Rat IFNγ ELISA Kit

ERIFNG   ERIFNG2   ERIFNG5

Number	Description
ERIFNG	**Rat Interferon Gamma (IFNγ) ELISA Kit,** sufficient reagents for 96 determinations
ERIFNG2	**Rat Interferon Gamma (IFNγ) ELISA Kit,** sufficient reagents for 2 × 96 determinations
ERIFNG5	**Rat Interferon Gamma (IFNγ) ELISA Kit,** sufficient reagents for 5 × 96 determinations

Kit Contents	ERIFNG	ERIFNG2	ERIFNG5
Antibody Coated Plate, 96-well plate	1 each	2 each	5 each
Lyophilized Recombinant Rat IFNγ Standard	2 vials	4 vials	10 vials
Sample Diluent	12mL	2 × 13mL	5 × 13mL
Biotinylated Antibody Reagent, contains 0.1% NaN_3	12mL	2 × 13mL	5 × 13mL
30X Wash Buffer	50mL	2 × 50mL	5 × 50mL
Streptavidin-HRP Concentrate	75μL	2 × 75μL	5 × 75μL
Streptavidin-HRP Dilution Buffer	14mL	2 × 14mL	5 × 14mL
TMB Substrate	13mL	2 × 13mL	5 × 13mL
Stop Solution, contains 0.16M sulfuric acid	13mL	2 × 13mL	5 × 13mL
Adhesive plate covers	6 each	12 each	30 each

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Storage: Upon receipt store at 2-8°C.

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Introduction

The Invitrogen™ Rat Interferon Gamma (IFNγ) ELISA is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of rat IFNγ in culture supernatants; EDTA, heparin and sodium citrate plasma; and serum.

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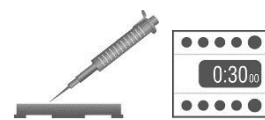
Procedure Summary

1. Add 50μL of Sample Diluent to each well. Add 50μL of standards or samples to each well in duplicate.
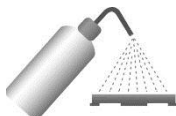
2. Cover plate and incubate at room temperature (20-25°C) for 1 hour.
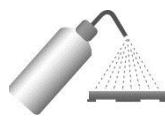
3. Wash plate THREE times.

4. Add 100μL of Biotinylated Antibody Reagent to each well. Cover plate and incubate at room temperature for 30 minutes.
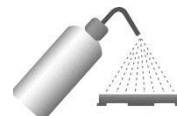
5. Wash plate THREE times.

6. Add 100μL of prepared Streptavidin-HRP Solution to each well.

7. Cover plate and incubate at room temperature for 30 minutes.

8. Wash plate THREE times.

9. Add 100μL of TMB Substrate Solution to each well.

10. Develop the plate in the dark at room temperature for 30 minutes.

11. Stop reaction by adding 100μL of Stop Solution to each well.

12. Measure absorbance on a plate reader at 450nm minus 550nm. Calculate results.

Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-1000μL and plastic pipettes to deliver 5-15mL
- A glass or plastic 2L container to prepare Wash Buffer
- A squirt wash bottle or an automated 96-well plate washer
- 1.5mL polypropylene or polyethylene tubes to prepare standards – do not use polystyrene, polycarbonate or glass tubes
- Disposable reagent reservoirs
- 15mL plastic tube to prepare Streptavidin-HRP Solution
- Microcentrifuge to prepare Streptavidin-HRP Solution
- A standard ELISA reader for measuring absorbance at 450nm and 550nm. If a 550nm filter is not available, the absorbance can be measured at 450nm only. Refer to the instruction manual supplied with the instrument being used.
- Graph paper or a computerized curve-fitting statistical software package

Precautions

- **All samples and reagents must be at room temperature (20-25°C) before use in the assay.**
- Review the instruction booklet carefully and verify all components against the Kit Contents list (page 1) before beginning the assay.
- Do not use a water bath to thaw samples. Thaw at room temperature.
- Sample Diluent must be used when diluting all samples.
- If using a multichannel pipettor, always use a new disposable reagent reservoir.
- To avoid cross-contamination use new disposable pipette tips for each transfer and a new adhesive plate cover for each incubation step.

