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Osteocalcin (Rat) ELISA Kit

09/19

(Catalog # E4764-100; 96 assays, Storage at 4°C)

I. Introduction:

Osteocalcin (OCN) is one of the key players in bone endocrinology. It is expressed and secreted solely by osteoblasts. Following protein synthesis, the mature peptide first undergoes several splicing events and then gets γ-carboxylated at three residues, resulting in a peptide with high affinity toward bone and the extracellular matrix. OCN also acts as a hormone impacting glucose metabolism and insulin secretion. BioVision's Osteocalcin (Rat) ELISA kit is based on Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Rat Osteocalcin alpha. Standards or samples are added to the micro ELISA plate wells that bind to the specific antibody. Then a biotinylated detection antibody specific for Rat Osteocalcin and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. The wells are washed, a TMB substrate solution is added to the wells and blue color develops in proportion to the amount of Osteocalcin bound. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Rat Osteocalcin. The concentration of Rat Osteocalcin in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

In vitro, quantitative determination of rat Osteocalcin
Detection Range: 0.78-50 ng/ml
Sensitivity: 0.47 ng/ml
Precision: Coefficient of variation is < 10%.

III. Sample Type:

Serum, plasma, other biological fluids

IV. Kit Contents:

Components	E4764-100	Part Number	Storage Temp
Micro ELISA Plate	8 wells x12 strips	E4764-100-1	-20°C
Reference Standard	2 vials	E4764-100-2	-20°C
Biotinylated Detection Antibody (100x)	120 µL	E4764-100-3	-20°C
HRP Conjugate (100x)	120 µL	E4764-100-4	-20°C (Away from light)
Reference Standard and Sample Diluent	20 ml	E4764-100-5	4°C
Biotinylated Detection Antibody Diluent	14 ml	E4764-100-6	4°C
HRP Conjugate Diluent	14 ml	E4764-100-7	4°C
Wash Buffer (25x)	30 ml	E4764-100-8	4°C
Substrate	10 ml	E4764-100-9	4°C (Away from light)
Stop Solution	10 ml	E4764-100-10	4°C
Plate Sealer	4	E4764-100-11	RT

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm and 650 nm
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards or sample dilutions

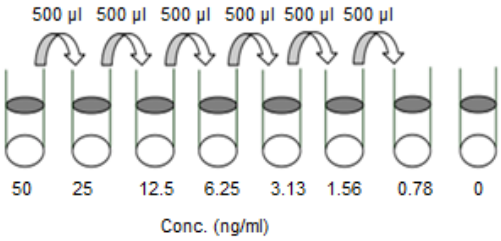
VI. Storage and Handling:

An unopened kit can be stored at 4 ºC for 1 month. If the kit is not used within 1 month, store the items separately according to the following conditions once the kit is received.

VII. Reagent and Sample Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Wash Buffer (25x):** Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40ºC water bath and mix it gently until the crystals have completely dissolved.
- **Biotinylated Detection Antibody:** Calculate the required amount before the experiment (100 µl/well). Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1x working solution with Biotinylated Detection Antibody Diluent.
- **HRP Conjugate:** Calculate the required amount before the experiment (100µl/well). Dilute the 100x Concentrated HRP Conjugate to 1x working solution with HRP Conjugate Diluent.
- **Standards:** Centrifuge the standard at 10,000xg for 1 min. Add 1.0 mL of Standard and Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 25 ng/ml.



Then make serial dilutions as needed. The recommended dilution gradient is: 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0 ng/ml. Prepare 7 tubes, add 500 µl of Standard & Sample Diluent to each tube. Pipette 500 µl of the 50 ng/ml stock solution to the first tube and mix up to produce a 25 ng/ml working solution. Transfer 500 µl of the solution into the other tube to form 2-fold serial dilutions of the highest standards to make the standard curve within the range of this assay.

VIII. Sample Preparation:

Note: Samples should be assayed within 7 days when stored at 4°C, otherwise aliquot and stored at -20°C (≤1 month) or -80°C (≤3 months). Avoid repeated freeze-thaw cycles.

