HUMAN OSTEOPROTEGERIN (OPG) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN OPG CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

PURCHASE INFORMATION:

ELISA Name	**HUMAN OSTEOPROTEGERIN (OPG) ELISA**
Catalog No.	SK00762-01
Lot No.	
Formulation	96 T
Standard range	62 - 4000 pg/ml
Sensitivity	7 pg/ml
Sample Volume	100 µl
Dilution factor	5 (***Optimal dilutions should be determined by each laboratory for each application***)
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Human OPG only
Calibration	Human OPG recombinant
Intra-assay Precision	6 - 8%
Inter-assay Precision	10 - 12%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Soluble OPG ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human OPG from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human OPG and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural OPG samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. An 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 biotinylated antibody specific for OPG is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin 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

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

COMPONENTS PROVIDED

Description	Code	Quantity
OPG Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against OPG.	**762-01-01**	**1 plate**
OPG Standard – 4000 pg/vial of recombinant human OPG in a buffered protein base with preservative; lyophilized.	**762-01-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial of 10-fold concentrate of biotinylated antibody against OPG with preservative; lyophilized.	**762-01-03**	**1 vial**
Positive Control - one vial of recombinant human OPG; lyophilized.	**762-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
Antibody Diluent Solution Concentrate – 11 mL of buffered protein based solution with preservative; lyophilized.	**DB20**	**1 tube**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 12 months. For longer storage, unopened Standard, Positive Control, Detection Antibody Concentrate and Antibody Diluent Solution Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard, Antibody Diluent Solution (DB20) and Detection Antibody concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrated solution and other components may be stored at 2 – 8° C for up to 12 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 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.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
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 EDTA, heparin, or citrate 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.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples may require a 5-fold dilution. A suggested 5-fold dilution is 50 µL sample + 200 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application.**
Use polypropylene test tubes.

REAGENT PREPARATION

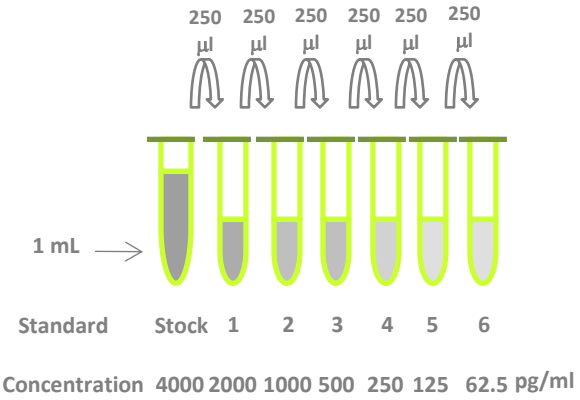
Bring all reagents to room temperature before use.

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 1x Wash Buffer.

Antibody Diluent Solution Concentrate – Reconstitute the Antibody Diluent Solution Concentrate with 11.0 mL of Dilution Buffer in provided 15 mL tube to prepare Antibody Diluent Solution (DB20).

OPG Standard - Reconstitute the OPG standard with 1.0 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 250 µL of Dilution Buffer into tubes #1 to #6. 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).

Tube	Standard	Dilution Buffer	Concentration
stock	Powder	1.0 ml	4000 pg/ml
# 1	250µl of stock	250µl	2000 pg/ml
# 2	250µl of 1	250µl	1000 pg/ml
# 3	250µl of 2	250µl	500 pg/ml
# 4	250µl of 3	250µl	250 pg/ml
# 5	250µl of 4	250µl	125 pg/ml
# 6	250µl of 5	250µl	62.5 pg/ml



Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note:** Positive Control could be reused within a few days if stored at -20° C to -70° C.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody Diluent Solution (DB20)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Antibody Diluent Solution (DB20) into another 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. **Note: Must be prepared 1 to 2 hours prior to use.**

Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days. **Protect from light.**

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL of **Dilution Buffer** to Blank wells.
4. Add 100 µL of **Standard dilutions**, **sample**, or **positive control** per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x 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 plate sealer. Incubate for 2 hours on microplate 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 60 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 3-10 minutes on microplate shaker 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 microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and samples, 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.

SPECIFICITY

Protein	Cross-reactivity (%)
Human OPG	100
Mouse OPG	17
Human CD40	0
Human sTNF RI	0
Human sTNF RII	0

LINEARITY

To assess the linearity of the assay, pooled research human **EDTA plasma** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
1X	1632.082	1632.082	100
5X	318.957	1594.785	97.7
10X	136.810	1368.10	83.8

To assess the linearity of the assay, pooled research human **serum** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
1X	1339.829	1339.829	100
5X	247.570	1237.85	92.4
10X	183.762	1837.62	137

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.081)
62.5	0.042
125	0.103
250	0.232
500	0.450
1000	0.869
2000	1.702
4000	2.677

- Lot No.:
- Positive Control:

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate 2 hours on the plate shaker at RT. Prepare Detection Antibody working solution.
↓
Aspirate and wash 4 times.
↓
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 60 minutes on the plate shaker at RT. **Protect from light**.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 3-10 min on the plate shaker at RT. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.