PrecisionBind Human Interferon Gamma (IFN gamma / IFN g) ELISA

REF : KB1053

Ver 1.0

RUO

NIAID Calibrated Assay

The standard used in the kit is are calibrated against an international standard from the National Institute of Allergy and infectious Diseases (NIAID), Bethesda, US.1 ng of supplied standard equals 39 U of GXg01-902-535 NIAID -standard.

Therefore 1000 pg/ml is equivalent to 39 U of IFNg as per NIAID.

ELISA for Accurate Quantitation of Human IFN-γ 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 KB1053 Σ 96 tests

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Introduction:

Interferon-γ is a potent multifunctional cytokine which is secreted primarily by activated NK cells and T cells. Originally characterized based on anti-viral activities, IFN-**γ** also exerts anti-proliferative, immunoregulatory, and proinflammatory activities. IFN-**γ** can up-regulate MHC class I and II antigen expression by antigen-presenting cells. Recombinant human IFN-**γ** is a 16.7 kDa protein containing 143 amino acids.

Long Name: Interferon gamma

Entrez Gene IDs: 3458 (Human); 15978 (Mouse); 25712 (Rat); 396991 (Porcine); 281237 (Bovine); 403801 (Canine); 493965 (Feline) 449517 (Primate); 100008602 (Rabbit).

Alternate Names: Interferon-γ, Interferon-gamma, IFN-γ, IFN-gamma, IFN-g, IFNg, IFG, IFI, IMD69 Immune interferon; interferon gamma; interferon, gamma.

Intended Use:

PrecisionBind Human Interferon Gamma (IFN gamma / IFN g) ELISA is specifically designed for the accurate quantitation of human IFN-γ 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 IFN-γ react with already coated affinity purified capture anti-human IFN-γ antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Biotinylated Anti-human IFN-γ is added leading to formation of a sandwich complex of solid phase anti-human IFN-γ-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 IFN-γ 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 IFN-γ present in the samples.

Materials Provided:

1. Anti-Human IFN-γ Coated Microtiter Plate (12x8 wells) – 1 no
2. Recombinant Human IFN-γ Standard (lyophilized) – 2 vials
3. Anti-Human IFN-γ Biotin Conjugated Detection Antibody – 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 at 2-8°C**. Upon reconstituting, aliquot recombinant protein 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.
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 IFN-γ. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human IFN-γ present in the sample.
3. Human IFN-γ 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 IFN-γ.
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 respective wells. Seal plate and incubate for 2 hours at 37°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 at for 1 hour at 37**°**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 37°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 37°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:

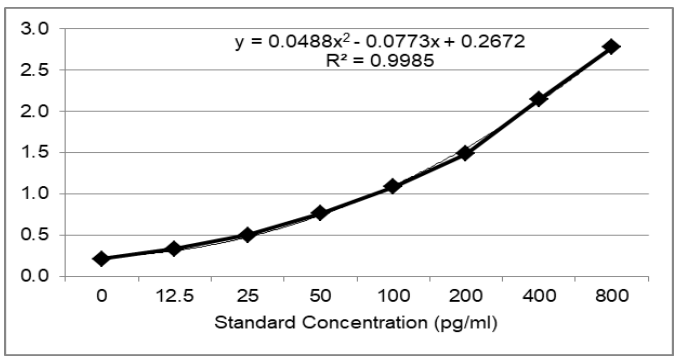
Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on standard graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the 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 Abs	Interpolated Concentration (pg/ml)	% Interpolated Concentration against Actual Concentration
0	0.207	0.7	0.7
12.5	0.332	10.9	10.9
25	0.500	24.1	24.1
50	0.762	54.8	54.8
100	1.086	104.7	104.7
200	1.488	188.9	188.9
400	2.144	406.5	406.5
800	2.781	799.4	799.4

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 **~3.6 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 ~**10.9 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 IFN gamma ELISA is ~**593 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 **≤ 10.9 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 ~**800 pg/ml.**

Specificity:
The antibodies used in the kit for capture and detection are monoclonal antibodies specific for human IFN gamma.

Calibration:
The standard used in the kit is are calibrated against an international standard from the National Institute of Allergy and infectious Diseases (NIAID), Bethesda, US.1 ng of supplied standard equals 39 U of GXg01-902-535 NIAID -standard.
Therefore 1000 pg/ml is equivalent to 39 U of IFNg as per NIAID.

Cross-Reactivity:
This assay recognizes natural and recombinant human IFN gamma. The markers listed below were prepared at 50 pg/ml in Assay Diluent and assayed for cross-reactivity.
They exhibited no cross-reactivity or interference.