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- Once reagents have been added to the plate, take care NOT to let plate DRY at any time during the assay.
- Avoid microbial contamination of reagents.
- Vigorous plate washing is essential.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Do not mix reagents from different kit lots. Discard unused kit components.
- Do not use glass pipettes to measure TMB Substrate Solution. Take care not to contaminate the solution. If the solution is blue before use, DO NOT USE IT.
- Individual components may contain antibiotics and preservatives. Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedures.
- Some components of this kit contain sodium azide. Please dispose of reagents according to local regulations.

Additional Precaution for the 2-plate and 5-plate Kits
- Dispense, pool, and equilibrate to room temperature only the reagent volumes required for the number of plates being used. Do not combine leftover reagents with those reserved for additional plates.

Sample Preparation
Sample Handling
- Serum, plasma (EDTA, heparin or sodium citrate) or culture supernatants may be tested in this ELISA.
- 50μL per well of serum, plasma or culture supernatant are required.
- Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -70°C.
- Avoid repeat freeze-thaw cycles when storing samples.
- Test samples and standards must be assayed in duplicate each time the ELISA is performed.
- Gradually equilibrate samples to room temperature before beginning assay. Do not use heated water baths to thaw or warm samples.
- Mix samples by gently inverting tubes.
- If samples are clotted, grossly hemolyzed, lipemic or microbially contaminated, or if there is any question about the integrity of a sample, make a note on the template and interpret results with caution.

Sample Dilution
- If the rat IFNγ concentration possibly exceeds the highest point of the standard curve (i.e., 500pg/mL), prepare one or more five-fold dilutions of the test sample. For example, a five-fold dilution is prepared by adding 50μL of test sample to 200μL of Sample Diluent. Mix thoroughly between dilutions before assaying. Prepare all sample dilutions using the Sample Diluent provided.

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Reagent Preparation

For procedural differences when using partial plates, look for **(PP)** throughout these instructions.

Wash Buffer

1. Label a clean glass or plastic 2L container "Wash Buffer." The 30X Wash Buffer may have a cloudy appearance.

2. Add the entire contents of the 30X Wash Buffer bottle (50mL) to the 2L container and dilute to a final volume of 1.5L with ultrapure water. Mix thoroughly.

   (PP) When using partial plates, store the reconstituted Wash Buffer at 2-8°C.

   Note: Wash Buffer must be at room temperature before use in the assay. Do not use Wash Buffer if it becomes visibly contaminated during storage.

Standards

- **(PP)** Reconstitute and use one vial of the lyophilized standard per partial plate.

- Prepare standards just before use and use within one hour of reconstitution. Do not store reconstituted standards.

1. Reconstitute standard with ultrapure water. Reconstitution volume is stated on the standard vial label. Mix by gently inverting the vial until the contents have completely dissolved. Use the Sample Diluent provided to prepare the standard curve serial dilutions.

2. Label eight tubes, one for each standard curve point: 500, 250, 125, 62, 31, 16, 8 and 0pg/mL, then prepare 1:2 serial dilutions for the standard curve as follows:

3. Pipette 150μL of Sample Diluent into each tube.

4. Pipette 150μL of the reconstituted standard into the first tube (i.e., 500pg/mL) and mix.

5. Pipette 150μL of this dilution into the next tube (i.e., 250pg/mL) and mix.

6. Repeat serial dilutions (using 150μL) five more times to complete the standard curve points. These concentrations, 500, 250, 125, 62, 31, 16, 8, and 0pg/mL, are the standard curve.

Serial dilutions using 150μL

| Reconstituted Standard | 500 pg/ml | 250 pg/ml | 125 pg/ml | 62 pg/ml | 31 pg/ml | 16 pg/ml | 8 pg/ml | 0 pg/ml |
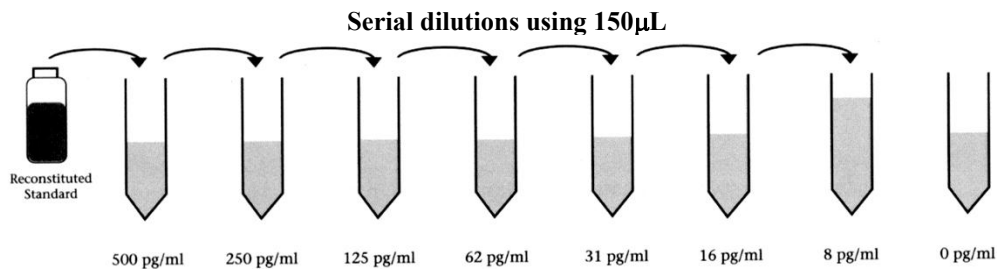
Assay Procedure

