Elecsys total P1NP

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REF		Σ	SYSTEM
07027940190	07027940500	100	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
TP1NP	10119

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 **cobas e** immunoassay analyzers.

Summary

Type I collagen is an important component of bone matrix and osteoblasts secrete its precursor procollagen molecule during bone formation.[7]

Type 1 procollagen contains both N-(amino) and C-(carboxy) terminal extensions. These extensions (propeptides) are cleaved by enzymes during bone matrix formation and released into the circulation. The propeptide measured by Elecsys total P1NP assay is from the amino terminal, hence P1NP procollagen-type 1 N-terminal-propeptide. P1NP is released during type 1 collagen formation and its subsequent incorporation into the bone matrix, and thus may be defined as a true bone formation marker.[8,9] 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.

Circulating levels of total P1NP demonstrate a significant change upon anti-resorptive[12,13] as well as anabolic[13] therapy within few months from treatment start. Suboptimal response to treatment may indicate non-compliance or the presence of secondary causes of osteoporosis which may need addressing.

Serum P1NP has been selected by the IOF-IFCC Bone Marker Standards Working Group as marker for bone formation, mainly based on the following criteria:

- it has been evaluated both for fracture prediction and monitoring osteoporosis therapies
- the assay is widely available, suitable for serum or plasma samples, with well documented sample handling and stability.[7]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 12 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 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 TP1NP.

M Streptavidin-coated microparticles, 1 bottle, 7.2 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-P1NP-Ab~biotin, 1 bottle, 10.3 mL:
Biotinylated monoclonal anti-P1NP antibody (mouse) 2.5 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

R2 Anti-P1NP-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 7.2 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 for laboratory 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 mist or vapours.

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.

Hazardous components:

- 2-methyl-2H-isothiazol-3-one hydrochloride

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 available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

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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
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 or tubes containing separating gel.

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

Li-heparin plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + intercept within ≤ ± 2x Limit of Blank + coefficient of correlation ≥ 0.95.

Stable for 24 hours at 15-25 °C, 5 days at 2-8 °C, 6 months at -20 °C (± 5 °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 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.

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 1.0 mL
- REF 05618860190, 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 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.

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 reference standards precisely defined by weighing native P1NP into an 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:

- every 12 weeks when using the same reagent lot
- every 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

Use Elecsys PreciControl Varia or other suitable controls for routine quality control procedures.

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 µg/L or ng/mL).

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	≤ 1112 µmol/L or ≤ 65 mg/dL
Hemoglobin	≤ 0.062 mmol/L or ≤ 100 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 205 nmol/L or ≤ 50 ng/mL
Rheumatoid factors	≤ 1000 IU/mL

Criterion: For concentrations of ≤ 15 ng/mL the deviation is ≤ 1.5 ng/mL. For concentrations > 15 ng/mL the deviation is ≤ 10 %.

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 P1NP concentrations up to 3900 µg/L (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
Ibandronate	6
Actonel (Risedronat)	150
Fosamax (Alendronate)	350
β-Estradiol	2.5
β-Estradiol-17-Valerate	2.5
β-Estradiol-3-Sulfate	2.5
Calciumcarbonate	2500
Vitamin D3	0.075

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 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 5 ng/mL. Values above the measuring range are reported as > 1200 ng/mL (or up to 2400 ng/mL for 2-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 2.5 ng/mL

Limit of Detection = 5 ng/mL

Limit of Quantitation = 10 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 P1NP concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:2 (either automatically by the 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 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

