

Datasheet for ABIN4884045  
**Osteomodulin ELISA Kit**[Go to Product page](#)**1** Validation

## Overview

Quantity: 96 tests

Target: Osteomodulin (OMD)

Reactivity: Human

Method Type: Sandwich ELISA

Application: ELISA

## Product Details

Purpose: Custom Human Osteoadherin (Osteomodulin) ELISA Kit.

Sample Type: Cell Culture Supernatant, Cell Lysate, Plasma, Serum, Tissue Lysate

Analytical Method: Quantitative

Detection Method: Colorimetric

Specificity: This ELISA antibody pair recognizes human Osteoadherin. Other species not yet determined.

Characteristics:

- Strip plates and additional reagents allow for use in multiple experiments
- Quantitative protein detection
- Establishes normal range
- The best products for confirmation of antibody array data

Components:

- Pre-Coated 96-well Strip Microplate
- Wash Buffer
- Stop Solution
- Assay Diluent(s)
- Lyophilized Standard
- Biotinylated Detection Antibody

## Product Details

- Streptavidin-Conjugated HRP
- TMB One-Step Substrate

### Material not included:

- Distilled or deionized water
- Precision pipettes to deliver 2 µL to 1 µL volumes
- Adjustable 1-25 µL pipettes for reagent preparation
- 100 µL and 1 liter graduated cylinders
- Tubes to prepare standard and sample dilutions
- Absorbent paper
- Microplate reader capable of measuring absorbance at 450nm
- Log-log graph paper or computer and software for ELISA data analysis

## Target Details

|                   |   |
|-------------------|---|
| Target:           | Osteomodulin (OMD)                                  |
| Alternative Name: | Osteoadherin ( <a href="#">OMD Products</a> )       |
| Gene ID:          | 4958  |
| UniProt:          | <a href="#">Q99983</a>                              |
| Pathways:         | <a href="#">Glycosaminoglycan Metabolic Process</a> |

## Application Details

|                |   |
|----------------|---|
| Sample Volume: | 100 µL  |
| Plate:         | Pre-coated  |
| Protocol:      | <ol style="list-style-type: none"><li>1. Prepare all reagents, samples and standards as instructed in the manual.</li><li>2. Add 100 µL of standard or sample to each well.</li><li>3. Incubate 2.5 h at RT or O/N at 4 °C.</li><li>4. Add 100 µL of prepared biotin antibody to each well.</li><li>5. Incubate 1 h at RT.</li><li>6. Add 100 µL of prepared Streptavidin solution to each well.</li><li>7. Incubate 45 min at RT.</li><li>8. Add 100 µL of TMB One-Step Substrate Reagent to each well.</li><li>9. Incubate 30 min at RT.</li><li>10. Add 50 µL of Stop Solution to each well.</li><li>11. Read at 450 nm immediately.</li></ol> |

|               |                       |
|---------------|-----------------------|
| Restrictions: | For Research Use only |
|---------------|-----------------------|

## Handling

---

|                  |  |
|------------------|--|
| Storage:         | -20 °C   |
| Storage Comment: | The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. |
| Expiry Date:     | 6 months   |



## Successfully validated (ELISA (ELISA))

by [Bone and Cartilage Research Unit, University of Liège](#)

