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EliKine™ Rat IFN-γ ELISA Kit Booklet

Item NO.	Product Name
KET7017	EliKine™ Rat IFN-γ ELISA Kit

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

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INTRODUCTION

Background

Produced by lymphocytes activated by specific antigens or mitogens. IFN-γ, in addition to having antiviral activity, has important immunoregulatory functions. It is a potent activator of macrophages, it has antiproliferative effects on transformed cells and it can potentiate the antiviral and antitumor effects of the type I interferons.

Assay principle

EliKine™ Rat IFN-γ ELISA Kit employs a two-site sandwich ELISA to quantitate Rat IFN-γ in samples. An antibody specific for Rat IFN-γ has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Rat IFN-γ present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for Rat IFN-γ is added to the wells. After washing, proprietary EliKine™ Streptavidin-HRP conjugates is added to the wells. Following a wash to remove any unbound streptavidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Rat IFN-γ bound in the initial step. The color development is stopped by Stop Solution and the intensity of the color is measured.

Characteristics

• This Kit allows for the determination of Rat IFN-γ concentrations in Rat serum, Plasma,cell culture supernates and other biological fluids.
• Detection range: 37.5 pg/mL - 2400 pg/mL.
• The minimum detectable dose (MDD) of Rat IFN-γ is typically less than 15 pg/mL.
• Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%)＜10%.
• Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components. The CV (%)＜12%.
• To assess linearity of the assay, samples containing and/or spiked with high concentrations of Rat IFN-γ were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.
• EliKine™ Rat IFN-γ ELISA Kit has high sensitivity and excellent specificity for detection of Rat IFN-γ. No significant cross-reactivity or interference between Rat IFN-γ and analogues was observed.
• The recovery of Rat IFN-γ spiked to different levels in samples throughout the range of the assay in various matrices was evaluated.
The recovery ranged from 98% to 116% with an overall mean recovery of 106%.

PRODUCT INFORMATION

Materials supplied & Storage conditions

Store kit reagents at 2-8 °C for 12 months. Remaining reagents should be returned to cold storage at 4 °C immediately after use.

Components	48T	96T	Storage Conditions
Rat IFN-γ microplate	48 wells	96 wells	2-8 °C
Rat IFN-γ standard	1(lyophilized)	2 (lyophilized)	2-8 °C
Sample Diluent	3 .5 mL(5×)	7 mL(5×)	2-8 °C
Assay Buffer	3 .5 mL(5×)	7 mL(5×)	2-8 °C
Rat IFN-γ Detect Antibody	60 uL(100×)	120 uL(100×)	2-8 °C
Streptavidin-HRP	60 uL(100×)	120 uL(100×)	2-8 °C
HRP Substrate (TMB)	5 mL	10 mL	2-8 °C
Stop Solution	5 mL	10 mL	2-8 °C
Wash Buffer	25 mL (20×)	50 mL (20×)	2-8 °C
Plate Covers	1	2	RT
Booklet	1	1	RT

Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.

Other Materials Required, Not Supplied

• Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
• Pipettes and pipette tips.
• Deionized or distilled water.
• Squirt bottle, manifold dispenser, or automated microplate washer.
• 500 mL graduated cylinder.

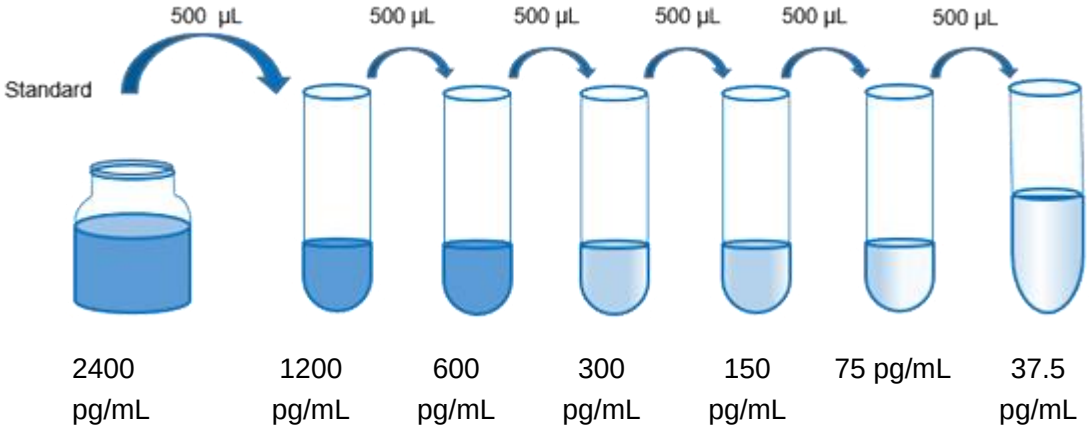
ASSAY PROTOCOL

Sample collection & storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.
Cell culture supernatants - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20˚C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20˚C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20˚C. Avoid repeated freeze-thaw cycles.
Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.
Samples should be aliquoted and must be stored at -20 ˚C to avoid loss of bioactive Rat IFN-γ. If samples are to be used within 24 hours, they may be stored at 2 to 8 ˚C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Reagent preparation

