Quantikine® ELISA

Mouse Oncostatin M (OSM) Immunoassay

Catalog Number MSM00

For the quantitative determination of mouse Oncostatin M (OSM) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Oncostatin M (OSM) is a 28-36 kDa member of the Class I helical cytokine family (alternatively, the IL-6 cytokine family) of molecules (1-3). It is so named for its ability to induce stasis, or inhibition, of A375 melanoma cell growth (4). OSM functions as a monomeric glycoprotein that participates in a number of developmental, skeletal and immunological processes (5-7). Structurally, the mouse OSM precursor is 263 amino acids (aa) in length (8, 9). It contains a 24 aa signal sequence, a 181 aa mature segment (aa 25-205), and a 58 aa cleavable, C-terminal propeptide (8). While the presence of the propeptide has little effect on receptor binding, OSM biological activity is markedly reduced (10). There are three potential N-linked glycosylation sites that typically account for 2-4 kDa of MW (10, 11). Although the existence of a signal sequence strongly suggests direct secretion, human OSM is known to be stored in neutrophil granules and released immediately upon degranulation (12). One isoform variant is reported that shows a deletion of aa 11-13 (13). Mature mouse OSM shares 50% and 72% aa sequence identity with mature human and rat OSM, respectively. Mouse cells known to express OSM include monocytes (8), eosinophils and neutrophils (14), Th1 $CD4^+$ T cells (15), osteoblasts and osteocytes (16), adipocytes (potentially) (17), Sertoli cells (18), bone marrow-derived dendritic cells (19) and select embryonic cell clusters such as those comprising the neuroepithelium and the aorta-gonad-mesonephros (or genital ridge) (20, 21).

The functional receptor for mouse OSM is a heterodimer composed of a 130 kDa signal transducing subunit termed gp130, and a 160 kDa ligand-binding subunit termed OSMR that also serves as a component of the IL-31 receptor complex (22-24). OSMR is a 948 aa type I transmembrane (TM) glycoprotein that is a member of the type 2 subfamily, type I cytokine receptor family, hematopoietic receptor superfamily of molecules. It contains a 714 aa extracellular region, a 21 aa TM segment, and a 213 aa cytoplasmic domain. The extracellular region is characterized by the presence of four fibronectin type III repeats (22, 24). The receptor system for OSM has remarkable species-cross-reactivity. Human OSM will act on both the human LIF receptor (gp130:LIFR) and human OSM receptor (gp130:OSMR). It will also act on both the mouse and rat LIF receptor, but not on either species' OSM receptor. Mouse OSM, by contrast, is able to activate both its own OSM receptor and that of rat, but is unable to activate the human OSM receptor, and shows no activity on any species' LIF receptor. Rat OSM has a mixed binding pattern. Like human OSM, it activates both its own OSM receptor and LIF receptor. But in cross-species studies, rat OSM activity is limited to the human LIF and mouse OSM receptors (24-28). Notably, mouse OSM activation of the mouse LIF receptor has been reported in a stromal cell line (16).

Mouse OSM is truly pleiotrophic in its effects. It induces CCL21 expression in endothelial cells (EC), promoting the migration of dendritic cells into lymph nodes (19). It also acts with IL-3 to expand the size of megakaryocyte colonies, thus increasing circulating platelet numbers (29). It appears to possess some autocrine activity, as it is both produced by, and promotes, Sertoli cell proliferation (18). Astrocytes are generated from neuroepithelium through the action of OSM, and hepatocyte stimulation by OSM results in acute phase protein production (20, 27). OSM has also been linked to pulmonary allergy. It induces eotaxin production and VCAM-1 expression by fibroblasts, and P-Selectin expression by EC, resulting in eosinophil migration into lung parenchyma (30, 31). Finally, OSM has been associated with autoimmunity. OSM is known to maintain thymic epithelial integrity. Functional OSM-responsive epithelium will make contact with $CD25^-CD44^+FcR^+$ thymic progenitors, generating $CD4^+$ macrophages that clear apoptotic thymocytes in a timely and efficient manner. Failure to do so initiates an antibody response to cell components that results in the production of anti-dsDNA antibodies (32, 33).

The Quantikine® Mouse Oncostatin M (OSM) Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse OSM levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse OSM and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse OSM accurately. Results obtained using natural mouse OSM showed dose-response curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse OSM.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse OSM has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any OSM present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mouse OSM is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of OSM bound in the initial step. The sample values are then read off the standard curve.

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.
- If samples generate values higher than the highest standard, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse OSM Microplate	894631	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse OSM.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse OSM Conjugate	894632	12 mL of a monoclonal antibody specific for mouse OSM conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse OSM Standard	894633	Recombinant mouse OSM in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse OSM Control	894634	Recombinant mouse OSM in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1W	895038	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:4 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 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 or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse OSM Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

