

# HUMAN ONCOSTATIN M (OSM) ELISA KIT

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



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

## PURCHASE INFORMATION:

ELISA NAME	HUMAN ONCOSTATIN M ELISA
Catalog No.	SK00509-01
Lot No.	
Formulation	96 T
Standard range	15.6 -1000 pg/mL
Sensitivity	7.8 pg/mL
Sample Volume	100 µl
Sample Type	Serum, EDTA plasma
Dilution factor	Optimal dilutions should be determined by each laboratory for each application
Specificity	Human OSM only
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2 °C-8 °C

## Order Contact:

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**INTRODUCTION**

Human Oncostatin M (OSM) immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human OSM in serum, cell culture supernates, and EDTA plasma. It contains recombinant human OSM and antibodies raised against this protein. It has been shown to accurately quantitate recombinant human OSM. Results obtained with naturally occurring OSM 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 human OSM.

**PRINCIPLE OF THE ASSAY**

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for OSM has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any OSM present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for OSM 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 OSM 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 the appropriate **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
<b>OSM Microplate</b> - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against OSM.	509-01-01	1 plate
<b>OSM Standard</b> – 1000 pg/vial of recombinant human OSM in a buffered protein base with preservatives; lyophilized.	509-01-02	1 vial
<b>Detection Antibody Concentrate</b> – 105 µL / vial, 100-fold concentrated of Biotinylated polyclonal antibody against OSM with preservatives; lyophilized.	509-01-03	1 vial
<b>Positive Control</b> - one vial of recombinant human OSM in a buffered protein base with preservatives; lyophilized.	509-01-04	1 vial
<b>Streptavidin-HRP Conjugate</b> -120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP	SAHRP	1 vial
<b>Dilution Buffer</b> - 60 mL/vial of a buffered protein base solution with preservatives	DB01	1 vial
<b>Wash Buffer</b> -50 ml/vial, 10-fold concentrated buffered surfactant, with preservative.	WB01	1 vial
<b>TMB Substrate Solution</b> - 11 ml / vial of TMB substrate solution	TMB01	1 vial
<b>Stop Solution</b> (0.5M HCl) , 11 ml /vial of 0.5M HCl	S-STOP	1 vial
<b>Plate Sealer</b>	EAPS	1

**STORAGE**

**Unopened Kit:** Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Detection Antibody Concentrated should be stored at -20 or -70 °C. Do not use past kit expiration date.

**Opened / Reconstituted Reagents:** Reconstituted Standard, Detection Antibody Solution SHOULD BE STORED at -20 °C or – 70°C for up to one months. 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.

**SAMPLE COLLECTION AND STORAGE**

**Plasma** - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Aliquot and store samples at -20 °C ~-70 °C. Avoid repeated freeze-thaw cycles. EDTA plasma samples require 50-100 fold dilution to perform assay.

**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. Serum samples require 50 or 100 fold dilution to perform assay. Optimal dilutions should be determined by each laboratory for each application.

**Use polypropylene test tubes.**

**REAGENT PREPARATION**

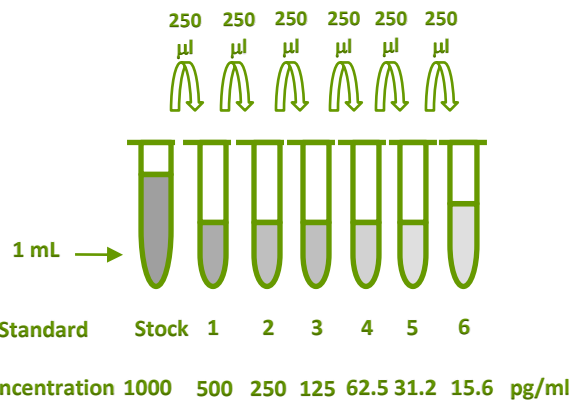
**Bring all reagents to room temperature before use.**

