Human IFN-γ ELISA Kit

Catalog Number KAC1231 (96 tests)

Pub. No. MAN0007823 **Rev.** 2.0

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™ Human IFN-γ ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human IFN-γ in serum, plasma, cell culture supernatant, and other biological fluids. The assay recognizes both natural and recombinant human IFN-γ.

IFN-γ (type II interferon) is IFN-γ is a lymphokine produced by activated T (and NK) cells. The IFN-γ gene encodes a 146 amino acid protein that is post-translationally processed into 20 kDa and 25 kDa species that differ by glycosylation. Native IFN-γ is pH2- labile, highly basic, and can aggregate to form dimers that are biologically active. IFN-γ plays a role in regulating cell growth, innate immunity, and adaptive immunity. IFN-γ is a principal macrophage activating factor (MAF), and it also regulates the pathway of differentiation of myeloid cells.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C. Store the Wash Solution Concentrate at room temperature. When stored as indicated, all reagents are stable until the expiration date.

Contents	Cat. No. KAC1231 (96 tests)	Color code
Zero Calibrator; in human serum, with benzamidine and thymol; lyophilized (see vial label for quantity and reconstitution volume)	2 vials	Black
Calibrators 1 to 5; in human serum, with benzamidine and thymol; lyophilized (see vial label for quantity and reconstitution volume)	5 vials	Yellow
Controls 1 and 2; in human serum, with benzamidine and thymol; lyophilized. Refer to vial label for reconstitution volume and range	2 vials	Silver
IFN-γ Antibody-Coated Wells, 96-well strip-well plate	1 plate	Blue
Anti-IFN-γ-HRP Conjugate; in Tris-Maleate buffer with bovine serum albumin and thymol	6 mL	Red
Wash Solution Concentrate (200X)	10 mL	Brown
Chromogenic TMB (tetramethylbenzidine) in DMF	12 mL	Brown
Stop Solution (1 N HCl)	12 mL	White

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at 450 nm, 490 nm, and 650 nm (polychromatic reading)
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Horizontal microplate shaker capable of 700 rpm ± 100 rpm
- Magnetic stirrer

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 2 mL of Wash Solution Concentrate (200X) with 398 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Use a magnetic stirrer to mix the solution.

   Note: Use 1X Wash Buffer on the same day it is prepared. Discard unused 1X Wash Buffer at the end of the day.

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.

Reconstitute Calibrators

Note: Calibrators are stable for 4 days at 2–8°C. For longer term storage, make aliquots and store at –20°C for up to 2 months. Avoid successive freeze thaw cycles.

1. Reconstitute the Zero Calibrator with distilled water. See the vial label for the exact volume.

   Note: Use the Zero Calibrator for sample dilutions.
2. Reconstitute Calibrators 1 to 5 by adding 0.5 mL of distilled water to each vial.

   Note: The calibrators are used to create a standard curve. 1 IU of calibrator is equivalent to 1 IU NIBSC 87/586. See the exact values of each calibrator on vial labels.

Reconstitute controls

Note: Controls are stable for 4 days at 2–8°C. For longer term storage, make aliquots and store at –20°C for up to 2 months. Avoid successive freeze thaw cycles.

Reconstitute Controls 1 and 2 by adding 0.5 mL of distilled water to each vial.

If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy can be determined.

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.

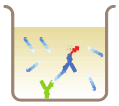
- Dilute the sample with reconstituted Zero Calibrator as needed.
- If samples generate values higher than the highest standard, dilute samples further and repeat the assay.

Perform ELISA (Total assay time: 2.5 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 50 µL of calibrators, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Add 50 µL of anti-IFN-γ HRP Conjugate solution into each well except the chromogen blanks.
c. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate 2 hours at room temperature on a horizontal shaker set at 700 rpm ± 10 rpm.
d. Thoroughly aspirate the solution and wash wells 3 times with 1X Wash Buffer.