A. Sample Incubation

- **(PP)** Determine the number of strips required. Leave these strips in the plate frame. Place remaining unused strips in the provided foil pouch with desiccant. Tightly seal the foil pouch and store at 2-8°C. After completing the assay, retain plate frame for second partial plate. When using the second partial plate, place strips securely in the plate frame.

- Use the Data Template provided to record the locations of zero standard (blank or negative control), rat IFNγ standards and test samples. Perform seven standard points and one blank in duplicate with each series of unknown samples.

- If the rat IFNγ concentration in any sample possibly exceeds the highest point on the standard curve, 500pg/mL, see Sample Preparation – Sample Dilution Section.

1. Add 50μL of Sample Diluent to each well.

2. Add 50μL of reconstituted standards or test samples in duplicate to each well. Mix well by gently tapping the plate several times.

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3. Carefully cover plate with a new adhesive plate cover. Ensure all edges and strips are sealed tightly by running your thumb over edges and down each strip. Incubate for one (1) hour at room temperature, 20-25°C.

4. Carefully remove adhesive plate cover and wash plate THREE times with Wash Buffer as described in the Plate Washing Section (section B).

B. Plate Washing

Note: Automated plate washers may produce sub-optimal results. For best results, perform a manual wash the first time the assay is performed. Automated plate washing may require validation to determine the number of washes necessary to achieve optimal results; typically, 3-5 washes are sufficient.

1. Gently squeeze the long sides of plate frame before washing to ensure all strips securely remain in the frame.

2. Discard plate contents. Use a squirt wash bottle to vigorously fill each well completely with Wash Buffer, then discard plate contents. Repeat procedure two additional times for a total of THREE washes. Blot plate onto paper towels or other absorbent material.

   Note: For automated washing, aspirate all wells and wash with Wash Buffer, overfilling wells. Blot plate onto paper towels or other absorbent material.

C. Biotinylated Antibody Reagent Incubation

- If using a multichannel pipettor, **use a new reagent reservoir and pipette tips** when adding the Biotinylated Antibody Reagent. Remove from the vial only the amount required for the number of strips being used.

1. Add 100μL of Biotinylated Antibody Reagent to each well containing sample or standard.

2. Carefully attach a new adhesive plate cover. Ensure all edges and strips are tightly sealed by running your thumb over edges and down each strip. Incubate plate for 30 minutes at room temperature, 20-25°C.

3. Carefully remove the adhesive plate cover and wash THREE times with Wash Buffer as described in the Plate Washing Section (section B).

D. Streptavidin-HRP Solution Preparation and Incubation

- Prepare Streptavidin-HRP Solution **immediately before use.** Do not prepare more Streptavidin-HRP Solution than required.

- Do not store prepared Streptavidin-HRP Solution.

- Use a 15mL plastic tube to prepare Streptavidin-HRP Solution.

- **Note:** If using a multichannel pipettor, **use new reagent reservoir and pipette tips** when adding the prepared Streptavidin-HRP Solution.

- Briefly centrifuge the Streptavidin-HRP Concentrate to force entire vial contents to the bottom.

1. **(PP)** Use only the Streptavidin-HRP Solution amount required for the number of strips being used. For each strip, mix 2.5μL of Streptavidin-HRP Concentrate with 1mL of Streptavidin-HRP Dilution Buffer. Store Streptavidin-HRP Concentrate reserved for additional strips at 2-8°C.

2. For one complete 96-well plate, add 30μL of Streptavidin-HRP Concentrate to 12mL of Streptavidin-HRP Dilution Buffer and mix gently.

3. Add 100μL of prepared Streptavidin-HRP Solution to each well.

4. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate plate for 30 minutes at room temperature, 20-25°C.

