Mouse IFN-γ (improved) ELISA Kit

Catalog Number KMC4021 (96 tests), KMC4022 (2 x 96 tests), KMC4021C (5 x 96 tests)

Pub. No. MAN0004158 **Rev.** 5.0 (32)

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Mouse IFN-γ (improved) ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of mouse IFN-γ in mouse serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant mouse IFN-γ .

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KMC4021 (96 tests)
Antibody Coated Wells. 96-well plate.	1 plate
Mouse IFN-g Biotin Conjugate. Contains 0.1% sodium azide.	11 mL
Mouse IFN-g Standard. Contains 0.1% sodium azide.	2 vials
Wash Buffer Concentrate (25X).	100 mL
Standard Diluent Buffer. Contains 0.1% sodium azide.	25 mL
Streptavidin-HRP (100X).	0.125 mL
Streptavidin-HRP Diluent. Contains 3.3 mM thymol.	25 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB).	25 mL
Stop Solution.	25 mL
Adhesive Plate Covers.	4

Materials required but not supplied

- Distilled or deionized water
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Pre-dilute samples

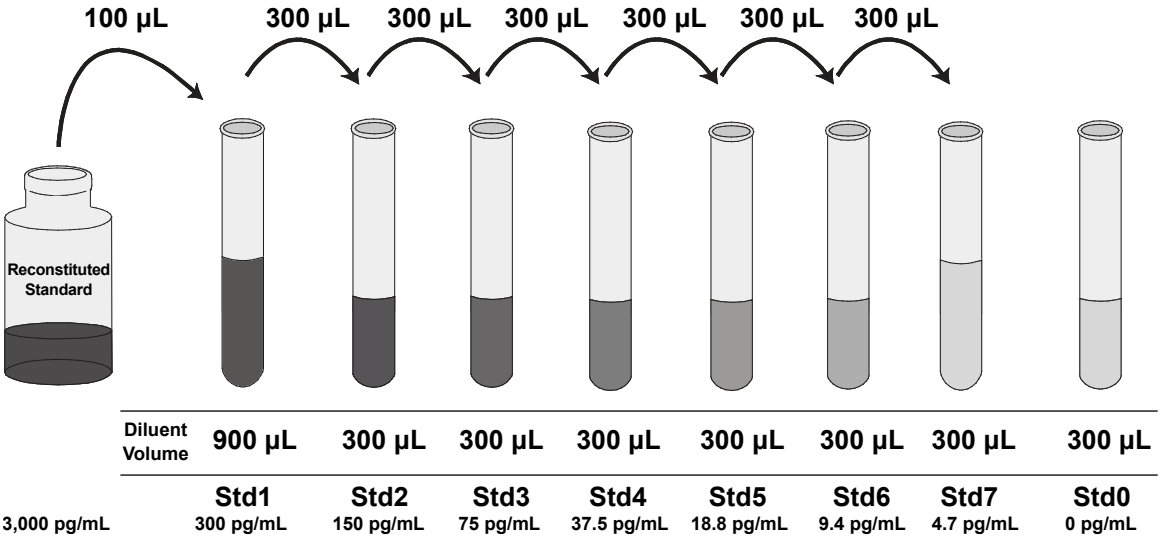
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Perform sample dilutions with Standard Diluent Buffer.
- Dilute serum, plasma, and cell culture samples 4-fold in Standard Diluent Buffer. Alternatively, samples may be diluted directly in the microtiter well by adding 75 µL of Standard Diluent Buffer to each well, followed by 25 µL of serum, plasma or cell culture sample.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Ms IFN-γ Standard to 3,000 pg/mL with Standard Dilution Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 3,000 pg/mL mouse IFN-γ . **Use the standard within 15 minutes of reconstitution.**
2. Add 100 µL Reconstituted Standard to one tube containing 900 µL Standard Diluent Buffer and mix. Label as 300 pg/mL mouse IFN-γ .
3. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 150, 75, 37.5, 18.8, 9.4, 4.7, and 0 pg/mL mouse IFN-γ .
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

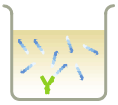
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen



a. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

a. Add 100 µL Ms IFN-γ Biotin Conjugate solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 1 hour at room temperature .
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 Add Streptavidin-HRP

a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

4 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution

Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

The following data were obtained for the various standards over the range of 0 to 300 pg/mL mouse IFN-γ .

