MOUSE INTERLEUKIN-3 (IL-3) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE-3 CONCENTRATIONS IN CELL CULTURE SUPERNATES, PLASMA AND SERUM

PURCHASE INFORMATION:

ELISA NAME	MOUSE IL-3 ELISA
Catalog No.	SK00771-03
Lot No.	
Formulation	96 T
Standard range	7.8-500 pg/mL
Sensitivity	5 pg/mL
Sample Volume	100 µL
Sample Type	cell culture supernates, plasma, serum
Specificity	Mouse IL-3
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2°C - 8°C

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

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INTRODUCTION

Mouse IL-3 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure mouse IL-3 in cell culture supernates, serum, and plasma. It contains recombinant mouse IL-3 and antibodies raised against this protein. It has been shown to accurately quantify recombinant mouse IL-3. Results obtained with naturally occurring IL-3 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-3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A Monoclonal antibody specific for IL-3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-3 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for IL-3 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is add 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 IL-3 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-3 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal purified IgG against mouse IL-3.	**771-03-01**	**1 plate**
IL-3 Standard – 0.5 ng/vial of recombinant mouse IL-3 in a buffered protein base with preservatives; lyophilized.	**771-03-02**	**1 vial**
Detection Antibody Concentrate– 120 µL / vial, 100-fold concentrated of Biotinylated antibody against mouse IL-3 with preservatives; lyophilized.	**771-03-03**	**1 vial**
Positive Control – one vial of recombinant mouse IL-3 , lyophilized	**771-03-04**	**1 vial**
Streptavidin-HRP Conjugate -75 µL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	**SAHRP**	**1 vial**
Dilution Buffer- 60mL/vial of buffered protein based solution with preservatives	**DB01**	**1 vial**
Wash Buffer - 50 mL/vial, 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 vial**
TMB Substrate Solution - 11 mL / vial of TMB substrate solution	**TMB01**	**1 vial**
Stop Solution - 11 mL /vial of 0.5N HCl	**S-STOP**	**1 vial**
Plate Sealer	**EAPS**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate as well as Dilution Buffer should be stored at -20°C or -70°C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard , Antibody 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 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.

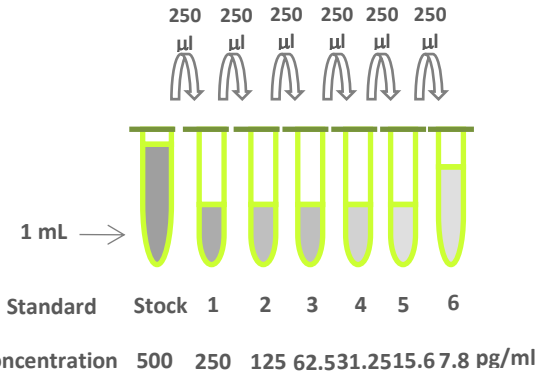
SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
Human IL-3 levels in cell culture supernatants may vary considerably. Optimal dilution has to be determined for each individual sample. For unknown cell culture samples it is useful to analyze undiluted as well as prediluted samples (e.g. 1:50 - 1:100) in parallel, thereby covering a wider range in one assay. Cell culturesupernatants with very high concentrations of human IL-3 require high dilutions (e.g. up to 1:2000) in order to be measured correctly. Such samples must be prediluted in the respective cell culture
medium.

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-3 Standard - Refer to vial label for reconstitution volume. Reconstitute the **IL-3** Standard with 1000 µL of Dilution Buffer. This reconstitution produces a stock solution of 500 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 500 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 mL	500 pg/ml
# 1	250µl of stock	250µl	250 pg/ml
# 2	250µl of 1	250µl	125 pg/ml
# 3	250µl of 1	250µl	62.5 pg/ml
# 4	250µl of 2	250µl	31.25 pg/ml
# 5	250µl of 3	250µl	15.6 pg/ml
# 6	250µl of 4	250µl	7.8 pg/ml



Detection Antibody- Reconstitute the **Detection Antibody concentrated** with 120 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. 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.

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: 1 x*

working solution of Streptavidin-HRP Conjugate should be used within a few days.

Positive Control- Reconstitute the positive control with 1mL of **Dilution Buffer** to make positive control solution.

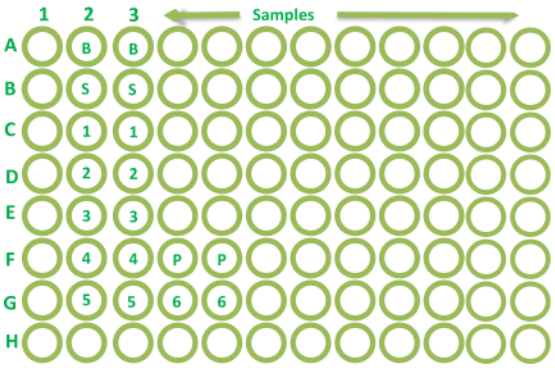
ASSAY PROCEDURE

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 plastic bag containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank wells (A2, A3).
4. Add 100 µL of Standard (from B2 to G3 , and G4 to G5), samples, or control (F4,F5) 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 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 45 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 5-10 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. 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. 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-3 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 mouse IL-3.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of IL-3 was 5 pg/mL.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Mouse IL-3	100%
Human IL-3	0
Mouse IL-1beta	0

TYPICAL DATA

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

IL-3 (pg/mL)	Average OD450 (Corrected)*
7.8	0.039
15.6	0.081
31.25	0.157
62.5	0.309
125	0.627
250	1.130
500	2.224

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100µL 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 to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Streptavidin-HRP conjugate to each well. Incubate 45 minutes on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate to each well. Incubate 10-20 min on the bench top. Protect from light.
↓
Add 100 µL Stop Solution to each well. Read 450nm within 15 min