Instruction For Use

【Product Name】

N-MID Osteocalcin (eCLIA)

【Order Information】

REF NO.	Package Size
0320510501	50T
0320510502	2×50T
0320510503	100T
0320510504	2×100T

【Intended Use】

Immunoassay for the in vitro quantitative determination of N-MID Osteocalcin in human serum and plasma. Determination of N-MID Osteocalcin is used for initial evaluation of various osteoporosis and bone synthesis after bone injury.

【Summary】

Osteocalcin, also known as γ-carboxyl-glutamate protein or bone-dependent vitamin K protein, is a specific non-collagenous protein secreted by osteoblasts and ubiquitous in the bones and teeth of all vertebrates. Osteocalcin contains 49 amino acids with a molecular weight of about 5800D and can contain up to 3 γ-carboxyl glutamate residues [1]. The synthesis of osteocalcin is dependent on vitamin K and vitamin D3. The use of vitamin K antagonist can reduce the osteocalcin content in bone, but does not affect the proline content and the mechanical strength of bone. Since arginine at position 43-44 of osteocalcin molecule is very unstable, it is easy to be rapidly degraded in blood circulation and serum after in vitro, forming osteocalcin fragments (1-43) and (44-49). Among them, N-MID macromolecular fragment (1-43) has good stability and reproducibility [2]. Osteocalcin is mainly used in clinical evaluation of osteoporosis and early bone synthesis after bone injury. Osteocalcin can maintain the normal mineralization rate of bone and inhibit the mineralization rate of cartilage. Serum osteocalcin can be used to understand the activity state of osteoblasts. The faster the bone renewal rate, the higher the osteocalcin value, and vice versa. Osteocalcin increases in primary osteoporosis and osteoporosis caused by menopause, but less in senile osteoporosis. It should be noted that osteocalcin is significantly elevated in parathyroidism[3-4].

【Test Principle】

Sandwich principle. Total duration of assay: 9 minutes.

20 µL of sample, biotinylated osteocalcin antibodies and osteocalcin antibodies labeled with ruthenium complex react to form a sandwich complex. 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 system reagent. 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 RFID.

【Main Components】

The reagent pack consists of MB, RA, RB, calibrators and control materials (optional). Different lots cannot be used at the same time or mixed up together.

Components	Ingredients	Volume (2×50T)	Volume (50T)	Volume (100T)	Volume (2×100T)
Magnetic Beads (MB)	Streptavidin-coated microparticles, 0.45mg/mL; ProClin300	2×1.8 mL	1×1.8 mL	1×3.5 mL	2×3.5 mL
Reagent B (RB)	Biotinylated monoclonal Osteocalcin antibodies, 1.3mg/L; PBS; ProClin300	2×3.8 mL	1×3.8 mL	1×7.5 mL	2×7.5 mL
Reagent A (RA)	Osteocalcin antibodies labeled with ruthenium, 1.5mg/L; PBS; ProClin300	2×3.8 mL	1×3.8 mL	1×7.5 mL	2×7.5 mL
Calibrators	Osteocalcin antigen; PBS; ProClin300	High: 1×1.0 mL Low: 1×1.0 mL			
Control Materials (Optional)	Osteocalcin antigen; PBS; ProClin300	High: 1×1.0 mL Low: 1×1.0 mL			

The calibration process of this kit can be traced to the manufacturer's selected measurement procedure.

See the control material value sheet for the target values and ranges of control materials.

Supporting instruments and materials required but not provided in this pack (provided by Lifotronic)

Additional materials for Automated ECL Immunoassay Analyzer eCL8000, eCL8000i, eCL8000p, eCL8000x:

- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- REF 679007, Buffer, 480 mL, or REF 679006, Buffer, 6×480 mL
- REF 679009, Concentrated Washing Buffer, 1 L, or REF 679008, Concentrated Washing Buffer, 6×1 L
- REF 679011, Assay Cup, 500 T, or REF 679012, Assay Cup, 5000 T

Additional materials for Automated ECL Immunoassay Analyzer eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p:

- REF 0320501804, Auffer, 2×1 L, or REF 0320501805, Auffer, 4×1 L
- REF 0320501904, Buffer, 2×1 L, or REF 0320501905, Buffer, 4×1 L
- REF 0320507502, PreClean, 2×800 mL, or REF 0320507503, PreClean, 4×800 mL
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 032120111, Assay Cup/Assay Tip, 12×2×105

Additional materials for Automated ECL Immunoassay Analyzer eCL9000, eCL9600, eCL9900, eCL9900i:

- REF 0320501801, Auffer, 2×2 L, or REF 0320501806, Auffer, 4×2 L
- REF 0320501901, Buffer, 2×2 L, or REF 0320501906, Buffer, 4×2 L
- REF 0320507501, PreClean, 2×2 L, or REF 0320507504, PreClean, 4×2 L
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 0320510002, Assay Cup/Assay Tip, 1×6×105

【Storage and Shelf Life】

Store at 2ºC-8°C.

