GENLISA® Mouse Interleukin 4 (IL-4 / IL4) ELISA

REF : KLM0051

Ver 3.0

RUO

NIBSC Calibrated Assay
*the standards used in this kit are calibrated against an international standard from the National Institute of Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK.
1 ng of supplied standard equals 25 U of 91/656 NIBSC-standard. Please note that the calibration is lot specific.

Enzyme Immunoassay for the Quantitative determination of Mouse Interleukin 4, IL-4 in serum, plasma and cell culture supernatant

RUO	**For Research Use Only**	REF	**Catalog Number**
	**Store At**	LOT	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KLM0051 Σ 96 tests

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Introduction:

The GENLISA® ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antibodies are used in this kit.

Long Name: Interleukin 4

Entrez Gene IDs: 3565 (Human); 16189 (Mouse); 287287 (Rat); 397225 (Porcine); 280824 (Bovine); 403785 (Canine); 574281 (Primate); 100302454 (Rabbit)

Alternate Names: B cell growth factor 1; BCDF; B-cell stimulatory factor 1; BCGF1; BCGF-1; binetrakin; BSF1; BSF-1; IL4; IL-4; IL-4B_cell stimulatory factor 1; IL4E12; interleukin 4; interleukin-4; Lymphocyte stimulatory factor 1; MGC79402; pitrakinra

Intended Use:

The GENLISA® Mouse Interleukin 4 (IL-4 / IL4) ELISA kit is used as an analytical tool for quantitative determination of Mouse Interleukin 4, IL-4 in serum, plasma and cell culture supernatant.

Principle:

This assay is based on the Sandwich ELISA procedure. Samples containing mouse IL-4 react with already coated affinity purified capture anti-mouse IL-4 antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Biotinylated Anti-mouse IL-4 is added leading to formation of a sandwich complex of solid phase antibody-mouse IL- 4-biotin labeled antibody. The wells are washed to remove any unbound reactants as per the wash procedure. Streptavidin:HRP conjugate is added which binds to Biotinylated Anti-mouse IL-4 complex. The wells are washed to remove any unbound reactants as per the wash procedure. The substrate 3, 3', 5, 5' Tetra Methyl Benzidine (TMB) is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader at 450 nm and it is directly proportional to the concentration of Mouse IL-4 present in the samples.

Materials Provided:

1. Anti-mouse IL-4 Antibody Coated Microtiter Plate (12 x 8 wells) – 1 no
2. Recombinant Mouse IL-4 Standard (lyophilized, concentrated, 370 ng/ml) – 2 vials
3. Anti-mouse IL-4 Biotin Conjugated Detection Antibody (lyophilized, concentrated, 15 ug/ml) – 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase – 1 vial
5. Streptavidin:HRP Diluent - 12 ml
6. (1X) Assay Diluent - 50 ml
7. (20X) Wash Buffer - 25 ml
8. TMB Substrate - 12 ml
9. Stop Solution - 12 ml
10. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Timer.
7. Absorbent Paper.

Handling/Storage:

1. All reagents should be stored at 2-8ºC for stability.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Store recombinant Standard and Detection at 2-8°C. Upon reconstituting, aliquot both standard and detection into polypropylene vials and store at -20°C as per assay requirements. Do not freeze-thaw for more than two times to ensure no loss of activity.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. For Research Use Only.

Sample Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
2. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20 minutes at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.
4. **Cell Culture Supernatant-** Collect sample in a sterile container. Centrifuge for 20 minutes at 2000-3000 rpm. Remove the supernatant carefully. When examining the components within the cell, dilute cell suspension with PBS (pH 7.2-7.4), if cell concentration is greater than 1 million/ml. Damage the cells by repeated freeze-thaw cycles to release intracellular components. Centrifuge for 20 minutes at 2000-3000 rpm. If precipitation appears, centrifuge again.

Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Reagent Preparation:
Please refer to lot specific instructions for preparation of the reagents.

