Human OSM-R ELISA Kit

Catalog Number
EK0484

For the quantitative determination of human OSM-R concentrations in cell culture supernates, serum, and plasma.

This instruction must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

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PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for OSM-R has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any OSM-R present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for OSM-R is added to the wells and binds to the combination of capture antibody- OSM-R in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A coloured product is formed in proportion to the amount of OSM-R present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven OSM-R standard dilutions and OSM-R sample concentration determined.

DETECTION RANGE

312.5pg/ml - 20000 pg/ml

PRECISION

The coefficient of variation of both intra-assay and inter-assay were less than 10%.

SENSITIVITY

The minimum detectable dose was 80pg/mL.

MATERIALS PROVIDED

1. Aluminium pouches with a Microwell Plate coated with antibody to human OSM-R (8X12)
2. 2 vials human OSM-R Standard lyophilized, 10000 pg/ml upon reconstitution
3. 2 vials concentrated Biotin-Conjugate anti-human OSM-R antibody
4. 2 vials Streptavidin-HRP solution
5. 4 bottle Standard /sample Diluent
6. 1 bottle Biotin-Conjugate antibody Diluent
7. 1 bottle Streptavidin-HRP Diluent
8. 1 bottle Wash Buffer Concentrate 20x (PBS with 1% Tween-20)

9. 1 vial Substrate Solution
10. 1 vial Stop Solution
11. 4 pieces Adhesive Films
12. package insert

STORAGE

Table 1: Storage of the kit

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 – 8℃. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="10">Opened/ Reconstituted Reagents</td><td>Standard /sample Diluent</td><td rowspan="8">May be stored for up to 1 month at 2 – 8℃.**</td></tr>
<tr><td>Concentrated Biotin-Conjugate</td></tr>
<tr><td>Streptavidin-HRP solution</td></tr>
<tr><td>Biotin-Conjugate antibody Diluent</td></tr>
<tr><td>Streptavidin-HRP Diluent</td></tr>
<tr><td>Wash Buffer Concentrate 20x</td></tr>
<tr><td>Substrate Solution</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at -20℃.
Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 – 8℃.**</td></tr>
</table>

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

THE REQUIRED ITEMS (not provided, but can help to buy):

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000μL.

3. 37 ℃ incubator, double-distilled water or deionized water, coordinate paper, graduated cylinder.

PRECAUTIONS FOR USE

1. Store kit regents between 2℃and 8℃. After use all reagents should be immediately returned to cold storage(2℃ to 8℃).
2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross contamination, please use disposable pipette tips.
4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.
5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.
6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.
7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.
8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.
10. Avoid microtiter plates drying during the operation.
11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

SAMPLE COLLECTION AND STORAGE

1. **Cell Culture Supernates** - Remove particulates by centrifugation.
2. **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.
3. **Plasma** - Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.
4. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
5. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor).

REAGENT PREPARATION

1. Bring all reagents to room temperature before use.
2. **Wash Buffer** - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard** - Reconstitute the Standard with 0.5mL of Standard /sample Diluent. This reconstitution produces a stock solution of 20000 pg /mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

   Pipette 250μL of Standard/sample Diluent into the 10000 pg/mL tube and the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 20000 pg/mL standard serves as the high standard. The Standard/ sample Diluent serves as the zero standard (0 pg/mL).

   If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.

4. Working solution of Biotin-Conjugate anti-human OSM-R antibody: Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

   The working solution should be used within one day after dilution.

5. Working solution of Streptavidin-HRP: Make a 1:100 dilution of the

concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

The working solution should be used within one day after dilution.