2 Add Chromogenic TMB

a. Add 100 µL of Chromogenic TMB to each well. The substrate solution begins to turn blue.
b. Incubate for 15 minutes at room temperature on a horizontal shaker set at 700 rpm ± 10 rpm in the dark.

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

3 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 3 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 obtained for calibrators 0 to 5 are for illustration only and should never be used in place of a real time standard curve.

Calibrator	Concentration (pg/mL)	Optical Density (450 nm)
5	30	3.11
4	10	1.35
3	5	0.70
2	2	0.34
1	1	0.17
0	0	0.03

Inter-assay precision

Samples were assayed 20 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2
Mean (IU/mL)	1.61	5.72
Standard Deviation	0.03	0.51
% Coefficient of Variation	5.8	8.8

Intra-assay precision

Samples of human IFN-γ were assayed in replicates of 10 to determine precision within an assay.

Parameters	Sample 1	Sample 2
Mean (IU/mL)	1.26	12.28
Standard Deviation	0.03	0.35
% Coefficient of Variation	3.2	3.8

Expected values

These values are given only for guidance and it is recommended that each laboratory establishes its own normal values.

Sample	Range (IU/mL)	Average (IU/mL)	Standard deviation
Serum (n=30)	0–0.77	0.28	0.15
Plasma (n=60)	0–0.89	0.08	0.12

High-dose hook effect

A sample spiked with human IFN-γ up to 500,000 IU/mL gives a response higher than that obtained for the last standard point.

Recovery

Sample	Added IFN-γ (IU/mL)	Recovery IFN-γ (IU/mL)	Recovery %
Serum	20.46	20.3	99
	9.85	10.12	103
	4.73	4.76	101
	2.41	2.25	94
	1.04	1.03	99
Plasma	20.46	20.33	99
	9.85	9.53	97
	4.73	4.81	102
	2.41	2.43	101
	1.04	1	96
Cell culture medium (1:4 dilution)	20.46	20.14	98
	9.85	10.37	105
	4.73	4.88	103
	2.41	2.58	107
	1.04	1.07	103

Sensitivity

The minimum detectable dose of human IFN-γ is 0.03 IU/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Specificity

No significant cross-reaction was observed in presence of 50 ng of IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, TNF-α, TNF-β, IFN-α, IFN-β, TGF-β, GM-CSF, OSM, MIP-1α, MIP-1β, LIF, MCP-1, G-CSF, and RANTES. This kit is specific for human natural and recombinant human IFN-γ.

Linearity of dilution

Dilution	Serum		Plasma		Cell culture medium (1:4 dilution)	
	Measured conc. (IU/mL)	Theor. conc. (IU/mL)	Measured conc. (IU/mL)	Theor. conc. (IU/mL)	Measured conc. (IU/mL)	Theor. conc. (IU/mL)
1/1	18	18	11.72	11.72	—	—
1/2	8.98	9	5.89	5.86	—	—
1/4	4.41	4.5	3.17	2.93	23.32	23.32
1/8	2.39	2.25	1.59	1.47	12.13	11.66
1/16	1.17	1.13	0.79	0.73	5.67	5.83
1/32	0.6	0.56	0.41	0.37	3.15	2.92
1/64	—	—	—	—	1.58	1.46
1/128	—	—	—	—	0.75	0.73

Time delay between dispensing last calibrator and last sample

IMPORTANT! To avoid drift, the time between pipetting of the first calibrator and the last sample must be limited to the time mentioned in this section.

Assay results remain accurate even when a sample is dispensed 30 minutes after the calibrators have been added to the coated wells.

Sample	Time delay			
	0 minutes	10 minutes	20 minutes	30 minutes
S1	2.7	2.7	2.7	2.7
S2	6.8	6.8	6.5	6.7
S3	4.2	4.1	4.0	4.0
N1	22.7	21.2	19.4	22.0
N2	24.9	23.7	21.7	20.1
N3	14.7	14.8	13.1	12.6
N4	17.9	15.3	13.8	13.9
N5	17.4	16.5	15.6	15.0

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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

Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.