Assay Procedure:

1. Bring Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X)**: dilute **25 ml of (20X) Wash Buffer in 475 ml of DI water**
4. **Standards Preparation:** Reconstitute the lyophilized vial in 25 ul Assay Diluent (1X) to get 370 ng/ml concentration. Dilute 13.5 ul of reconstituted Standard (370 ng/ml) with 486.5 ul of Assay diluent (1X) to generate a 10 ng/ml mid stock solution. Prepare the Standard stock by diluting the mid stock as per the below table. Thus the Mouse IL-4 Standards are 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml and 15.6 pg/ml. Assay Diluent (1X) serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
370 ng/ml	Reconstituted Standard	Lyophilized Standard provided in the Kit + 25 ul of Assay Diluent (1X)
10 ng/ml	Mid Stock	13.5 ul Reconstituted Standard + 486.5 ul Assay Diluent (1X)
1000 pg/ml	Standard No.7	100 ul Mid stock + 900 ul Assay Diluent (1X)
500 pg/ml	Standard No.6	500 ul Standard No.6 + 500 ul Assay Diluent (1X)
250 pg/ml	Standard No.5	500 ul Standard No.5 + 500 ul Assay Diluent (1X)

Standard Concentration	Standard No	Dilution Particulars
125 pg/ml	Standard No.4	500 ul Standard No.4 + 500 ul Assay Diluent (1X)
62.5 pg/ml	Standard No.3	500 ul Standard No.3 + 500 ul Assay Diluent (1X)
31.25 pg/ml	Standard No.2	500 ul Standard No.2 + 500 ul Assay Diluent (1X)
15.6 pg/ml	Standard No.1	500 ul Standard No.2 + 500 ul Assay Diluent (1X)
0 pg/ml	Standard No.0	Only Assay Diluent (1X)

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Mouse IL-4. High Dose Hook Effect is due to excess of antibody for very high concentrations of Mouse IL-4 present in the sample.
3. Mouse IL-4 concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Mouse IL-4.
5. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
   Make a work list in order to identify the location of Standards and Samples

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.

2. Add 100 ul of **Standards** and **Samples** to the plate, seal plate and incubate for 2 hours at Room Temperature (18-25°C).

3. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

4. Add 100 ul of diluted **Biotinylated Detection Antibody** solution to each well, seal plate and incubate for 2 hours at Room Temperature (18-25°C).

5. Wash plate 4 times with **Wash Buffer (1X)** as mentioned in step 3.

6. Add 100 ul of diluted **Streptavidin:HRP Conjugate** to each well, seal plate and incubate for 30 minutes at Room Temperature (18-25°C).

7. Wash plate 4 times with **Wash Buffer (1X)** as mentioned in step 3.

8. Add 100 ul of **TMB Substrate** to all wells and Incubate for 30 minutes at Room Temperature. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.

9. Stop reaction by adding 100 ul of **Stop Solution** to each well. Positive wells should turn from blue to yellow.

10. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Graph Paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Mouse Interleukin 4, IL-4 concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Mouse Interleukin 4, IL-4 Concentration.

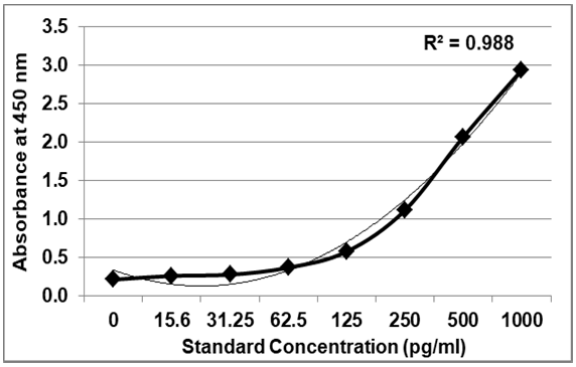
If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.