Standard Mouse IFN-γ (pg/mL)	Optical Density (450 nm)
300	3.41
150	2.26
75	1.23
37.5	0.60
18.8	0.31
9.4	0.17
4.7	0.09
0	0.02

Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	149.3	35.1	10.0
Standard Deviation	5.7	2.3	0.9
% Coefficient of Variation	3.8	6.7	9.0

Intra-assay precision

Samples of known mouse IFN-γ concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	145.0	37.0	10.9
Standard Deviation	5.1	1.2	0.3
% Coefficient of Variation	3.5	3.1	3.1

Cross-reactivity

The cross-reactivity with recombinant **Rat** IFN-γ was determined to be 1.7%.

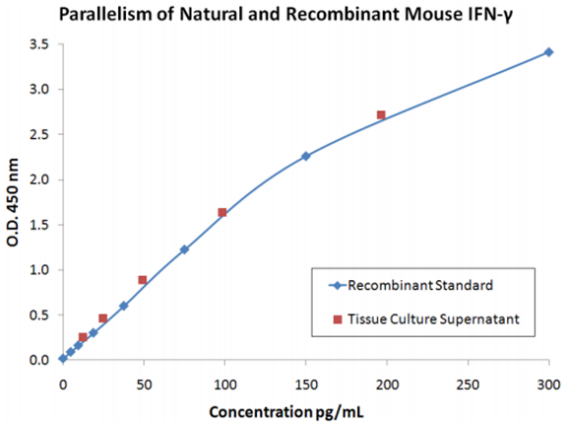
Linearity of dilution

Mouse serum and cell culture medium containing 10% fetal bovine serum were spiked with mouse IFN-γ and serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

Dilution	Cell Culture			Serum		
	Measured (pg/mL)	Expected		Measured (pg/mL)	Expected	
		(pg/mL)	%		(pg/mL)	%
1/4	329.5	392.8	84	49.9	61.0	82
1/8	197.7	196.4	101	29.0	30.5	95
1/16	101.6	98.2	103	15.8	15.3	104
1/32	53.4	49.1	109	9.1	7.6	119
1/64	16.3	24.5	118	3.8	3.8	100

Parallelism

Natural mouse IFN-γ from mouse splenocytes stimulated with 10 µg/mL PHA was serially diluted in Standard Diluent Buffer. The optical density of the expected value of each dilution was plotted against the mouse IFN-γ standard curve. The standard accurately reflects mouse IFN-γ content in samples.



Recovery

The recovery of mouse IFN-γ added to mouse serum averaged 88.9%. The recovery of mouse IFN-γ added heparin, and citrate mouse plasma averaged 106.0% and 96.6%, respectively. The recovery of mouse IFN-γ added to cell culture medium containing 10% fetal bovine serum averaged 100.3%.

Sensitivity

The analytical sensitivity of this assay is <2 pg/mL mouse IFN-γ . This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times, and calculating the corresponding concentration.

Specificity

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Mouse IFN-γ (improved) ELISA Kit. The following substances were tested and found to have no cross-reactivity: **Human** IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IFN-α , IFN-γ , TNF-α , GM-CSF, MIP-1 α , MIP-1 β , IP-10, MIG, Eotaxin, RANTES, and MCP-1; **rat** IL-1 β , IL-2, IL-4, IL-10, IL-13, MCP-1, and TNF-α ; **mouse** IL-1 β , IL-4, IL-5, IL-10, IL-17, GM-CSF, KC, TNF-α , and VEGF.

Limited product warranty

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