ms_03141071190V11.0

total P1NP

cobas®

total procollagen type 1 amino-terminal propeptide

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
03141071 190	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

Intended use

Immunoassay for the in vitro quantitative determination of total P1NP in human serum and plasma.

This assay is intended for use in monitoring therapy following the diagnosis of osteoporosis[1,2,3] in post-menopausal women and in patients diagnosed with Paget's disease of the bone.[4,5,6]

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

Summary

More than 90 % of organic bone matrix consists of type 1 collagen, which is preferentially synthesized in bone.[7] Type 1 collagen is derived from type 1 procollagen which is synthesized by fibroblasts and osteoblasts. Type 1 procollagen contains both N-(amino) and C-(carboxy) terminal extensions. These extensions (propeptides) are removed by specific proteases during the conversion of procollagen to collagen and its subsequent incorporation into the bone matrix. The extension measured by this assay is the amino terminal, hence P1NP-type 1 procollagen amino-terminal-propeptide. This marker, P1NP, is therefore a specific indicator of type 1 collagen deposition and thus may be defined as a true bone formation marker.[8,9] P1NP is released during type 1 collagen formation into the intracellular space and eventually into the blood stream. P1NP appears to be released as a trimeric structure (derived from the trimeric collagen structure) but is rapidly broken down to a monomeric form by thermal degradation effects.[10,11] This Elecsys P1NP assay detects both fractions present in blood and is therefore called total P1NP.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample and a biotinylated monoclonal P1NP-specific antibody are incubated together.
- 2nd incubation: After addition of streptavidin labeled microparticles and a monoclonal P1NP-specific antibody labeled with a ruthenium complex[a)], a sandwich complex is formed which 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 TP1NP.

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

R1 Anti-P1NP-Ab~biotin (gray cap), 1 bottle, 10 mL:
Biotinylated monoclonal anti-P1NP antibody (mouse) 2.5 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

R2 Anti-P1NP-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 mL:
Monoclonal anti-P1NP antibody (mouse) labeled with ruthenium complex 2.5 mg/L; phosphate buffer 100 mmol/L, pH 7.2; 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.

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	8 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 or tubes containing separating gel.

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.

Stable for 24 hours at 15-25 °C, 5 days at 2-8 °C, 6 months at -20 °C. Samples may be frozen and thawed up to 5 times without adverse effects.

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 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.

Note: Avoid hemolysis! Samples showing visible signs of hemolysis may cause interference.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 03141080190, total P1NP CalSet, for 4 x 1 mL
- REF 05618860190, PreciControl Varia, for 2 x 3 mL each of PreciControl Varia 1 and 2

- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or
  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 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 11298500316, 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.

Bring the cooled reagents to approx. 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 reference standards precisely defined by weighing native P1NP into an 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 1 month (28 days) 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 µg/L or ng/mL).

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), hemolysis (Hb < 0.062 mmol/L or < 0.1 g/dL; do not use samples with visible signs of hemolysis), lipemia (Intralipid < 2000 mg/dL) and biotin (< 205 nmol/L or < 50 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

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 2490 IU/mL.

There is no high-dose hook effect at P1NP concentrations up to 3900 µg/L (ng/mL).

In vitro tests were performed on 28 commonly used pharmaceuticals. No interference with the assay was found.

The pharmaceuticals tested included drugs common to the management of osteoporosis, i.e. Ibandronate, Risedronate and Alendronate as well as oestrogen-based therapies ("HRT") and also calcium and vitamin D supplementation therapies; none showed any evidence of assay interference.

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.

Bone metabolism may be affected by the use of cytotoxic agents. Results obtained from patients treated with such therapies should be interpreted with caution.

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

5-1200 µg/L or ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 5 µg/L (ng/mL). Values above the measuring range are reported as > 1200 µg/L (ng/mL).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: < 5 ng/mL (µg/L)

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 P1NP concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:2 (either automatically by the MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers or manually). The concentration of the diluted sample must be > 100 µg/L (ng/mL).

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.

Non-linear dilution behaviour may be seen when using sera from patients diagnosed with renal insufficiency.

Expected values

Sera taken from 573 healthy female volunteers who had been enrolled in a study of determinants of bone loss (OFELY[12,13]) were measured for total P1NP levels. The following results were obtained (µg/L or ng/mL):[14]

total P1NP

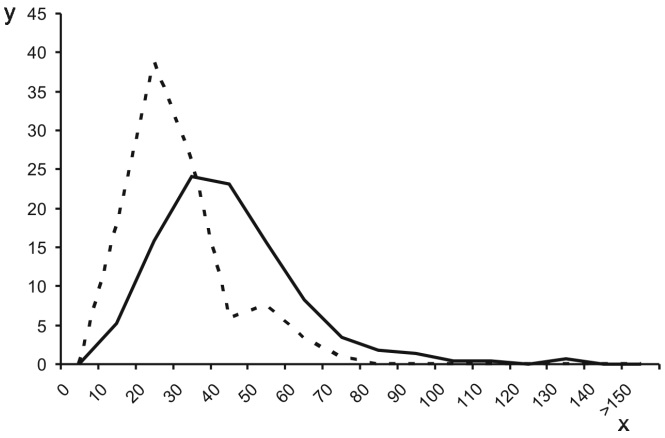
total procollagen type 1 amino-terminal propeptide