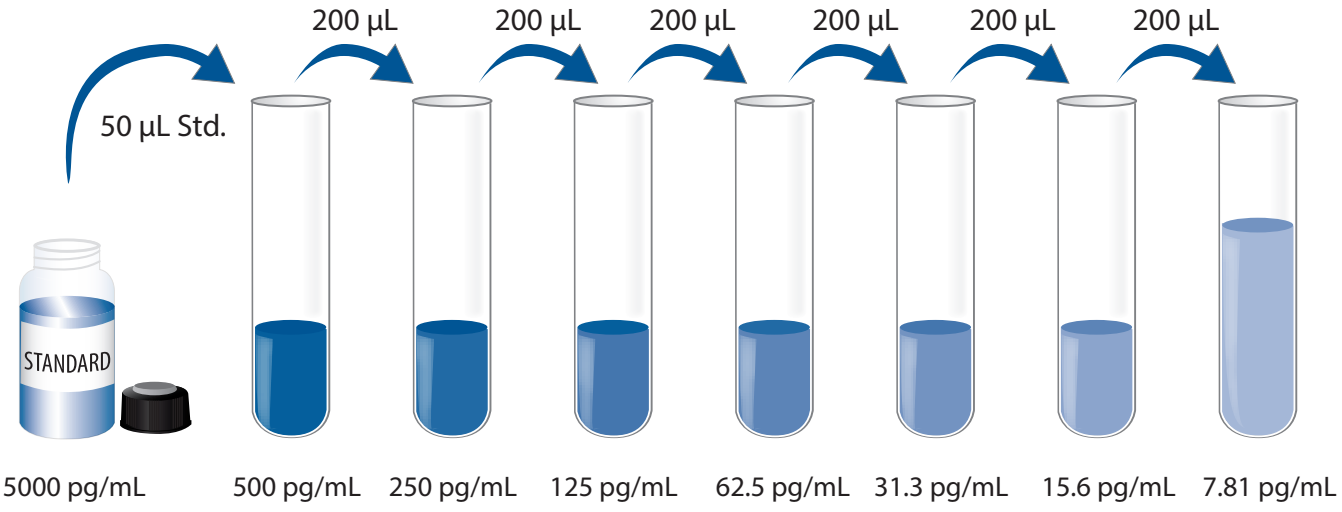
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Calibrator Diluent RD5-26 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

Mouse OSM Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse OSM Standard with deionized or distilled water. This reconstitution produces a stock solution of 5000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle mixing prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5-26 (diluted 1:4) into the 500 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the 500 pg/mL standard to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 500 pg/mL standard serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

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

1. Prepare all reagents, standards, control, and samples as directed by the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours 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 Wash Buffer (400 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 Mouse OSM Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

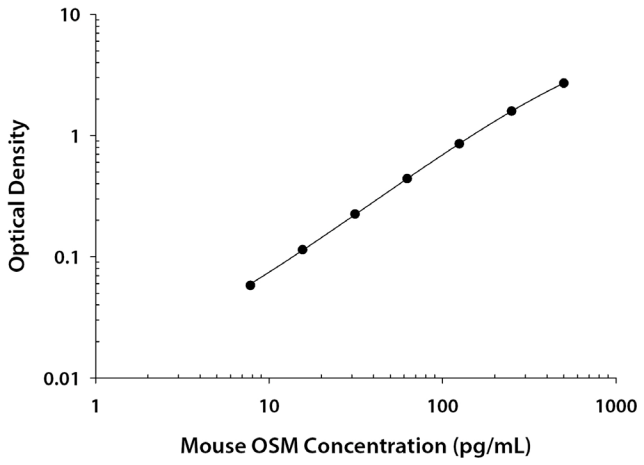
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 mouse 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

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



(pg/mL)	O.D.	Average	Corrected
0	0.018 0.019	0.019	—
7.81	0.075 0.078	0.077	0.058
15.6	0.127 0.138	0.133	0.114
31.3	0.240 0.247	0.244	0.225
62.5	0.458 0.460	0.459	0.440
125	0.873 0.874	0.874	0.855
250	1.606 1.610	1.608	1.589
500	2.708 2.734	2.721	2.702

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	36.7	62.1	127	36.5	65.3	135
Standard deviation	2.36	1.32	3.64	4.53	6.20	9.20
CV (%)	6.4	2.1	2.9	12.4	9.5	6.8

RECOVERY

The recovery of mouse OSM spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	97	92-103%
Serum (n=4)	100	95-109%
EDTA plasma (n=4)	95	92-100%
Heparin plasma (n=4)	99	94-106%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of mouse OSM were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	102	106	106	108
	Range (%)	101-103	104-107	105-109	107-111
1:4	Average % of Expected	103	111	110	113
	Range (%)	102-106	110-113	106-115	111-115
1:8	Average % of Expected	105	112	111	115
	Range (%)	98-111	110-115	108-118	111-118
1:16	Average % of Expected	100	110	110	115
	Range (%)	81-117	108-112	105-119	110-118

SENSITIVITY

One hundred-six assays were evaluated and the minimum detectable dose (MDD) of mouse OSM ranged from 0.219-4.70 pg/mL. The mean MDD was 1.20 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant mouse OSM produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - 11 serum, 6 EDTA plasma, and 6 heparin plasma samples were evaluated for the presence of mouse OSM in this assay. No detectable levels were observed.

Cell Culture Supernates - P388D1 mouse lymphoma cells (2.35 x 10^4 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were grown unstimulated or stimulated with 20 ng/mL of recombinant mouse IL-3 for 3 days. Aliquots of the cell culture supernates were removed and assayed for mouse OSM.

Condition	(pg/mL)
Unstimulated	479
Stimulated	828

SPECIFICITY

This assay recognizes natural and recombinant mouse OSM.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant mouse OSM control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

CT-1
EPO
GM-CSF
gp130
IL-2
IL-3
IL-6
IL-11
IL-27
LIF
LIF Rα
OSM Rβ

Recombinant human:

CNTF
OSM

Recombinant rat:

CNTF

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