 ng/mL and within ± 10 % of initial value for samples > 20 ng/mL.

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 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 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 (study protocol No. 9905) with the Elecsys N-MID Osteocalcin assay have revealed the following ranges in ng/mL:

	Number	N-MID osteocalcin	
		50 <sup>th</sup> perc.	5-95 <sup>th</sup> perc.
Healthy women			
• Premenopausal, > 20 yrs.	108	23	11-43
• Postmenopausal (no HRT)	102	27	15-46
Osteoporosis patients	120	27	13-48
Healthy men			
• 18- < 30 yrs.	183	40	24-70
• 30-50 yrs.	179	25	14-42
• > 50-70 yrs.	125	24	14-46

In patients with renal failure the osteocalcin values can be elevated, both directly, due to impaired clearance and indirectly, due to renal osteodystrophy.<sup>11</sup>

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 duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
Sample	Mean ng/mL	Repeatability		Intermediate precision	
		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	1.9	5.56	3.3
Human serum 5	8.11	0.091	1.1	0.159	2.0
PreciControl Varia 1	19.3	0.164	0.9	0.267	1.4
PreciControl Varia 2	93.2	1.01	1.1	1.65	1.8

cobas e 601 and cobas e 602 analyzers					
Sample	Mean ng/mL	Repeatability		Intermediate precision	
		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	1.1	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: 185

Passing/Bablok<sup>12</sup> Linear regression

$$y = 1.29x - 2.79$$

$$y = 1.43x - 6.24$$

$$r = 0.866$$

$$r = 0.987$$

The sample concentrations were between 10 and 210 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.

#### References

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# Elecsys N-MID Osteocalcin



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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).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

## 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):

	Contents of kit
	Analyzers/Instruments on which reagents can be used
	Reagent
	Calibrator
	Volume after reconstitution or mixing
	Global Trade Item Number

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