Bring all reagents to room temperature before use. If crystals have formed in the Buffer Concentrates, warm them gently until they completely dissolved.
Wash Buffer - Pour entire contents (50 ml) of the Wash Buffer (20×) into a clean 1000 ml graduated cylinder. Bring to final volume of 1000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2 to 25˚C. Please note that Wash Buffer (1×) is stable for 30 days.
Sample Diluent/ Assay Buffer - Pour the entire contents (7 ml) of the Diluent (5×) into a clean 100 ml graduated cylinder. Bring to final volume of 35 ml with distilled water. Mix gently to avoid foaming. Store at 2 to 8˚C. Please note that the Diluent (1×) is stable for 30 days.
HRP Substrate - The reagents should be ready within 15 minutes of use. Protect from light. 100 µL of the solution is required per well.
Standard - Reconstitute the Rat IFN-γ standard in 1ml of Sample Diluent for a concentration of 2400 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.
Add 500 µL of Sample Diluent to each of 6 tubes labeled 1200, 600, 300, 150, 75 and 37.5 pg/mL of Rat IFN-γ standard just as below.



Rat IFN-γ Detect Antibody - Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated detect antibody solution with Assay buffer in a clean plastic tube as needed according to the standards and samples. Detect antibody should be used within 30 minutes after dilution.
Streptavidin-HRP - Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated Streptavidin-HRP with Assay buffer in a clean plastic tube as needed according to the standards and samples. Streptavidin-HRP should be used within 30 minutes after dilution.
Sample Diluent - If your samples need to be diluted, Sample Diluent is used for dilution of serum/plasma samples, while cell culture medium is used for dilution of cell culture supernates.

Assay procedure

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of diluted standard and sample per well. Add 100 µL Sample Diluent to Blank well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided for a record of standards and samples assayed.
4. Aspirate each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash buffer (250 µL) using a squirt bottle, multi-channel pipette, manifold dispenser, or automatic washer. 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.
5. Add 100 µL of diluted Rat IFN-γ detect antibody to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.
6. Repeat the aspiration/wash as in step 4.

7. Add 100 µL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 30 minutes at room temperature. Avoid placing the plate in direct light.
8. Repeat the aspiration/wash process for five times as in step 4.
9. Add 100 µL of HRP substrate solution to each well. Incubate for 15 minutes at room temperature. Protect from light.
10. Add 50 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.
11. Determine the optical density of each well within 30 minutes, using a microplate 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.

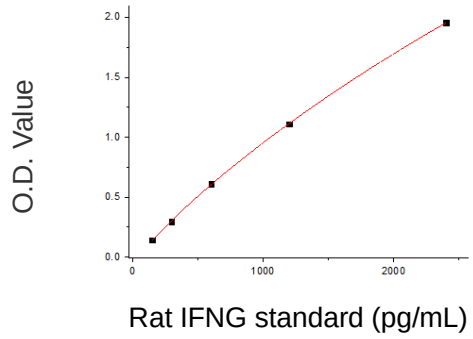
DATA ANALYSIS

Calculation of results

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the concentration on the x-axis against the mean absorbance for each standard on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Rat IFNG 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.

Typical data

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.
Detection range: 37.5 pg/mL - 2400 pg/mL



OTHER INFORMATION

Precautions

• Do not mix or substitute reagents with those from other lots or sources.
• It is important that the calibrator diluent 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 the appropriate calibrator diluent 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 other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.
• Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment.
• Stop Solution contains 2N Sulfuric Acid (H_2SO_4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.
• When mixing or reconstituting protein solutions, always avoid foaming.
• To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
• To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
• When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
• Substrate solution should remain colorless until added to the plate. Keep Substrate solution protected from light. Substrate solution should change from colorless to gradations of blue.
• Stop Solution should be added to the plate in the same order as the Substrate solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate solution.

Troubleshooting

Problem	Cause	Suggested Solution
Poor standard curve	Inaccurate Pipetting.	Check pipettes.
	Improper standard dilution.	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing.
Low Signal	Incubation times too short.	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation.
	Inadequate reagent volumes or improper dilution.	Check pipettes and ensure correct preparation.
	Incubation times with TMB too short.	Ensure sufficient incubation time until blue color develops prior addition of Stop solution.
Large CV	Plate is insufficiently Washed.	Review manual for proper wash technique. If using a plate washer, Large CV check all ports for obstructions.
	Contaminated wash buffer.	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit.	Store your reconstituted standards at -20°C (avoid repeated freeze-thaw cycles), all other assay components 4°C. Keep HRP Substrate (TMB) protected from light.
	Stop solution.	Stop solution should be added to each well before measurement.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37ºC.
High background	Plate is insufficiently washed.	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer.	Make fresh wash buffer.