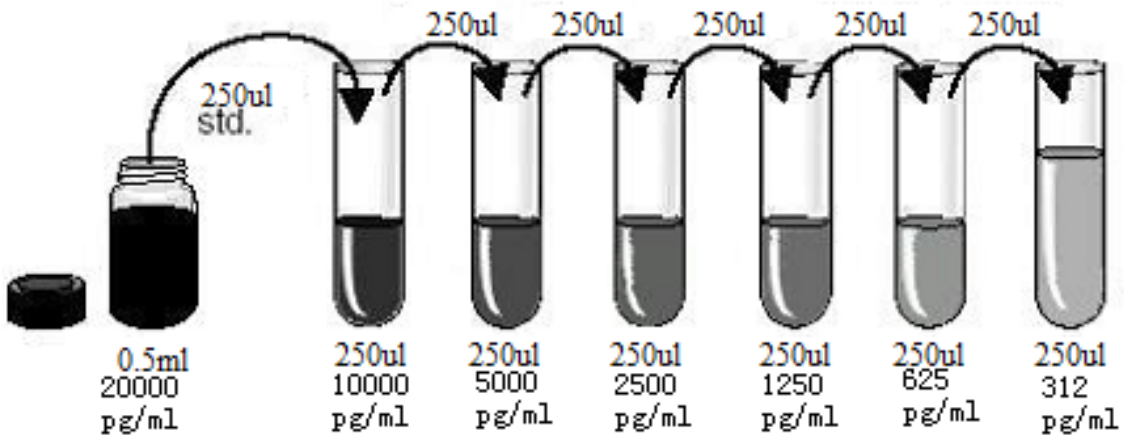

Figure 1: Preparation of OSM-R standard dilutions

GENERAL ELISA PROTOCOL

1. Prepare all reagents and working standards as directed in the previous sections.
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8℃ sealed tightly.
3. Add 100µL of Standard, control, or sample, per well, then add 50µL of the working solution of Biotin-Conjugate to each well. Cover with the adhesive strip provided and incubate 2 hours at RT. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350µL) using a squirt bottle, manifold dispenser or auto-washer. 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.

5. Add 100 μL of the working solution of Streptavidin-HRP to each well. Cover with a new adhesive strip and incubate for 60 minutes at RT Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 4.
7. Add 100μL of Substrate Solution to each well. Incubate for 15-20 minutes at RT. Avoid placing the plate in direct light.
8. Add 100μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.(optionally 650nm as the reference wave length;610-650nm is acceptable)

ASSAY PROCEDURE SUMMARY

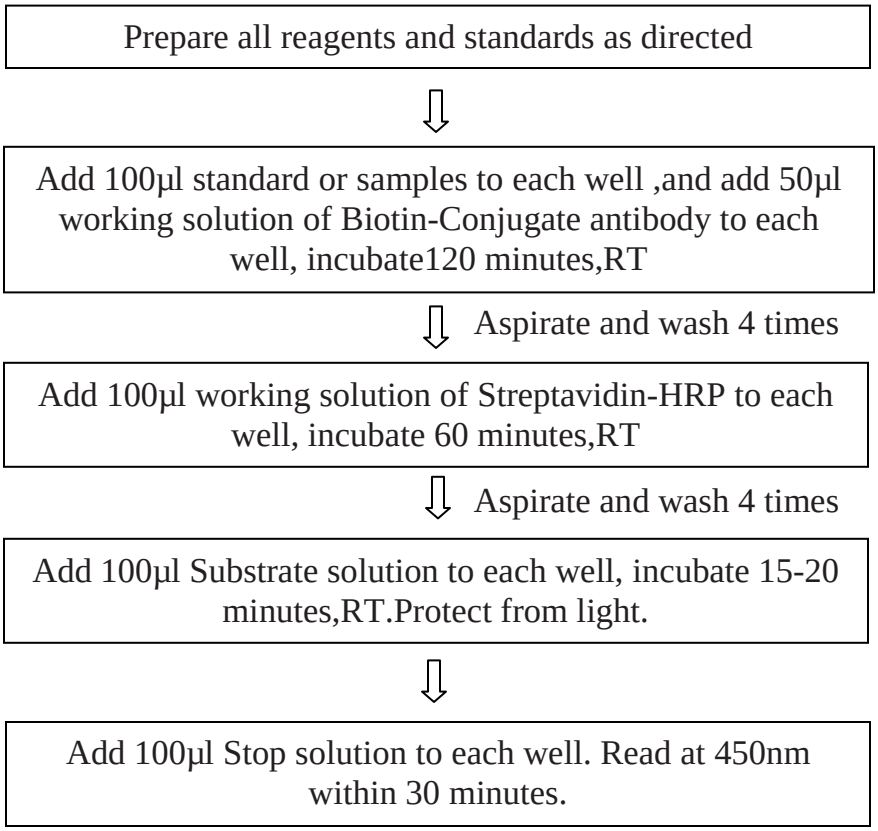

Figure 2: Assay procedure summary

TECHNICAL HINTS

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. 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.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