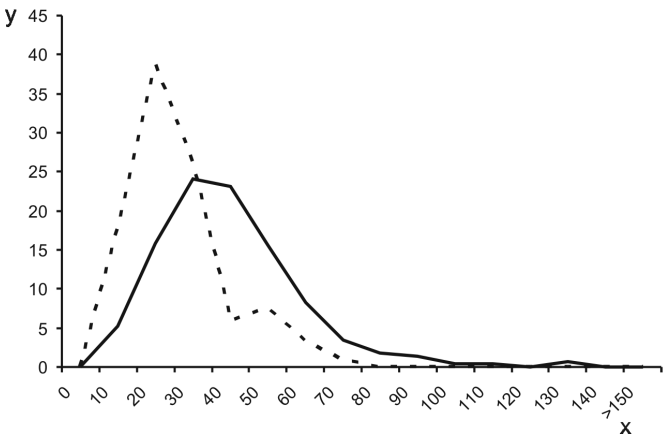
Sera taken from 573 healthy female volunteers who had been enrolled in a study of determinants of bone loss (OFELY[14,15]) were measured for total P1NP levels. The following results were obtained (µg/L or ng/mL):[16]

	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.

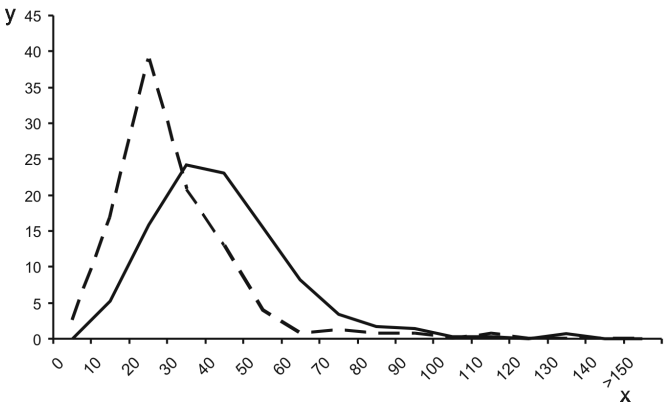
Figure 1: Frequency of total P1NP concentrations (µg/L or ng/mL) observed in normal, untreated, pre- (n = 129) and post- (n = 290) menopausal women



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 (approximately 6 %)[17,18] and food intake or diet show no detectable influence upon serum levels.[19,20]

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.[21,22,23] Diseases associated with secondary bone disease may have an effect upon levels of total P1NP.[24,25]

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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 (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 µg/L (ng/mL)	SD µg/L (ng/mL)	CV %	SD µg/L (ng/mL)	CV %
Human serum 1	6.24	0.137	2.2	0.144	2.3
Human serum 2	15.6	0.246	1.6	0.313	2.0
Human serum 3	577	10.2	1.8	13.8	2.4
Human serum 4	596	11.8	2.0	13.8	2.3
Human serum 5	1163	41.2	3.5	44.5	3.8
PC[c)] Varia1	29.7	0.480	1.6	0.599	2.0
PC Varia2	211	3.78	1.8	4.48	2.1

c) PC = PreciControl

Method comparison

a) A comparison of the Elecsys total P1NP assay, REF 07027940190 (**cobas e** 801 analyzer; y) with the Elecsys total P1NP assay, REF 03141071190 (**cobas e** 601 analyzer; x) gave the following correlations (ng/mL):

Number of samples measured: 139

Passing/Bablok[26]	Linear regression
y = 0.986x - 1.73	y = 0.980x - 2.30
τ = 0.975	r = 0.999

The sample concentrations were between 7.24 and 1161 ng/mL.

b) A comparison of the Elecsys total P1NP assay, REF 07027940190 (**cobas e** 402 analyzer; y) with the Elecsys total P1NP assay, REF 07027940190 (**cobas e** 801 analyzer; x) gave the following correlations (ng/mL):

Number of samples measured: 135

Passing/Bablok[26]	Linear regression
y = 0.978x - 1.18	y = 1.03x -5.81
τ = 0.983	r = 0.998

The sample concentrations were between 6.09 and 1076 ng/mL.

Analytical specificity

No cross-reactivities were seen with the following analytes: β-CrossLaps, N-MID Osteocalcin, parathyroid hormone (PTH), and 25-hydroxy vitamin D.

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For further information, please refer to the appropriate user guide or operator's manual for the analyzer concerned, the respective application sheets 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:

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

CE 0123

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