	Post-menopausal			Pre-menopausal
	All	HRT[b)] yes	HRT no	All
N	444	154	290	129
5th percentile	16.27	14.28	20.25	15.13
Median	37.09	28.48	42.94	27.80
Mean	40.43	31.74	45.05	30.10
95th percentile	73.87	58.92	76.31	58.59

b) HRT = patients receiving hormone replacement therapy

Below are frequency plots showing total P1NP concentration ranges with normal, untreated pre- versus untreated post-menopausal women (figure 1) and below (figure 2) P1NP ranges in post-menopausal women receiving HRT therapy versus those not receiving therapy.

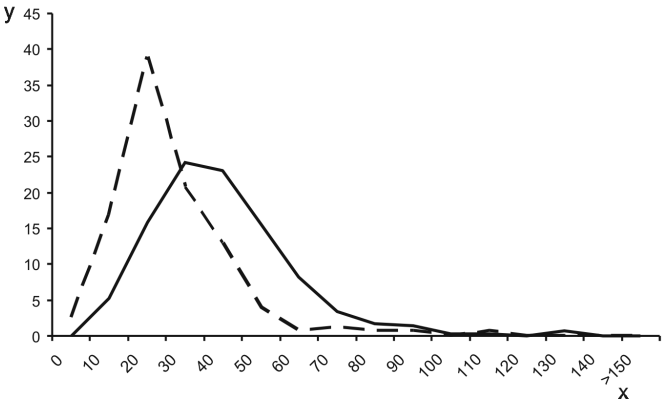
Figure 1: Frequency of total P1NP concentrations (µg/L or ng/mL) observed in normal, untreated, pre- (n = 129) and post- (n = 290) menopausal women



x: total P1NP (µg/L or ng/mL) - -: pre-menopausal

y: Frequency (%) —: post-menopausal

Figure 2: The effect of hormone replacement therapy on total P1NP (µg/L or ng/mL) concentration frequency distribution in treated ("HRT yes"; n = 154) and untreated ("HRT no"; n = 290) post-menopausal women



x: total P1NP (µg/L or ng/mL) – –: HRT yes

y: Frequency (%) —: HRT no

The measurement of total P1NP shows minimal circadian or seasonal variation (approx. 6 %)[15,16] and food intake or diet show no detectable influence upon serum levels.[17,18]

Significantly elevated serum total P1NP levels are associated with the presence of metastatic bone disease and may also be seen in patients with renal insufficiency. Diseases associated with secondary bone disease may have an effect upon levels of total P1NP.

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, samples 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 µg/L (ng/mL)	SD µg/L (ng/mL)	CV %	SD µg/L (ng/mL)	CV %
Human serum 1	12.8	0.340	2.6	0.531	4.1
Human serum 2	57.2	1.04	1.8	1.34	2.3
Human serum 3	527	6.93	1.3	11.7	2.2
Human serum 4	33.4	0.891	2.7	0.960	2.9
Human serum 5	1140	34.1	3.0	37.6	3.3
PreciControl Varia 1	30.5	0.488	1.6	0.629	2.1
PreciControl Varia 2	166	2.06	1.2	2.83	1.7

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers		**Repeatability**		**Intermediate precision**	
Sample	Mean µg/L (ng/mL)	SD µg/L (ng/mL)	CV %	SD µg/L (ng/mL)	CV %
Human serum 1	14.4	0.275	1.9	0.527	3.7
Human serum 2	57.9	1.15	2.0	1.47	2.5
Human serum 3	496	8.42	1.7	11.3	2.3
Human serum 4	34.2	0.816	2.4	1.10	3.2
Human serum 5	1090	34.8	3.2	37.2	3.4
PreciControl Varia 1	30.7	0.524	1.7	0.797	2.6
PreciControl Varia 2	158	2.29	1.4	3.04	1.9

Method comparison

A comparison of the Elecsys total P1NP assay (y) with a commercially available PINP test (x) using clinical samples gave the following correlations (µg/L or ng/mL):

Number of samples measured: 76

Passing/Bablok[19]	Linear regression
y = 1.01x + 1.73	y = 1.02x - 5.55
τ = 0.879	r = 0.981

The sample concentrations were between 19.7 and 319.5 µg/L (ng/mL).

Analytical specificity

Cross-reactivities of < 1 % were seen with the following analytes: β-CrossLaps, N-MID Osteocalcin, parathyroid hormone (PTH), and 25-OH vitamin D.

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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.

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

CE

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