Recombinant human:
IFN-β IFN-γ R1

Other recombinants:
bovine IFN-γ canine IFN-γ cotton rat IFN-γ equine IFN-γ feline IFN-γ mouse IFN-γ porcine IFN-γ rat IFN-γ

A sample containing 12.5 ng/ml of recombinant rhesus macaque IFN-γ reads as 137 pg/ml (1.1% cross-reactivity)

Assay Range:
12.5 pg/ml to 800 pg/ml.

Parallelism and Matrix Effect:
Sample Dilution factor – Human Serum, Human Plasma and Human CSF samples have been tested. Sample dilution Factor for all three matrices is 1:50 dilution.

Neat Human Serum, Human Plasma and Human CSF were spiked with 400 pg/ml Human IFN gamma and ELISA assay was run.

Sample	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery
Neat Human CSF	2.733	Out of range	-
Neat Human Plasma	2.615	Out of range	-
Neat Human Serum	2.899	Out of range	-

A) Serum

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:100 dilution	800	3.177	--	0.0	--
1:200 dilution	400	2.564	619.0	154.7	64.6
1:400 dilution	200	1.949	322.0	161.0	62.1
1:800 dilution	100	1.139	114.0	114.0	87.7
1:1600 dilution	50	0.810	61.7	123.4	81.1
1:3200 dilution	25	0.516	25.9	103.8	96.4
1:6400 dilution	12.5	0.349	10.1	80.7	124.0

Results:

i. Parallelism is generally maintained across the 1:800 to 1:6400 dilutions.
ii. % Recovery for most dilutions falls within the acceptable range of 80%–120%.
iii. No significant matrix effect observed at higher dilutions.

B) Plasma

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:100 dilution	800	1.702	852.5	106.6	93.8
1:200 dilution	400	1.028	451.7	112.9	88.6
1:400 dilution	200	0.614	232.8	116.4	85.9
1:800 dilution	100	0.555	202.7	202.7	49.3
1:1600 dilution	50	0.368	108.6	217.3	46.0
1:3200 dilution	25	0.245	46.7	186.6	53.6
1:6400 dilution	12.5	0.191	18.7	149.2	67.0

Results:

i. Parallelism is generally maintained across the 1:100 to 1:400 dilutions.
ii. % Recovery for most dilutions falls within the acceptable range of 80%–120%.
iii. No significant matrix effect observed at higher dilutions.

C) Cerebrospinal Fluid

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:100 dilution	800	1.782	904.8	113.1	88.4
1:200 dilution	400	1.242	472.1	118.0	84.7
1:400 dilution	200	0.642	247.1	123.5	80.9
1:800 dilution	100	0.572	211.4	211.4	47.3
1:1600 dilution	50	0.400	124.7	249.4	40.1

Dilution	Expected Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration (pg/ml)	% Recovery	% Deviation
1:3200 dilution	25	0.267	57.8	231.4	43.2
1:6400 dilution	12.5	0.194	20.2	161.9	61.8

Results:

i. Parallelism is generally maintained across the 1:100 to 1:400 dilutions.
ii. % Recovery for most dilutions falls within the acceptable range of 80%–120%.
iii. No significant matrix effect observed at higher dilutions.

The PrecisionBind Human IFN gamma ELISA 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 IFN-γ 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 KB1053 PrecisionBind Human IFN-γ 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.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- 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 → 

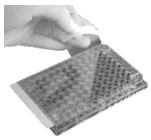
Thaw at Room Temperature (18-24ºC) → USE NOW

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

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

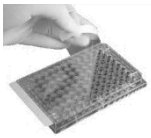
4. Pipette **100 ul Samples** into the sample wells.

5. Cover plate  and incubate for 

at 37°C.

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

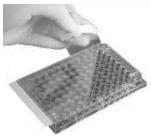
7. Pipette **100 ul** diluted **Biotinylated Detection Antibody** to all wells.

8. Cover plate  and incubate for 

at 37°C.

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

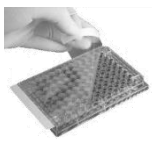
10. Pipette **100 ul** of diluted **Streptavidin:HRP** to all wells.

11. Cover plate  and incubate for 

at 37°C.

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 37°C.

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.

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SYMBOLS KEY

MTP	Anti-Human IFN-γ Coated Microtiter Plate (12x8 wells)
STD	Recombinant Human IFN-γ Standard, Lyophilized
BIO CONJ	Anti-Human IFN-γ Biotin Conjugated Detection Antibody
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