Do not freeze.

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

Damaged, expired or contaminated reagents should be discarded.

Stability of the reagents	
Unopened at 2~8°C	18 months
Store at 2~8°C after opening	8 weeks
on the analyzers at 2~15°C	8 weeks

The calibrators and control materials are stable up to the stated expiration date.

Stability of the calibrators and control materials	
Unopened at 2~8°C	18 months
Store at 2~8°C after opening	8 weeks

Store calibrators and control materials upright in order to prevent the calibrator solution and control material solution from adhering to the cap of the vial.

【Applicable Instrument】

Automated ECL Immunoassay Analyzers: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

【Specimen collection, handling and storage】

It is recommended to use human serum samples or plasma samples collected with EDTA-K3, EDTA-K2, Heparin lithium, Heparin sodium blood collection tubes.

Blood samples should be collected in accordance with the standard operation of venipuncture. After the sample is completely coagulated, centrifugation should be performed to remove residual cellular substances. The sample should be free of air bubbles during testing. Lipid layer covering the sample after centrifugation should be removed. It is not recommended to use hemolyzed samples.

Samples can be stored for 8 hours at 18~28ºC, 3 days at 2~8ºC, and 3 months at -15ºC or below. Freezing and thawing can be performed once.

【Assay Procedure】

Testing procedures and precautions

Previous to operation, users should read the operational manual carefully to obtain system operation procedure, sample processing, security precaution, maintenance and other related information.

Set up N-MID Osteocalcin test according to the operational manual.

Put the N-MID Osteocalcin reagent pack into the correct analyzer slot to automatically suspend magnetic beads at least 30 min before testing.

Recommended ambient temperature: 10ºC~30ºC; Relative humidity: ≤ 80%.

Calibration

Calibration should be performed using lot-matching reagent and calibrators.

Before calibration, the reagent and main curve information should be imported into the analyzer via radio frequency identification reagent card (refer to instrument user manual). The analyzer adjusts the main curve to produce working curve according to the calibrator results, and identifies validity of the working curve automatically.

Recalibration is recommended when:

(1) The reagents of different lot are used;

(2) The same lot of reagents were used on the analyzer beyond 4 weeks;

(3) Quality control findings outside the defined limits.

(4) The Buffer of different lots are used.

Quality control

For quality control, use N-MID Osteocalcin Control Materials.

In addition, other suitable control material can be used.

Control materials 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.
Please make sure that the correct values are used.

Calculation

The system software automatically calculates the analyte concentration using particular algorithm, and the result unit is ng/mL, μg/L.
Conversion factors: ng/mL = μg/L.

The sample dilution

Samples with Osteocalcin concentrations above the test range can be diluted with sample diluent. The recommended dilution is 1:5 (automatic dilution by instrument or manual dilution). If manually diluted, the result should be multiplied by the dilution. If the instrument is automatic dilution, the instrument will automatically calculate the result.

【Biological Reference Intervals】

The reference intervals of 5%-95% was calculated by testing 826 samples:

Test object		N-MID Osteocalcin（ng/mL）	
		Sample cases	5%~95% confidence interval
Healthy women	Premenopause, >20 years old	146	11.32～42.89
	Post-menopause	145	14.67～45.66
	Premenopause, Osteoporosis patients	131	13.49～48.23
Healthy men	18～30 years old	136	24.31～70.57
	30～50 years old	135	14.63～42.18
	50～70 years old	133	13.84～46.25

Due to the differences in geography, race, gender and age, it is recommended that each laboratory determine the applicability of reference range through tests, and establish the reference range of this laboratory if necessary.

【Interpretation of results】

When interpreting the test results, it is necessary to refer to the patient's overall clinical situation, including: symptoms, medical history as well as other corresponding data and information.

