MOUSE OSTEOPROTEGERIN (OPG) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE OPG CONCENTRATIONS IN CELL CULTURES, SERUM AND PLASMA.

PURCHASE INFORMATION:

ELISA NAME	MOUSE OSTEOPROTEGERIN (OPG) ELISA
Catalog No.	SK00762-03
Lot No.	
Formulation	96 T
Standard Range	31.25 - 4000 pg/ml
Sensitivity	15 pg/ml
Sample Volume	100 µl
Sample Type	Serum, plasma, cell cultures
Specificity	Mouse OPG
Sample Dilution	*Optimal dilutions should be determined by each laboratory for each application*
Intra-assay Precision	4-8%
Inter-assay Precision	8-12%
Storage	2 °C-8 °C

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Order Contact:
AVISCERA BIOSCIENCE INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.com

INTRODUCTION

Mouse OPG immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure Mouse OPG in urine, serum, and plasma. It contains recombinant Mouse OPG and antibodies raised against this protein. It has been shown to accurately quantify recombinant Mouse OPG. Results obtained with naturally occurring OPG samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the Immunoassay kit can be used to determine relative mass values for natural Mouse OPG.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for OPG has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any OPG present is bound by the immobilized antibody. After washing away any unbound substances, a polyclonal antibody Biotiylated specific for OPG is added to the wells. Following a wash to remove any unbound Antibody, Streptavidin HRP Conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of OPG bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Mouse OPG Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against OPG.	762-03-01	1 plate
OPG Standard – 4000 pg/vial of recombinant Mouse OPG in a buffered protein base with preservatives; lyophilized.	762-03-02	1 vial
Detection Antibody Conjugate – 105 µL / vial, 100-fold concentrated of polyclonal antibody HRP conjugate against mouse OPG with preservatives;	762-03-03	1 vial
Positive Control - one of Mouse OPG, lyophilized	762-03-04	1 vial
Dilution Buffer- 60 ml/vial, 10-fold concentrated buffered surfactant, with preservative.	DB01	1 vial
Streptavidin-HRP Conjugate -120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP	SAHRP	1 vial
Wash Buffer -50 ml/vial, 10-fold concentrated buffered surfactant, with preservative.	WB01	1 vial
TMB Substrate Solution-11 ml / vial of TMB substrate solution	TMB01	1 vial
Stop Solution - 11 ml /vial of 0.5M HCl	S-STOP	1 vial
Plate Sealer	EAPS	1

STORAGE

Unopened Kit: Store at 2 - 8° C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted standard and Detection Antibody 100-fold

Concentrate should be stored for up to two weeks at -20~-70°C . Diluted standard working solution and Positive Control should be prepared and used immediately. Streptavidin-HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 6 months at 2 - 8° C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted Hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.

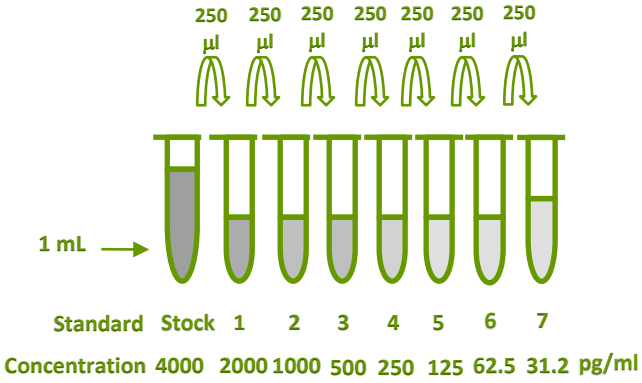
Optimal dilutions should be determined by each laboratory for each application.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

OPG Standard - Refer to vial label for reconstitution volume. Reconstitute the **OPG** Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

Standard	Standard	Dilution Buffer	Concentration
Stock	Powder	1000 µl	4000 pg/ml
# 1	250 µl of stock	250 µl	2000 pg/ml
# 2	250 µl of 1	250 µl	1000 pg/ml
# 3	250 µl of 2	250 µl	500 pg/ml
# 4	250 µl of 3	250 µl	250 pg/ml
# 5	250 µl of 4	250 µl	125 pg/ml
# 6	250 µl of 5	250 µl	62.5 pg/ml
# 7	250 µl of 6	250 µl	31.25 pg/ml



Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 105µl of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 105 µl of 100-fold

concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of Dilution Buffer into the 15 ml centrifuge tube and transfer 120 µl of 100-fold concentrated stock solution to prepare working solution.
Note: 1x working solution of Streptavidin HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the **Positive Control** with 1 mL of Dilution Buffer to prepare Positive Control working solution. Allow it to sit for a minimum of 15 minutes with gentle agitation. That should be prepared and used immediately.

ASSAY PROCEDURE

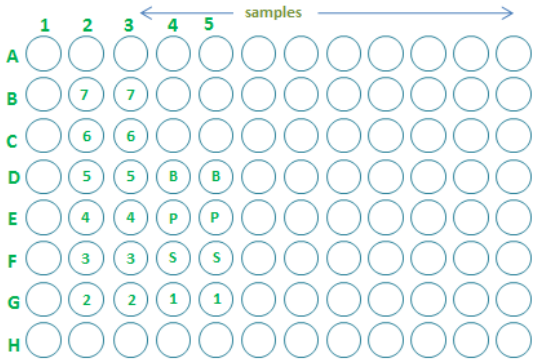
Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch containing the desiccant pack, reseal.
3. Add 100 µL of **Dilution Buffer** to Blank well (D4, D5).
4. Add 100 µL of **Standard** (B2, B3 to G2, G3 and F4, F5 to G4, G5), **sample**, or positive control (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **Detection Antibody working solution** to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 45 minutes on micro-plate shaker at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 5-15 minutes at room temperature. **Protect from light.**
11. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the OPG concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.



TYPICAL DATA

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	CORRECTED (450NM)
Blank	0.100
31.25	0.049
62.5	0.083
125	0.165
250	0.339
500	0.678
1000	1.349
2000	2.501
4000	3.306

- **Lot No.:**
- **Positive Control: 286.21-477.02 pg/ml**

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed Mouse OPG.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of OPG was 15 pg/mL.

SPECIFICITY

This assay recognizes natural serum mouse OPG. The factors listed below were prepared at 50ng/mL in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 50ng/mL in a mid-range mouse OPG control were assayed for interference. No significant cross-reactivity or interference was observed.

PROTEINS	CROSS-REACTIVITY(%)
Mouse OPG	100
Mouse RANK/Fc Chimera	0
Human OPG/Fc Chimera	11

Mouse RANKL did not cross-react in mouse OPG assay however that did interfere at greater than 0.78 ng/ml.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

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Add 100µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.

↓

Aspirate and wash 4 times.

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Add 100 µl **Detection Antibody working solution** to each well. Incubate 2 hours on the plate shaker at RT.

↓

Aspirate and wash 4 times.

↓

Add 100 µl **Streptavidin HRP Conjugate working solution** to each well. Incubate 45 min on the plate shaker at RT.

↓

Aspirate and wash 4 times.

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Add 100 µl **Substrate Solution** to each well. Incubate 5-15 min on the bench top. **Protect from light.**

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Add 100 µl **Stop Solution** to each well. Read 450nm within 15 min