5. Carefully remove the adhesive plate cover and wash THREE times as described in the Plate Washing Section.

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E. Substrate Incubation and Stop Step

- Use new disposable reagent reservoirs when adding TMB Substrate and Stop Solution.

- Dispense from bottle ONLY amount required, 100μL per well, for the number of wells being used. Do not use a glass pipette to measure the TMB Substrate.

- **(PP)** Do not combine leftover substrate with that reserved for the second partial plate. Take care not to contaminate remaining TMB Substrate Solution.

1. Pipette 100μL of TMB Substrate into each well.

2. Allow enzymatic color reaction to develop at room temperature in the dark for 30 minutes. Do not cover plate with aluminum foil or a plate sealer. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.

3. After 30 minutes, stop the reaction by adding 100μL of Stop Solution to each well.

F. Absorbance Measurement

Note: Evaluate the plate within 30 minutes of stopping the reaction.

Measure the absorbance on an ELISA plate reader set at 450nm and 550nm. Subtract 550nm values from 450nm values to correct for optical imperfections in the microplate. If an absorbance at 550nm is not available, measure the absorbance at 450nm only. When the 550nm measurement is omitted, absorbance values will be higher.

G. Calculation of Results

- The standard curve is used to determine rat IFNγ amount in an unknown sample. Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding rat IFNγ concentration (pg/ml) on the horizontal (X) axis.

- Calculate results using graph paper or curve-fitting statistical software. The rat IFNγ amount in each sample is determined by interpolating from the absorbance value (Y axis) to concentration (X axis) using the standard curve.

- If the test sample was diluted, multiply the interpolated value obtained from the standard curve by the dilution factor to calculate pg/ml of rat IFNγ in the sample.

- Absorbance values obtained for duplicates should be within 10% of the mean value. Carefully consider duplicate values that differ from the mean by greater than 10%.

Rat IFNγ (pg/mL)
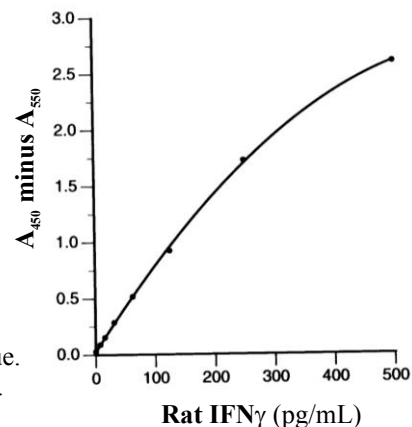
Performance Characteristics

Sensitivity: < 2pg/mL
The sensitivity or Lower Limit of Detection (LLD)[1] is determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD. This value is the smallest dose that is not zero with 95% confidence.

Assay Range: 8-500pg/mL
Suggested standard curve points are 500, 250, 125, 62, 31, 16, 8, and 0pg/mL.

Reproducibility:
Reproducibility of the Rat IFNγ ELISA Kit was evaluated in each sample matrix. To determine intra-assay precision, 20 replicates of samples containing two levels of recombinant rat IFNγ were evaluated on a single plate.

To evaluate inter-assay precision, three operators tested samples by performing at least three separate assays on more than one day. Twelve duplicate sample values were used to calculate inter-assay precision data for each level of rat IFNγ. Data are reported in Table 1.

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Table 1. Intra-assay and inter-assay precision of the Rat IFNγ ELISA Kit.

Sample	Level	Intra-assay Precision			Inter-assay Precision		
		Mean (pg/mL)	SD (pg/mL)	CV (%)	Mean (pg/mL)	SD (pg/mL)	CV (%)
Serum	1	77.9	7.5	9.6	71.0	5.5	7.7
	2	366.4	30.9	8.4	310.1	25.2	8.1
EDTA Plasma	1	98.9	9.3	9.4	98.6	8.2	8.3
	2	271.9	24.2	8.9	250.3	19.4	7.8
Citrate Plasma	1	87.5	6.6	7.5	76.2	5.1	6.7
	2	311.0	29.6	9.5	297.2	31.1	8.1
Heparin Plasma	1	86.3	8.5	9.9	81.9	8.9	10.9
	2	402.8	37.3	9.3	384.1	31.1	8.1
Cell Culture Supernatant	1	72.2	4.2	5.9	63.5	6.4	10.0
	2	366.2	23.2	6.3	289.9	32.3	11.1

Specificity:
This ELISA is specific for the measurement of natural and recombinant rat IFNγ. This ELISA does not cross-react with the following cytokines: rat IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, MIP-1α, RANTES, or TNFα, or human or pig IFNγ. When preparations of NIH/NIBSC mouse IFNγ (lot # Gg02-901-533) were evaluated in the Rat IFNγ ELISA Kit, 6250pg/mL mouse IFNγ was equivalent to 258pg/mL rat IFNγ (4.1% cross-reactivity).