**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 Wash Buffer.

**OSM Standard - Refer to vial label for reconstitution volume.** Reconstitute the **OSM Standard** with 1 ml of **Dilution Buffer**. This reconstitution produces a stock solution of **1000 pg/mL**. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of the appropriate Sample Solution into the tube #2 to # 6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. The appropriate Sample Solution serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1000 µl	1000 pg/ml
# 1	250 µl of 1	250 µl	500 pg/ml
# 2	250 µl of 2	250 µl	250 pg/ml
# 3	250 µl of 3	250 µl	125 pg/ml
# 4	250 µl of 4	250 µl	62.5 pg/ml
# 5	250 µl of 5	250 µl	31.25 pg/ml
# 6	250 µl of 5	250 µl	15.6 pg/ml



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

transfer 120 µl of 100-fold concentrated stock solution to prepare working solution. *Note: 1 x 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 make Positive Control working solution.

**ASSAY PROCEDURE**

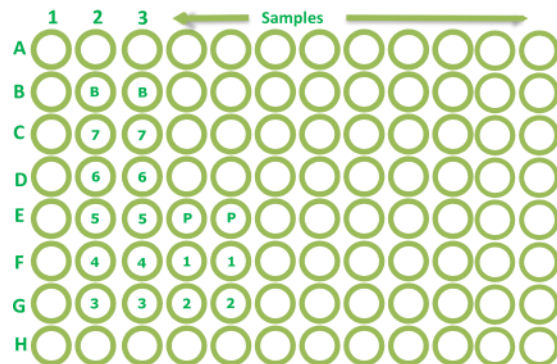
**Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.**

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 foil pouch containing the desiccant pack, reseal.
3. Add 100 µL of **Dilution Buffer** to Blank well (B2, B3).
4. Add 100 µL of Standard (from C2 to G3, G4 to F5), samples, or control per well (E4, E5). Cover with the 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 35 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 13-15 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 OSM 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.

OSM (PG/ML)	CORRECTED (450NM)
Blank	0.103
15.6	0.040
31.25	0.080
62.5	0.150
125	0.281
250	0.501
500	0.973
1000	1.848

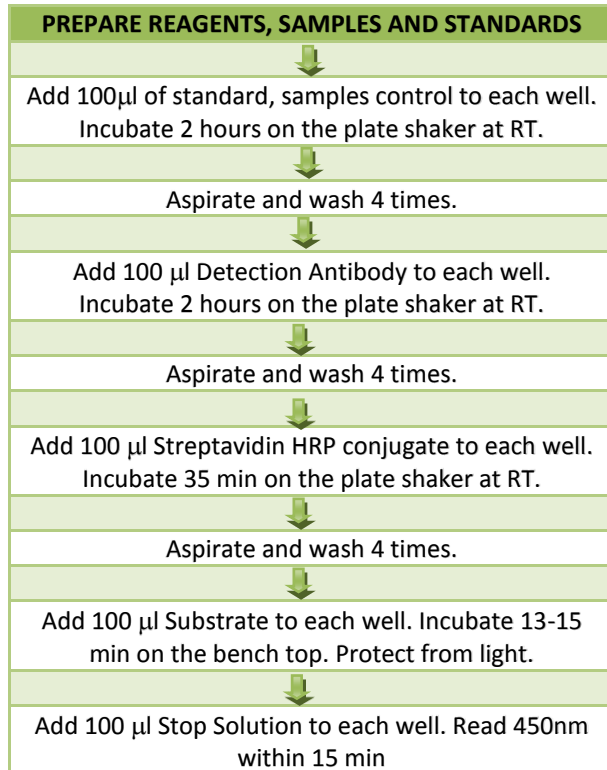
**CALIBRATION**

This immunoassay is calibrated against a highly purified recombinant human OSM.

**SENSITIVITY**

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of OSM was 7.8 pg/mL.

**SUMMARY OF ASSAY PROCEDURE**



**SPECIFICITY**

PROTEINS	CROSSREACTIVITY (%)
Human OSM	100
Human CNTF	0
Human Periostin	0
Human IL-6	0
Human gp130	0
Human CLCF1	0