Typical Data (representative only)

Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.211	-	-
15.6	0.259	13.5	86.6
31.25	0.275	26.1	83.6
62.5	0.367	66.6	106.6
125	0.576	125.9	100.7
250	1.116	248.2	99.3
500	2.070	501.6	100.3
1000	2.941	998.6	99.9

Typical Graph (representative only)



Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit:

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

This kit has been validated as per guidelines. Please view the details herein below.

Sensitivity:
Limit Of Quantification:
It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be 15 pg/ml.

Limit Of Detection:
It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2*SD. 10 replicates of '0' standards were evaluated and the LOD was found to be less than 12 pg/ml.

Assay Range:
15.6 pg/ml – 1000 pg/ml

Specificity:
The antibodies used in the kit for capture and detection are monoclonal antibodies specific for Mouse IL-4. The standard used in the kit is calibrated against an international standard from the National Institute of Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK. 1 ng of supplied standard equals 25 U of 91/656 NIBSC-standard. Please note that the calibration is lot specific.

Precision:
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Linearity:
The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Mouse IL-4 and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8
serum (n=5)	84-107%	87-108%	82-112%
EDTA plasma (n=5)	83-102%	83-115%	83-118%
heparin plasma (n=5)	83-99%	80-95%	82-93%

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (<0.1% w/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

Components (2-8ºC) → 30 mins RT Before Use → 

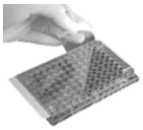
Thaw at Room Temperature (18-24ºC) → USE NOW

2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components → AVOID → COOL (2-8ºC) → THAW (22±4ºC) → COOL (2-8ºC) → THAW (22±4ºC) → AVOID → NO CHANGE IN RESULTS

3. Pipette **100 ul Standards** into respective Standard wells.

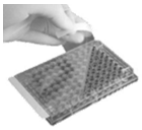
4. Pipette **100 ul Samples** into the sample wells.

5. Cover plate  and incubate for 

at room temperature.

6. Aspirate and wash wells 4 times with **Wash Buffer (1X).**

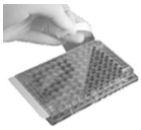
7. Pipette **100 ul** diluted **Biotinylated Detection Antibody** to all wells.

8. Cover plate  and incubate for 

at room temperature.

9. Aspirate and wash wells 4 times with **Wash Buffer (1X).**

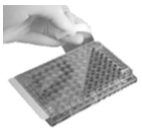
10. Pipette **100 ul** of diluted **Streptavidin:HRP** to all wells

11. Cover plate  and incubate for 

at room temperature

12. Aspirate and wash wells 4 times with **Wash Buffer (1X).**

13. Pipette **100 ul TMB Substrate** into each wells

14. Cover plate  and incubate for 

at room temperature.

15. Pipette **100 ul Stop Solution** into each well.

16. Read absorbance at 450 nm with a  microplate reader within 

of stopping reaction.

LIMITED WARRANTY

Krishgen Biosystems Private Limited does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems Private Limited, or against damages resulting from such non-Krishgen Biosystems Private Limited made products or components. Krishgen Biosystems Private Limited passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components.

This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems Private Limited.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems Private Limited shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems Private Limited shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems Private Limited be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems Private Limited with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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THANK YOU FOR USING KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS PRIVATE LIMITED | OUR REAGENTS | YOUR RESEARCH |

SYMBOLS KEY

MTP	**Anti-mouse IL-4 Antibody Coated Microtiter Plate (12x8 wells)**
STD	**Recombinant Mouse IL-4 Standard, Lyophilized**
BIO CONJ	**Anti-mouse IL-4 Biotin Conjugated Detection Antibody, Lyophilized**
STRP HRP	**Concentrated Streptavidin Horseradish Peroxidase**
STRP HRP DIL	**Streptavidin:HRP Diluent**
1X ASY DIL	**(1X) Assay Diluent**
20X WASH BUF	**(20X) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalogue Number**
	**Expiration Date**
	**Storage Temperature**