Calibration:
The standard in this ELISA is calibrated to a rat IFNγ reference standard.

Expected Values:
Serum and plasma samples collected from 18 apparently healthy Wistar rats were evaluated in this assay. The levels of rat IFNγ in each sample type are reported in Table 2.

Table 2. Rat IFNγ levels from apparently healthy rats.

Sample Type	Mean (pg/mL)	Median (pg/mL)	Range (pg/mL)
Serum	2.4	1.3	0-17.7
EDTA Plasma	2.9	1.5	0-29.3
Citrate Plasma	0.8	0.7	0-2.2
Heparin Plasma	2.2	2.3	0-6.2

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Spike and Recovery:
Recovery of rat IFNγ is evaluated using the Rat IFNγ ELISA Kit. Individual serum and plasma samples from Wistar rats and Sample Diluent controls were spiked with recombinant rat IFNγ. Endogenous rat IFNγ levels were determined by evaluating non-spiked samples with spiked aliquots of the same samples. Expected values were calculated by adding endogenous rat IFNγ levels to those of spiked diluent controls. Percent (%) recovery was obtained by dividing observed by expected values. Results for representative individual samples and populations are reported in Table 3.

Table 3. Spike and recovery values from representative individual samples and a sample population.

| Sample | Level | Representative Sample | | | Sample Population | | | |
		Expected (pg/mL)	Observed (pg/mL)	Recovery (%)	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)	n
Serum	1	429.8	406.9	94.7	378.1	367.5	97.2	6
	2	44.0	44.6	101.4	36.8	37.4	101.6	6
EDTA Plasma	1	391.6	355.1	90.7	301.9	276.6	91.6	6
	2	24.6	25.7	104.5	34.0	33.4	98.2	6
Citrate Plasma	1	306.5	289.8	94.6	334.0	333.7	99.9	6
	2	27.6	29.6	107.2	41.1	41.7	101.5	6
Heparin Plasma	1	362.4	378.8	104.5	377.6	394.5	104.5	6
	2	43.1	41.4	96.1	43.6	41.5	95.2	6

Linearity of Dilution:
Serum and plasma samples from Wistar rats are spiked with recombinant rat IFNγ, serially diluted in Sample Diluent and evaluated in the Rat IFNγ ELISA Kit. Dilution linearity for plasma is assessed for each anticoagulant. Representative data from EDTA plasma samples are reported in Table 4. Results for heparin and citrate plasma are similar to those for EDTA plasma. Observed values are compared to expected values to calculate percent (%) recovery and demonstrate the assay's dilution linearity.

Table 4. The dilution linearity of the Rat IFNγ ELISA Kit.

Sample	Dilution	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
Serum	Neat	392.9	392.9	-
	1:2	196.5	200.3	101.9
	1:4	98.2	92.6	94.3
	1:8	49.1	52.3	106.5
	1:16	24.6	26.4	107.3
EDTA Plasma	Neat	321.8	321.8	-
	1:2	160.9	160.1	104.8
	1:4	80.5	84.4	104.8
	1:8	40.2	41.6	103.5
	1:16	20.1	24.0	119.4
Cell Culture Supernatant	Neat	468.4	468.4	-
	1:2	234.2	227.4	97.1
	1:4	117.1	119.0	101.6
	1:8	58.6	59.6	101.8

Cited Reference

1. *Immunoassay: A Practical Guide,* Chan and Perlstein, Eds., 1987, Academic Press: New York, p71.

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Limited product warranty

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code	⅃	Temperature limitation	⧗	Use by	▰	Manufacturer	🛈	Consult instructions for use	⚠	Caution, consult accompanying documents

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Data Templates

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
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