- **Serum**
  Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 min at 1000×g at 2~8°C. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and endotoxin free.
- **Plasma**
  Collect plasma using EDTA-Na_2 as anticoagulant. Centrifuge samples for 15 min at 1000x g at 4°C within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysed samples are not suitable for ELISA assay.
- **Cell lysates:**
  For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1000×g. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each 1×10^6 cells, add 150-250 µl of pre-cooled PBS to keep the cells suspended. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 10 min at 1500xg at 4°C. Remove the cell fragments, collect the supernatant for assay. Avoid repeated freeze-thaw cycles.
- **Tissue homogenates:**
  It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolysed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000xg to get the supernatant.
- **Cell culture supernatant or other biological fluids:** Centrifuge samples for 20 min at 1000xg at 2~ 8°C. Collect the supernatant for assay

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

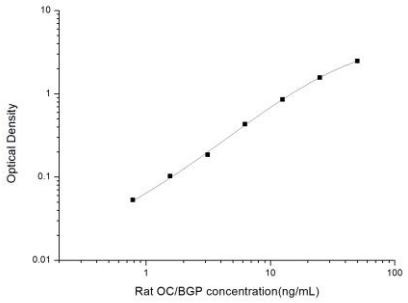
1. Add 100 µl of each **standard or samples** into appropriate wells. Cover the plate with the plate sealer provided in the kit and incubate for 90 min at 37ºC. **Note:** solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and bubble formation as much as possible.
2. Remove the liquid out of each well. Do not wash. Immediately add 100 µl of **Biotinylated Detection Antibody** working solution to each well. Cover the plate with the sealer provided in the kit. Gently mix and incubate for 1 hr. at 37°C.
3. Aspirate the solution from each well add 350 µl of **1x wash buffer** to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.

   Note: a microplate washer can be used in this step and other wash steps.
4. Add 100 µl of **HRP Conjugate** working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.
5. Aspirate the solution from each well, repeat the wash process for five times as conducted in step 3.
6. Add 90 µl of **Substrate** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
7. Add 50 µl of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
8. Read the absorbance in micro plate reader set to 450 nm.

X. Calculation:

Determine the average of the duplicate readings for each standard and samples then subtract the average zero standard optical density. Plot a four-parameter logistic with standard concentration on the x-axis and OD values on the y-axis. If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample is under the lowest limit of the standard curve, retest the samples with appropriate dilution. The actual concentration is the concentration obtained by calculated multiplied by the dilution factor.

Typical standard curve and data is provided below for reference only. A standard curve must be run with each assay.



Concentration(ng/mL)	50	25	12.5	6.25	3.13	1.56	0.78	0
OD	2.537	1.636	0.918	0.498	0.251	0.169	0.119	0.066
Corrected OD	2.471	1.57	0.852	0.432	0.185	0.103	0.053	-

Precision:
Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level Human S100B were tested 20 times on one plate, respectively.
Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level Human S100B were tested on 3 different plates, 20 replicates in each plate.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean(ng/mL)	2.70	4.70	20.10	2.80	5.10	19.80
Standard deviation	0.10	0.30	1.00	0.20	0.20	0.80
C V (%)	3.70	6.38	4.98	7.14	3.92	4.04

Recovery
The recovery of Human S100B spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=5)	85-98	91
EDTA plasma (n=5)	86-96	91
Cell culture media (n=5)	95-109	102

Linearity
Samples were spiked with high concentrations of Human S100B and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

		Serum (n=5)	EDTA plasma(n=5)	Cell culture media(n=5)
1:2	Range (%)	86-99	100-115	89-102
	Average (%)	91	106	95
1:4	Range (%)	99-113	83-93	94-107
	Average (%)	107	88	101
1:8	Range (%)	98-113	83-98	91-107
	Average (%)	105	90	99
1:16	Range (%)	101-114	81-93	98-111
	Average (%)	107	88	104

XI. RELATED PRODUCTS:

- Osteocalcin (Mouse) ELISA Kit (E4763)
- Osteocalcin (Human) ELISA Kit (E4762)

FOR RESEARCH USE ONLY! Not to be used on humans.