KRISHGEN BioSystems
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PrecisionBind Human Interleukin 4 (IL-4 / IL4) ELISA

REF : KB1066

Ver 1.0

RUO

NIBSC Calibrated Assay

* 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 14 U of 88/656 NIBSC-standard.
Therefore 1000 pg/ml is equivalent to 14 U of IL-4 as per NIBSC.

ELISA for Accurate Quantitation of Human IL-4 from Cell Culture Supernatant, Serum, Plasma, or Other Bodily Fluids

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

For Research Purposes Only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Krishgen Biosystems Private Limited is strictly prohibited.

REF KB1066 **Σ 96 tests**

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Introduction:

Interleukin-4, abbreviated IL-4, is a cytokine that induces differentiation of naive helper T cells (Th0 cells) to Th2 cells. Upon activation by IL-4, Th2 cells subsequently produce additional IL-4. It has many biological roles, including the stimulation of activated B-cell and T-cell proliferation, and the differentiation of CD4+ T-cells into Th2 cells. It is a key regulator in humeral and adaptive immunity. IL-4 induces B-cell class switching to Ice, and up-regulates MHC class II production. Overproduction of IL-4 is associated with allergies.

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:

PrecisionBind Human Interleukin 4 (IL-4 / IL4) ELISA is specifically designed for the accurate quantitation of human IL-4 from cell culture supernatant, serum, plasma or other bodily fluids. It is ready-to-use, accurate, and sensitive.

Principle:

This assay is based on the Sandwich ELISA procedure. Samples containing human IL-4 react with already coated affinity purified capture Anti-Human IL-4 antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Biotinylated Anti-human IL-4 is added leading to formation of a sandwich complex of solid phase antibody-human 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-human 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 Human IL-4 present in the samples.

Materials Provided:

1. Anti-human IL-4 Coated Microtiter Plate (12X8wells) – 1 no.
2. Recombinant Human IL-4 Standard (lyophilized) – 2 vials
3. Anti-Human IL-4 Biotin Conjugated Detection Antibody (lyophilized; concentrated) – 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase - 1 vial
5. (1X) Assay Diluent – 50 ml
6. (20X) Wash Buffer – 25 ml
7. TMB Substrate – 12 ml
8. Stop Solution – 12 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Semi-Log graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

Storage Information:

1. Store main kit components at 2-8°C.
2. Store recombinant **Standard and Detection at 2-8°C.** Upon reconstitution, aliquot recombinant protein and detection antibody into polypropylene vials and store at -20°C as per assay requirements. Do not freeze thaw for more than two times.
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

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. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

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

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at temperature <-20°C. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x *g*. Remove serum layer and assay immediately or store serum samples at temperature <-20°C. Avoid repeated freeze/thaw cycles.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at temperature <-20°C. Avoid repeated freeze/thaw cycles.

Reagent Preparation:

Please refer to lot-specific instructions for preparation of the reagents mentioned in the Reagent Preparation Sheet. *Note each reagent sheet is specific for a particular Lot only and is not to be interchanged amongst different lots*.

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 Human IL-4. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human IL-4 present in the sample.
3. Human 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 Human 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.
9. 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 each well, 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 hour at Room Temperature (18-25°C).

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

6. Add **100 ul** of diluted **Streptavidin:HRP** solution 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 in step 3.

8. Add **100 ul** of **TMB Substrate** solution and incubate in the dark for 30 minutes at Room Temperature (18-25°C). 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:

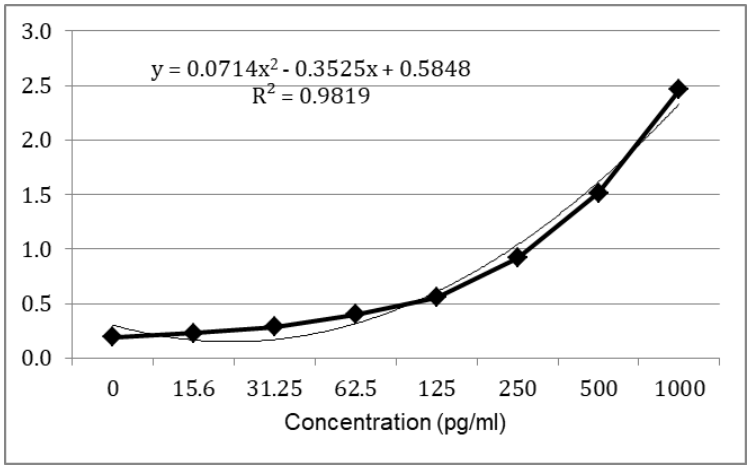
Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on standard graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the unknown cytokine concentrations, find the unknowns 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 cytokine concentration. If samples were diluted, multiply by the appropriate dilution factor.

Computer based curve-fitting software may be preferred. Software which is able to generate a cubic spline curve-fit or a polynomial regression to the 2nd order is best recommended for automated results.

Typical Data (representative only)

Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery
0	0.195	1.8	--
15.6	0.227	12.0	77.1
31.25	0.284	30.4	97.2
62.5	0.398	67.8	108.5
125	0.555	121.1	96.8
250	0.916	252.0	100.8
500	1.513	499.2	99.8
1000	2.465	1000.2	100.0

Typical Graph (representative only)



Performance Characteristics:

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.

Sensitivity:
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 is ~**9.66 pg/ml**.

Limit of Quantitation (LOQ): It is defined as the lowest concentration of an analyte that can be measured with acceptable precision and accuracy, 10 replicates of '0' standards were evaluated and the LOQ is **~12.50 pg/ml**.

