

Osteocalcin ELISA



Product Name	N-MID [®] Osteocalcin ELISA	REF	AC-11F1R
Abbreviated Product Name	Osteocalcin ELISA		

INTENDED USE

Not for use in clinical or diagnostic procedures.

The N-MID[®] Osteocalcin ELISA is an enzyme immunological test for the quantitative measurement of osteocalcin. Results are to be used for performance evaluation / investigational / research use only.

Summary and explanation of the test

Osteocalcin, or bone Gla protein (BGP), is the major non-collagenous protein of bone matrix. It has a molecular weight of approximately 5800 Dalton and consists of 49 amino acids, including three residues of gamma-carboxyglutamic acid. Osteocalcin is synthesized in bone by osteoblasts. After production, it is partly incorporated into the bone matrix and partly delivered to the circulatory system. The precise physiological function of osteocalcin is still unclear.

Principle of the procedure

The N-MID[®] Osteocalcin ELISA is based upon the application of two highly specific monoclonal antibodies (Mabs) against human osteocalcin. An antibody recognizing the midregion (amino acids 20-29) is used as the capture antibody and for detection a peroxidase conjugated antibody recognizing the N-terminal region (amino acids 10-16) is used. In addition to intact osteocalcin (amino acid 1-49) the N-terminal-Mid fragment (amino acids 1-43) is also detected.

Standards, control and unknown samples are pipetted into the appropriate microtitre wells coated with streptavidin. Then a mixture of a biotinylated antibody and a peroxidase conjugated antibody is added. Following incubation for 2 hours at room temperature the wells are washed and a chromogenic substrate is added and the colour reaction is stopped with sulfuric acid. Finally, the absorbance is measured.

PRECAUTIONS

The following precautions should be observed in the laboratory:

- Do not eat, drink, or smoke where immunodiagnostic materials are being handled
- Do not pipette by mouth
- Wear gloves when handling immunodiagnostic materials and wash hands thoroughly afterwards
- Cover working area with disposable absorbent paper

WARNINGS

The N-MID[®] Osteocalcin ELISA is for performance evaluation / investigational / research use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in these Instructions For Use (IFU). Immunodiagnostic Systems Limited (IDS) will not be held responsible for any loss or damage (except as required by statute), howsoever caused, arising out of non-compliance with the instructions provided.

Storage

- Store the N-MID[®] Osteocalcin ELISA kit upon receipt at 2-8°C. Under these conditions the kit is stable up to the expiry date stated on the box.
- Following reconstitution the **Standards** and the **Controls** should be stored below -18°C for up to 3 months, and should only be frozen and thawed twice. When the components of the **Antibody Solution** are mixed, the remaining solution should be stored at 2-8°C for no longer than 1 month or frozen below -18°C. The remaining reagents and immunostrips should be stored at 2-8°C.

Note:

The specimens' storage and stability information stated above are general recommendations for use in a variety of settings of laboratories. Each laboratory should follow the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations to establish its own specimens handling and storage stability. For guidance on

appropriate practices, please refer to the CLSI GP44-A4, Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline - Fourth Edition

MATERIALS

Specimen collection

Collect blood by venipuncture taking care to avoid haemolysis. Separate the serum from the cells within 3 hours after collection of blood. It is recommended to freeze (<-18°C) samples immediately.

When analysing plasma, both heparin and EDTA plasma may be used.

Materials supplied

Before opening the kit, read the section on **Precautions**. The kit contains reagents sufficient for 96 determinations. For reconstitution of lyophilized material, add appropriate volume of distilled water and leave for 10 minutes before mixing. Make sure to avoid foam.

Streptavidin coated microtitre plate **MICROPLAT**

Microwell strips (12 x 8 wells) pre coated with streptavidin. Supplied in a plastic frame.

Osteocalcin Standard 0 **CAL 0**

One vial (lyophilized) containing a PBS-buffered solution with protein stabilizer and preservative. Reconstitute with 5.0 mL of distilled water. The standard must be stored below -18°C after use.

Osteocalcin Standards - **CAL 1 - 5**

Five vials (lyophilized) containing synthetic human osteocalcin in a PBS-buffered solution with protein stabilizer and preservative. Reconstitute with 0.5 mL of distilled water. The exact value of each Standard is printed on the QC Report. The standards must be stored below -18°C after use, and should only be frozen and thawed twice.

Controls - **CTRL 1-2**

Two vials (lyophilized) containing synthetic human osteocalcin in a PBS-buffered solution with protein stabilizer and preservative. Reconstitute with 0.5 mL of distilled water. Controls must be stored below -18°C, and should only be frozen and thawed twice. Please refer to enclosed QC Report for control range.

Peroxidase Conjugated Antibody **ENZYMCONJ**

One vial (min. 0.25 mL) of a concentrated solution of a peroxidase conjugated murine monoclonal antibody specific against the N-terminal region of osteocalcin in a TRIS-buffered solution with protein stabilizer, detergent and preservative. Prior to use, add 10 mL **Conjugate Diluent Solution**.

