MOUSE SOLUBLE INTERLEUKIN 6 RECEPTOR ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE SOLUBLE IL-6R CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM, AND PLASMA.

PURCHASE INFORMATION:

ELISA Name	MOUSE SOLUBLE IL-6R ELISA
Catalog No.	SK00155-09
Lot No.	
Formulation	96 T
Standard range	6.25-400 pg/mL
Sensitivity	3 pg/mL
Sample Volume	100 µl
Dilution factor	100 (*Optimal dilutions should be determined by each laboratory for each application*)
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Mouse Soluble IL-6R
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2-8 °C

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

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INTRODUCTION

Mouse IL-6R immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure mouse IL-6R in cell culture supernates, serum, and plasma. It contains recombinant mouse IL-6R and antibodies raised against this protein. It has been shown to accurately quantify recombinant mouse IL-6R. Results obtained with naturally occurring IL-6R 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 IL-6R.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-6R has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6R present is bound by the immobilized antibody. After washing away any unbound substances, the HRP conjugated polyclonal antibody specific for IL-6R is added to the wells. After washing away any unbound antibody HRP conjugated, a substrate solution is added to the wells and color develops in proportion to the amount of IL-6R 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
IL-6R Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against IL-6R.	**155-09-01**	**1 plate**
IL-6R Standard – 400 pg/vial of recombinant mouse IL-6R in a buffered protein base with preservatives; lyophilized.	**155-09-02**	**1 vial**
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrated of HRP conjugated polyclonal antibody against IL-6R with preservatives; lyophilized.	**155-09-03**	**1 vial**
Positive Control - one vial of recombinant mouse IL-6R, lyophilized	**155-09-04**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservatives	**DB11**	**2 bottles**
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 – 11mL 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, Detection Antibody Concentrate Solution and Antibody Diluent Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrate 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 and seal along entire edge of zip-seal. 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, 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

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.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) 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 100-fold dilution. A suggested 10-fold dilution is 5µL sample + 495 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application. Use polypropylene test tubes.**

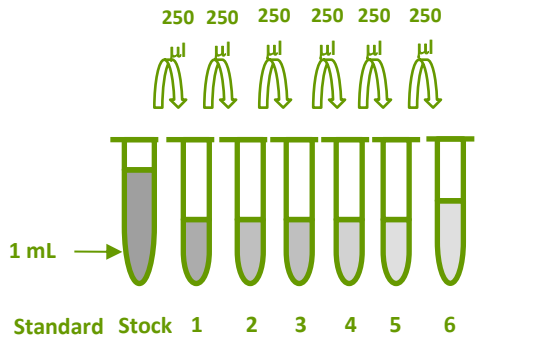
REAGENT PREPARATION

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 Wash Buffer.

IL-6R Standard - Refer to vial label for reconstitution volume. Reconstitute the **IL-6R** standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 400 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 400 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	400 pg/ml
# 1	250µl of stock	250µl	200 pg/ml
# 2	250µl of 1	250µl	100 pg/ml
# 3	250µl of 2	250µl	50 pg/ml
# 4	250µl of 3	250µl	25 pg/ml
# 5	250µl of 4	250µl	12.5 pg/ml
# 6	250µl of 5	250µl	6.25 pg/ml



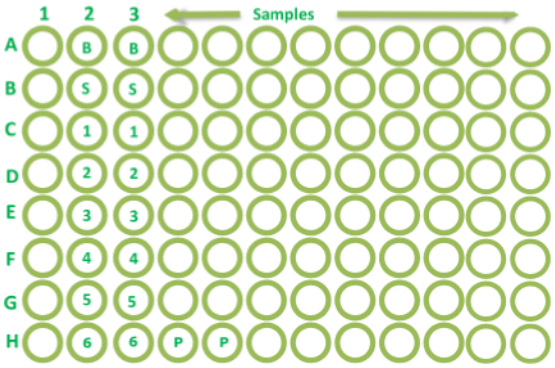
Detection Antibody - Reconstitute the Detection Antibody Concentrate with 105 µl of **Dilution Buffer** to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Antibody Diluent Solution into another 15 ml centrifuge tube and transfer the 105 µl of 100-fold concentrated stock solution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

Positive Control - Reconstitute the positive control with 1.0 mL of Dilution Buffer to make positive control solution. **Note:** Positive Control should be used immediately.

ASSAY PROCEDURE

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

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 with the desiccant pack.
3. Add 100 µL of **Dilution Buffer** to Blank wells (A2, A3).
4. Add 100 µL of **Standard** (B2, B3 to H2, H3), **sample**, or **positive control** (H4, H5) 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 plate sealer. Incubate for 1 hours on micro-plate shaker at room temperature. **Protect from light.**
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Substrate Solution** to each well. Incubate for 2-4 minutes at room temperature. **Protect from light.**
9. 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.
10. 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, positive 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 IL-6R 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.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant Soluble mouse IL-6R.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of IL-6R was 3 pg/mL.

SPECIFICITY

Protein	Cross-reactivity (%)
Mouse soluble IL-6R	100
Mouse sgp 130	0
Mouse IL-6	0

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 (0.098)
6.25	0.032
12.5	0.079
25	0.189
50	0.371
100	0.745
200	1.434
400	2.622

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 1 hour on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 2-4 min on the plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min