IC_{50}: The half-maximal inhibitory concentration (IC_{50}) in a sandwich ELISA measures the concentration of an inhibitor (such as a drug, molecule, or antibody) required to reduce the binding of a target antigen to the capture/detection antibody pair by 50%. The IC_{50} for PrecisionBind Human IL-4 ELISA is ~**498.2 pg/ml**.

Lower Limit of Quantification: The lowest concentration of an analyte that can be quantitatively measured with acceptable accuracy and precision. 10 replicates of '0' standards were evaluated and the LLOQ is **≤ 12.50 pg/ml.**

Upper Limit of Quantification: The highest concentration of an analyte that can be quantitatively measured with acceptable accuracy and precision in an assay. 10 replicates of '0' standards were evaluated and the ULOQ is **~1000 pg/ml.**

Specificity:
The antibodies used in the kit for capture and detection are monoclonal antibodies specific for human IL-4.

Cross Reactivity:
This assay recognizes natural and recombinant human IL-4. The markers listed below were prepared at 50 pg/ml in Assay Diluent and assayed for cross-reactivity. No significant cross-reactivity or interference was observed.

Recombinant human:
This kit shows the cross reactivity for IL-4 Rα , IL-4

Calibration:
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 14 U of 88/656 NIBSC-standard.
Therefore 1000 pg/ml is equivalent to 14 U of IL-4 as per NIBSC.

Assay Range:
15.6 pg/ml to 1000 pg/ml.

Parallelism and Matrix Effect:
Sample Dilution factor – Human Serum, Human Plasma and Human CSF samples have been tested. Neat samples can be run directly.
Neat Human Serum, Human Plasma and Human CSF were spiked with 1000 pg/ml Human IL-4 and ELISA assay was run.

Sample	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery
Neat CSF samples	2.789	1211.2	121.1
Neat Plasma	2.722	1165.5	116.6
Neat Human Serum	2.849	1253.1	125.3

A) Serum

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:1 dilution	1000	2.468	1002.0	100.2	99.8
1:2 dilution	500	1.602	539.9	108.0	92.6
1:4 dilution	250	1.174	353.7	141.5	70.7
1:8 dilution	125	0.654	155.7	124.6	80.3
1:16 dilution	62.5	0.475	93.7	149.9	66.7
1:32 dilution	31.25	0.306	37.5	120.1	83.3
1:64 dilution	15.6	0.258	22.0	140.9	71.1

B) Plasma

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:1 dilution	1000	2.389	954.1	95.4	104.8
1:2 dilution	500	1.678	575.5	115.1	86.9
1:4 dilution	250	1.102	324.5	129.8	77.0
1:8 dilution	125	0.567	125.2	100.2	99.8
1:16 dilution	62.5	0.410	71.8	114.9	87.0
1:32 dilution	31.25	0.322	42.8	136.8	73.1
1:64 dilution	15.6	0.235	14.6	93.5	107.1

C) Cerebrospinal Fluid

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:1 dilution	1000	2.416	970.3	97.0	103.1
1:2 dilution	500	1.602	539.9	108.0	92.6
1:4 dilution	250	1.128	335.0	134.0	74.6
1:8 dilution	125	0.581	130.1	104.1	96.1
1:16 dilution	62.5	0.433	79.5	127.2	78.6
1:32 dilution	31.25	0.354	53.2	170.4	58.7
1:64 dilution	15.6	0.267	24.9	159.5	62.8

Results:

i) Parallelism is maintained across the 1:1 to 1:16 dilutions.
ii) % Recovery for most dilutions falls within the acceptable range of 80%–120%.
iii) No significant matrix effect observed at higher dilutions.

iv) The PrecisionBind Human IL-4ELISA kit was tested for matrix effect on human serum, plasma, CSF and physiological buffer 7.4 to mimic tear fluid samples.

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 Human 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%

Limitations of Method:

Any diagnosis should not be based on the results of in-vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis. The KB1066 PrecisionBind Human IL-4 ELISA is a research use kit only and is not licensed for In-Vitro Diagnostic Use.

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

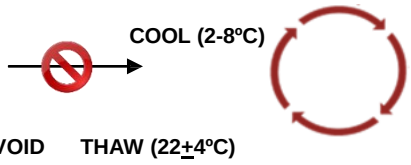
1. Remove all components, 30 minutes before adding into the assay plate.

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.

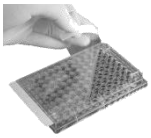
Kit Components RESULTS



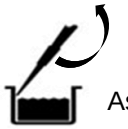
NO CHANGE IN

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

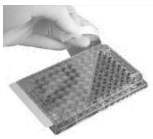
4.  Pipette **100 ul Samples** into the sample wells.

5. Cover plate  and incubate for 

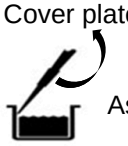
at room temperature.

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).**

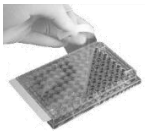
10.  Pipette **100 ul** of diluted **Streptavidin:HRP** to all wells.

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 product; 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 product 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 product 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, 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 product. 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-human IL-4 Coated Microtiter Plate (12x8 wells)
STD	Recombinant Human IL-4 Standard, Lyophilized
BIO CONJ	Anti-Human IL-4 Biotin Conjugated Detection Antibody, Lyophilized
STRP HRP	Concentrated Streptavidin Horseradish Peroxidase
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