Human IFN-gamma (Interferon Gamma) ELISA Kit

Cat #: orb654845 (manual)

Enzyme Immunoassay for the estimation of Human Interferon Gamma (IFNgamma / IFNg) in human serum, plasma, cell culture supernatant and other biological samples. Validated for citrated/EDTA plasma only.

Introduction:

The 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.

Intended Use:

The Human Interferon Gamma (IFNgamma / IFNg) ELISA kit is used as an analytical tool for quantitative determination of the analyte in human serum, plasma and other biological samples.

Principle:

The method employs sandwich ELISA technique. Monoclonal antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and the analyte present in the standard and sample are bound by the antibodies. Biotin labeled antibody is added and followed by Streptavidin:HRP is pipetted and incubated to form a complex. After washing microwells in order to remove any unbound proteins, TMB substrate solution is added to microwells and color develops proportionally to the amount of analyte present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

- Coated Microtiter Plate (12 x 8 wells) - 1 no
- Standard (lyophilized, concentrated) - 2 vials
- Biotinylated Detection Antibody (concentrated) - 120 ul
- Streptavidin: HRP Conjugate (concentrated) - 120 ul
- Standard Diluent - 20 ml
- Biotin Antibody Dilution Buffer - 12 ml
- HRP Conjugate Dilution Buffer - 12 ml
- (25X) Wash Buffer - 20 ml
- TMB Substrate - 12 ml

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- Stop Solution - 12 ml
- Instruction Manual

Materials to be provided by the End-User:

- Microtiter Plate Reader able to measure absorbance at 450 nm.
- Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
- Deionized (DI) water
- Wash bottle or automated microplate washer
- Clean tubes and Eppendorf tubes
- Precision single and multi-channel pipette and disposable tips.
- 37°C incubator
- Timer.

Handling/Storage:

- All reagents should be stored as indicated on the component label.
- All the reagents and wash solutions should be used within 12 months from manufacturing date.
- Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
- The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

- Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
- 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.

- 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.
- **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
- **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.
- **Cell Culture Supernatant-** Collect sample in a sterile container. Centrifuge for 20-mins 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-min at 2000-3000 rpm. If precipitation appears, centrifuge again.

- **Tissue Samples-** Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes and collect the supernatant carefully.
- **Urine-** Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20˚C. Avoid repeated freeze-thaw cycles.
- **Saliva-** Collect Saliva using a collection device or equivalent. Centrifuge samples at 1000 x g at 2-8˚C for 15 minutes. Remove particulates and assay immediately or store samples in aliquot at ≤ -20˚C. Avoid repeated freeze-thaw cycles.
- **Feces-** Dry feces were collected as much as possible, weighing more than 50 mg. The feces were washed three times with PBS (w:v = 1:9, e.g. 900 µl lysis buffer is added in 100 mg feces), sonicated (or mashed) and centrifuged at 5000Xg for 10 minutes, where the supernatant was collected for testing.
- **Cell Culture supernatants and other biological fluids-** Centrifuge samples at 1000 x g for 20 minutes. Collect the supernatant and assay immediately or store samples in aliquot at -20˚C or -80˚C for later use. Avoid repeated freeze-thaw cycles.
- **Cerebrospinal fluid (CSF)-** Remove the particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20˚C. Avoid repeated freeze-thaw cycles.

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

Reagent Preparation (all reagents should be diluted immediately prior to use):

- Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
- Bring all reagents to Room temperature before use.
- To make **Wash Buffer (1X) 500 ml;** dilute **20 ml of (25X) Wash Buffer in 480 ml of DI water**.
- **Streptavidin: HRP Conjugate & Biotinylated Antibody Working Solution** - Briefly spin or centrifuge the Streptavidin: HRP Conjugate & Biotinylated eEF2 Antibody before use. Dilute them to the working concentration 100-fold with HRP Conjugate Dilution Buffer & Biotin Antibody Dilution Buffer, respectively.
- **Standards Preparation**: Reconstitute original lyophilized standard with 1.0 ml of Standard Diluent. Keep the standard for 10 mins with gentle agitation before making further dilutions. Prepare the additional Standards by serially diluting the standard stock solution as per accompanying sheet available in the kit.

Assay Range:

15.63 - 1000 pg/ml

Sensitivity:

5.9 pg/ml

Procedural Notes:

- In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
- High Dose Hook Effect may be observed in samples with very high concentrations of the analyte. High Dose Hook Effect is due to excess of antibody for very high concentrations of the analyte present in the sample.
- If the analyte concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
- Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of the analyte.
- It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
- Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
- 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.
- 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:

- It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.
- Add **100 ul Standard Diluent** to blank wells.
- Add **100 ul prepared Standards and Samples** to respective wells.
- Cover the plate with a sealer and incubate for 80 minutes at 37°C.
- Aspirate and wash plate 4 times with diluted 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.
- Pipette **100 ul Biotinylated Antibody Working Solution** to all wells.
- Cover the plate with a sealer and incubate for 50 minutes at 37°C.
- Aspirate and wash as per Step (5) above.
- Pipette **100 ul Streptavidin: HRP Conjugate Working Solution** to all wells. Mix well.
- Cover the plate with a sealer and incubate for 50 minutes at 37°C.
- Aspirate and wash as per Step (5) above.
- Pipette **100 ul TMB Substrate** in all the wells.
- Incubate the plate at **37°C** for **10 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
- Pipette **100 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.
- Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Note: It is strictly recommended to follow the instructions and procedures as per the IFU (instructions for use) / data sheet accompanying the kit and not use the website or a previous version in hand. This may be due to the fact that there may have been changes in the kit which may not have been reflected in the previous version(s) of the IFU / data sheet.

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 450nm) 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 analyte 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 analyte concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a 4 -PL (2^{nd} order) or 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.

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:

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

Standard Calibration Range:

15.63 - 1000 pg/ml

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 5.9 pg/ml

Specificity:

This assay has high sensitivity and excellent specificity for detection of the analyte. No significant cross-reactivity or interference between the analyte and analogues was observed.

Recovery

Matrices listed below were spiked with a known concentration of the analyte and the recovery rates were calculated by comparing the measured value to the expected amount of the analyte in the samples.

Matrix	Recovery Range (%)	Average (%)
serum(n=5)	87-99	93
EDTA plasma(n=5)	81-95	88
heparin plasma(n=5)	80-95	87

Precision:

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of the analyte and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8	1:16
serum(n=5)	89-97%	93-102%	81-96%	93-106%
EDTA plasma(n=5)	85-94%	92-101%	87-96%	82-90%
heparin plasma(n=5)	96-105%	88-96%	87-98%	93-101%

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/w) 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.

LIMITED WARRANTY

Biorbyt 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 Biorbyt, or against damages resulting from such non-Biorbyt made products or components. Biorbyt passes on to customer the warranty it received (if any) from the maker thereof of such non Biorbyt 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 Biorbyt.

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