【Limitations】

The test results are only for clinical reference and cannot be used alone as the basis for diagnosis or exclusion of cases.
The detection range of the kit is 0.079～300 ng/mL. Values below the lower detection limit are reported as <0.079 ng/mL; Values above the upper detection limit are reported as > 300 ng/mL (5-fold diluted sample is reported as > 1500 ng/mL).
When the sample containing triglyceride ≤ 1000 mg/dL, bilirubin ≤ 55 mg/dL, biotin ≤ 50 ng/mL, and HAMA ≤ 100 ng/mL, the interference deviation of the measured results lies within ± 10.0%. When the rheumatoid factor concentration reached 1500 IU/mL, the relative recovery of the test results is between 90.0% - 110.0%.
Samples containing Calcitonin CT (100 ng/mL) and Parathyroid hormone PTH (100 ng/mL) were tested, and the determination results were not higher than 0.5 ng/mL.
The measured result was unaffected by the high-dose hook effect at N-MID Osteocalcin concentrations up to 4200 ng/mL.

【Analytical Performance】

Lower Limits of Measurement

Limit of Blank = 0.079 ng/mL.
Limit of Detection = 0.5 ng/mL.

Accuracy

The relative deviation shall not exceed ±10%.

Linearity

The correlation coefficient is not less than 0.9900 in the interval of 0.5 ~ 300 ng/mL.

Within-run precision (repeatability)

The coefficient of variation (CV) is less than or equal to 6.0%.

Between-lot precision

The coefficient of variation (CV) is less than or equal to 10.0%.

Homogeneity of calibrators and control materials

Within-bottle homogeneity: the coefficient of variation (CV) is less than or equal to 6.0%.

Accuracy of calibrators

The relative deviation shall not exceed ±10%.

Measured values of quality control

The measured results of supporting assigned quality control of the kit should be within the quality control range.

Method comparison

A comparison of the Lifotronic N-MID Osteocalcin assay (y) with the Roche Elecsys N-MID Osteocalcin Method (x) using clinical samples gave the following correlations:
Number of serum samples measured:125
Linear regression:
Y=1.0018X+0.4811
r =0.996
The sample concentrations were between approx 2.52 and 258.7 ng/mL.

【Precaution and Warning】

The kit is only used for in vitro diagnostics.
Exercise the normal precautions required for handling all laboratory reagents.
All reagents and samples including specimen, reagents, calibrators and control materials should avoid foaming before and during test.
Safety data sheet available for professional user on request.
This product contains animal-derived substances, and may have potential biological risks. All samples and reaction wastes should be treated as the source of infection, and all wastes must be disposed of in accordance with local regulations.
Any serious incident that would be 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.
The preservative ProClin™300 which contain Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1): May cause an allergic skin reaction. thus, proper personal protection should be adopt while handling the reagent: avoid breathing dust/fume/gas/mist/vapours /spray, wear protective gloves and contaminated work clothing should not be allowed out of the workplace. If skin irritation or rash occurs: get medical advice/attention, take off contaminated clothing and wash it before reuse, and dispose of contents/container to an approved waste disposal plant.

【Literature References】

1. Lina Liu, Yanmei Mao, Channan Zhao, et al. Circulating Levels of Osteoprotegerin, Osteocalcin and Osteopontin in Patients with Rheumatoid Arthritis: A Systematic Review and Meta-Analysis. Immunological Investigations. 2019;48(2): 107-120.
2. Liu Y, Liu X, R Lewis J, et al. Correction: Relationship between serum osteocalcin/undercarboxylated osteocalcin and type 2 diabetes: a systematic review meta-analysis study protocol. BMJ open, 2019; 9(6).
3. Somaye Fatahi, Ehsan Ghaedi, Seyed Mohammad Mousavi, et al. The Endocrine Function of Osteocalcin Regulated by Bone Resorption: A Lesson from Reduced and Increased Bone Mass Diseases. International Journal of Molecular Sciences. 2019;20(18):1661-6596.
4. Michela Rossi, Giulia Battafarano, Jessica Pepe, et al. The Association Between Osteocalcin and C-Reactive Protein. A Relation of Bone with Inflammation: A Systematic Review and Meta-Analysis.Hormone and Metabolic Research. 2019;51:353-361.

【Symbols Explanation】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Use-by date	IVD	In vitro diagnostic medical device
LOT	Batch code	Σ	Sufficient for <n> tests
SN	Serial number		Biological risks
	Temperature limit		This way up
EC REP	Authorized representative in the European Community	CE	Indicates this device is in compliance with Europe Directive.
REF	Catalogue number	P/N	Material code
	Warning		Date of manufacture
UDI	Unique device identifier		

【Manufacturer】

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【Version and Revision】

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