Biotinylated Antibody **Ab BIOTIN**

One vial (min. 0.25 mL) of a concentrated conjugate solution of biotinylated murine monoclonal antibody against the mid-region of osteocalcin in a TRIS-buffered solution with protein stabilizer, detergent and preservative. Prior to use, add 10 mL **Conjugate Diluent Solution**.

Conjugate Diluent Solution **BUF**

One vial (min. 22 mL) of a PBS-buffered solution with protein stabilizer, detergent and preservative.

Substrate Solution **SUBS TMB**

One vial (min. 12 mL) of a ready for use tetramethylbenzidine (TMB) substrate in an acidic buffer. Please note that the chromogenic substrate might appear slightly bluish.

Stopping Solution **H2SO4**

One vial (min. 12 mL) of ready for use 0.18 mol/L sulfuric acid.

Washing Solution **WASHBUF 50x**

One vial (min. 20 mL) of a concentrated washing buffer with detergent and preservative. Dilute 1+50 times in distilled water before use.

Sealing tape

Adhesive film for covering wells during incubation.

Materials required — not supplied

- Containers for preparing the Antibody Solution and the Washing Solution.
- Precision micropipettes to deliver 20 µL
- Distilled water
- Precision 8- or 12-channel multipipette to deliver 100 µL and 150 µL.
- Microtiter plate reader with both 450 nm and 650 nm filters

ASSAY PROCEDURE

For optimal performance of the assay it is important to comply with the instructions given below

Assay Procedure

- Prior to use, prepare and equilibrate all solutions to room temperature. **Perform the assay at 18-22°C.**
- Determine the number of strips needed for the assay.
- It is recommended to test all samples in duplicate. In addition, for each run a total of 16 wells are needed for the standards and controls.
- Place the appropriate number of strips in the plastic frame. Store unused immunostrips in the tightly closed foil bag with desiccant capsules.

1 Preparation of the Antibody Solution:

The **Antibody Solution** is prepared by 1) adding 10 mL of **Conjugate Diluent Solution** **BUF** to both the **Peroxidase Conjugated Antibody Solution** **ENZYMCNJ** and the **Biotinylated Antibody Solution** **Ab biotin**, and 2) mixing the two conjugate solutions in equal volumes.

2 Incubation in Immuno Strips

Pipette 20 µL of either **Standards** **CAL 0 - 5** -, **Controls** **CTRL 1 - 2** - or unknown samples into appropriate wells followed by 150 µL of the **Antibody Solution**. Cover the immunostrips with sealing tape and incubate for 120±5 minutes at 18-22°C (without any mixing).

3 Washing

Wash the immunostrips 5 times manually with 300 µL diluted **Washing Buffer** (**WASHBUF 50x** diluted 1+50 in distilled water). Using an automated plate washer, follow the instructions of the manufacturer or the guidelines of the laboratory. Usually 5 washing cycles are adequate. Make sure that the wells are **completely emptied** after each manual or automatic washing cycle.

4 Incubation with chromogenic substrate solution

Pipette 100 µL of the **Substrate Solution** **SUBS TMB** into each well and incubate for 15±2 minutes at 18-22°C in the dark (without any mixing). Use sealing tape. Do not pipette directly from the vial containing TMB substrate but transfer the needed volume to a clean reservoir. Remaining substrate in the reservoir should be discarded and not returned to vial TMB.

5 Stopping of colour reaction

Pipette 100 µL of the **Stopping Solution** **H2SO4** into each well.

6 Measurement of absorbance

Measure the absorbance at 450 nm with 650 nm as reference within two hours.

Limitations of the procedure

If the absorbance of a sample exceeds that of **Standard 5**, the sample should be diluted in **Standard 0** and re-analysed.

RESULTS

Calculation of results

A four-parametric logistic curve fit can be used.

Alternatively, calculate the mean of the duplicate absorbance determinations. Construct a standard curve on graph paper by plotting the mean absorbances of the six standards (ordinate) against the corresponding osteocalcin concentrations (abscissa). Determine the osteocalcin concentration of the controls and each patient sample by interpolation.

Example of results obtained

Standards/ Controls/ Samples	Osteocalcin (ng/mL)	A _{450nm-650nm} Obs 1/ Obs 2	Mean A _{450nm-650nm}	Interpolated Osteocalcin (ng/mL)
Standard 0	0.0	0.019 / 0.020	0.020	
Standard 1	4.2	0.108 / 0.096	0.102	
Standard 2	7.6	0.183 / 0.177	0.180	
Standard 3	19.4	0.627 / 0.656	0.642	
Standard 4	35.4	1.271 / 1.278	1.275	
Standard 5	56.1	1.988 / 1.873	1.931	
Control 1		0.474 / 0.459	0.467	15.1
Control 2		1.147 / 1.132	1.140	31.8
Sample I		0.099 / 0.105	0.102	4.6
Sample II		0.899 / 0.850	0.875	25.1
Sample III		1.412 / 1.375	1.394	38.7

Please note:

The data above are for illustration only and should not be used to calculate the results of another assay.



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