Report Number: 103081

Date: Oct 04 2018

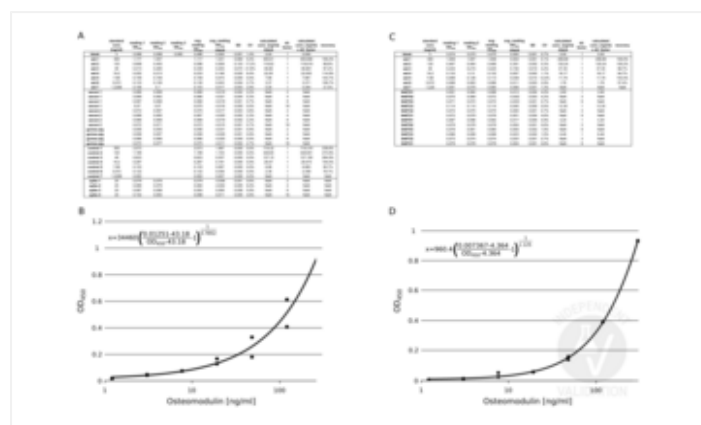
|                   |  |
|-------------------|--|
| Target:           | Osteomodulin   |
| Lot Number:       | 070218 2424  |
| Method validated: | ELISA (ELISA)  |
| Positive Control: | <p>ELISA using diluent A recommended for serum:</p> <p>Recombinant full length Osteomodulin (R&amp;D systems, 2884-AD-050, lot NFV031506A) at the same concentration as the standard curve</p> <p>Two human serum samples in duplicate, diluted 1:2, 1:4, 1:8, and 1:16 (suggested 1:2)</p> <p>ELISA using diluent C recommended for culture supernatant:</p> <p>Four supernatants of osteoblasts culture, in duplicate, diluted 1:1, 1:3, and 1:9</p>   |
| Negative Control: | <p>Sample diluent</p> <p>one guinea pig serum sample in duplicate, diluted 1:2, 1:4, 1:8, and 1:16 (cross-reactivity unknown)</p>  |
| Spike Control:    | Recombinant full length Osteomodulin (R&D systems, 2884-AD-050, lot NFV031506A)  |
| Notes:            | ABIN4884045 recognizes human osteomodulin in culture supernatant and recombinant human osteomodulin but not in human serum.  |
| Protocol:         | <ul style="list-style-type: none"> <li>• Reagent preparation and the assay are carried out according to the manufacturer's instructions.</li> <li>• For the spike control prepare a 40ng/ml solution of standard protein using diluent A. Dilute standard twofold in undiluted, 1:2, 1:4 and 1:8 diluted serum to achieve a final serum dilution 1:2, 1:4, 1:8, and 1:16 in 100µl total volume using diluent A.</li> <li>• Add 100µl of the standard or the sample to each well.</li> <li>• Incubate for 2.5h at RT shaking at 500rpm.</li> <li>• Aspirate the solution in the wells and wash wells 4x with 350µl wash buffer.</li> <li>• Add 100µl of the biotinylated detection antibody to each well and incubate for 1h at RT shaking at 500rpm.</li> <li>• Aspirate the solution in the wells and wash wells 4x with 350µl wash buffer.</li> <li>• Add 100µl of the HRP conjugate to each well and incubate for 45min at RT shaking at 500rpm.</li> <li>• Aspirate the solution in the wells and wash wells 4x with 350µl wash buffer.</li> </ul> |

- Add 100µl of the substrate reagent in dim light and incubate plate for 30min at RT protected from light.
- Add 50µl of the stop solution to each well in the same order the substrate reagent was added.
- Read OD<sub>450</sub> immediately using a Multiskan GO (Thermofisher Scientific).

#### Experimental Notes:

- The standard curves in the different diluents don't look like the figure in the kit insert: OD<sub>450</sub> were lower, but are good, with good duplicate reproducibility (A).
- The curve with recombinant full length Osteomodulin from R&D Systems gives higher ODs than the standard protein from the kit.
- ABIN4884045 allows detection of Osteomodulin in culture supernatants, in undiluted wells with values around 3-12ng/ml. This concentration appears very low but possible.
- Serum values remain below the blank values and are not detected by the detecting antibody as confirmed with western blot, contrarily to standard protein, recombinant protein and culture supernatant (C).
- The ABIN4884045 detection antibody recognizes osteomodulin from the kit standard and human recombinant osteomodulin which in a western blot. It also detects osteomodulin from human osteoblast culture supernatant but the signal is weak. However, in serum there is no signal after immunodetection although we can see that there is the correct transfer of total protein on the blot. The same western blot procedure with a different commercial antibody (R&D systems, BAF2884) gives good results in these human serum samples ( [Sanchez C \(2018\)](#)).
- Furthermore, a substantial matrix effect of serum makes the spiking of 20ng/ml recombinant Osteomodulin undetectable, even in 1:16 serum dilution with diluent A before adding the Osteomodulin. Recovery of Osteomodulin in serum samples is 0% even at a 1:16 dilution.

#### Image for Validation report #103081



#### Validation image no. 1 for Osteomodulin (OMD) ELISA Kit (ABIN4884045)

A. Standard curve (std), human (serum) and guinea pig (guinea pig) serum samples, recombinant Osteomodulin (control), and spike control (spike) measurements using diluent A. B. The standard curve for diluent A was fitted with a 4-Parameter Logistic model ( $R^2=0.975$ ). C. Standard curve (std), human osteoblast serum free supernatant (T0: pre-osteoblasts, T2: mature osteoblasts) measurements using diluent C. D. Standard curve for diluent C fitted with a 4-Parameter Logistic model ( $R^2=0.999$ ).