Manual

Osteonectin ELISA

For the in vitro determination of osteonectin in serum

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EAGLE BIOSCIENCES, INC.
20A NW BLVD. SUITE 112 NASHUA, NH 03063
P: 617-419-2019 F: 617-419-1110
WWW.EAGLEBIO.COM — INFO@EAGLEBIO.COM

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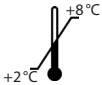

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Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: + 49 6251 849430
e.mail: info@immundiagnostik.com www.immundiagnostik.com

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1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of osteonectin in serum. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Osteonectin (SPARC, BM-40) is a 32,700 molecular weight, Ca^{2+}-binding glyco-protein which is a synthetic product of osteoblasts, endothelial cells, and megakaryocytes. Osteonectin is involved in tissue remodeling both from the perspective of bone metabolism and endothelial cell proliferation and repair. Therefore, the quantitative analysis of this protein could serve as a useful marker for the researcher studying the biochemical processes.

Possible research areas

- vascular wound repair
- platelet activation
- skeletal metabolism

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR4231	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	1 x 100 ml
KR4231	AB	Antibody, (rabbit anti-osteonectin), ready-to-use	1 x 12 ml
KR4231	CONJ	Conjugate (anti-rabbit), peroxidase-labelled, ready-to-use	1 x 22 ml
KR4231	STD	Standards, ready-to-use (0; 0.04; 0.12; 0.37; 1.11; 3.33 µg/ml)	1 x 6 vials
KR4231	CTRL1	Control, ready-to-use, (see specification for range)	1 x 1 vial
KR4231	CTRL2	Control, ready-to-use, (see specification for range)	1 x 1 vial
KR4231	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 100 ml
KR0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	2 x 15 ml

Cat. No.	Label	Kit components	Quantity
KR0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 2 times within the expiry date stated on the label.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- The **standards (STD), controls (CTRL) and antibody (AB)** can be stored at 2–8 °C for 2 weeks. **For a storage for more than two weeks the components have to be stored at -20 °.**
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. PREPARATION OF SAMPLES

Dilution of samples

Serum

Serum samples must be diluted **1:10** before performing the assay,

e.g. **25 µl** serum + **225 µl** sample dilution buffer (SAMPLEBUF), mix well.

For analysis, pipet **100 µl** of the diluted sample per well.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of osteonectin.

The test principle is based on a competition between antigen in the sample or standards and the antigen coated on the wells of microplate. Standards or samples are transferred with the primary antibody against osteonectin directly into the precoated microtiter plate. The antigen in the samples competes with the antigen immobilised on the microtiter plate for the free binding site of the specific antibodies against osteonectin. A peroxidase-conjugated antibody is used for detection and quantification, and tetramethylbenzidine (TMB) as a peroxidase substrate. The enzymatic reaction is terminated by acidic stop solution.

A dose response curve of absorbance unit (optical density, OD at 450 nm) vs. concentration is generated using the values obtained from standard. The colour change is inversely proportional to the amount of analyte (sample or control). Osteonectin, present in the samples is determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	**Before use**, wash the wells **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each **100 µl standards/controls/diluted samples** into the respective wells.
3.	Add **100 µl antibody** (AB) into each well.
4.	Cover the strips and incubate **over night** at 2–8 °C on a **horizontal shaker***.
5.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
6.	Add **200 µl conjugate** (CONJ) into each well.
7.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
8.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
9.	Add **200 µl substrate** (SUB) into each well.
10.	Incubate for **10–20 min**** at room temperature (15–30 °C) in the **dark**.
11.	Add **50 µl stop solution** (STOP) into each well and mix well.
12.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm as a reference.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm.

1. **4 parameter algorithm**

   It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e.g. 0.001).

2. **Point-to-point calculation**

   We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. **Spline algorithm**

   We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Serum

The obtained results have to be multiplied by the **dilution factor of 10** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result by the dilution factor used.

9. LIMITATIONS

Samples with an OD lower than the OD of the highest standard can be further diluted and re-assayed. For the following analysis, the changed dilution factor has to be taken into consideration.

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range

We recommend each laboratory to establish its own reference range.

11. PRECAUTIONS

- All reagents in the kit package are for research use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still should be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

12. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure,

which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.

- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

14. REFERENCES

General literature

1. Whitson, S.W., et al., J. Cell Biol. 99, 607 (1984).
2. Otsuka, K., et al., J. Biol. Chem. 259, 9805 (1984).
3. Kuwata, F., et al., J. Biol. Chem. 260, 6993 (1985).
4. Sage, H., et al., J. Biol. Chem. 259, 3993 (1984).
5. Sage, H., et al., J. Cell. Physiol. 127, 373 (1986).
6. Kelm, Jr., R.J., et al., Blood 80, 3112 (1992).
7. Sage, E.H. and Bornstein, P. J. Biol. Chem. 266, 14831 (1992).

Used symbols:


Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet

Immundiagnostik AG

Stubenwald-Allee 8a
D-64625 Bensheim

Tel.: +49 (0) 62 51/70 19 00
Fax: +49 (0) 62 51/84 94 30

info@immundiagnostik.com
www.immundiagnostik.com