CALCULATION OF RESULTS

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. 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.
3. The data may be linearized by plotting the log of the OSM-R 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.
4. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Table 2:Typical data using the OSM-R ELISA (Measuring wavelength:450nm,Reference wavelength:650nm)

Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.020	0.018	0.019	——
312.5	0.115	0.110	0.113	0.094
625	0.203	0.198	0.201	0.182
1250	0.378	0.372	0.375	0.356
2500	0.775	0.768	0.759	0.740
5000	1.447	1.441	1.444	1.425
10000	2.238	2.226	2.232	2.213
20000	3.254	3.238	3.246	3.227

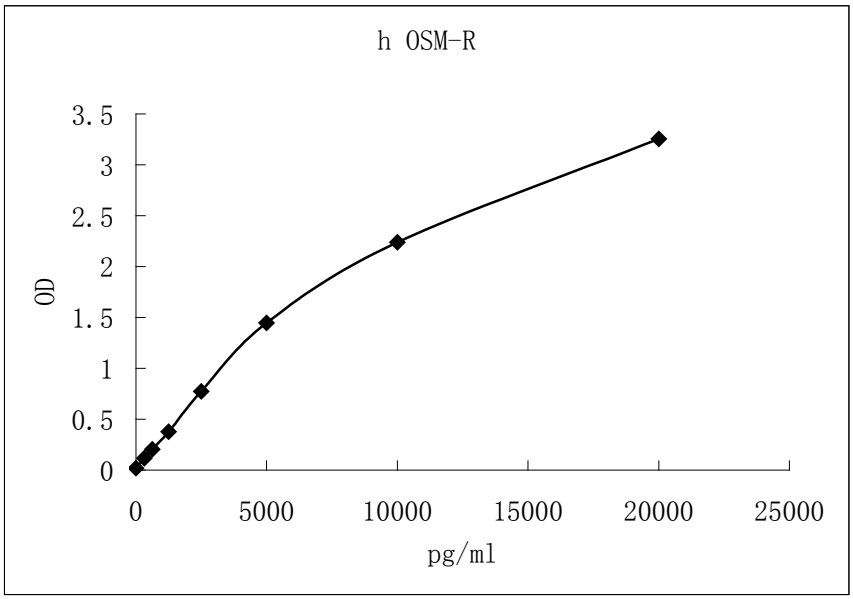

Figure 3:Representative standard curve for OSM-R ELISA. OSM-R was diluted in serial two-fold steps in Sample Diluent.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

BACKGROUND

OSM is a cytokine originally isolated from medium conditioned by PMAtreated U 937 human histiocytic leukemia cells based on its ability to inhibit growth of A375 melanoma cells. The human OSM cDNA encodes a 252 amino acid preproOSM polypeptide with a 25 residue hydrophobic signal peptide and a hydrophilic Cterminaldomain that are proteolytically processed to generate the 196 residue mature form of OSM. Although both mature and proOSM are equally active in radioreceptor assays, the mature OSM is 5to 60 fold more active in growth inhibition assays. Thus, proteolytic

processing of the proOSM peptide may be important in regulating the in vivo activities of OSM.

OSM is a pleiotropic cytokine that initiates its biological activities by binding to specific cell surface receptors. The gp130, a signal transducing component (subunit) of the IL6, LIF and CNTF receptor complexes, was identified as a lowaffinity OSM receptor that does not transduce OSM signals. The low affinity LIF receptor (LIF R, a gp130related protein) has now been identified to be a component of a highaffinity OSM receptor that will transduce OSM signals. Since OSM is also active on cells that do not express LIF R, a specific OSM receptor that does not involve LIF R must also exist. Besides its growth inhibitory activities on human A375 melanoma and mouse M1 myeloid leukemic cells, as well as on other solid tumor cells, OSM also has growth stimulatory activities on normal fibroblasts, AIDSKaposi's sarcoma cells, and a human erythroleukemia cell line, TF1.

Other OSMmediated activities reported to date include: stimulation of plasminogen activator activity in cultured bovine aortic endothelial cells regulation of IL6 expression in human endothelial cells and stimulation of LDL uptake and upregulation of cell surface LDL receptors in HepG2 cells.

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