Human IFN-γ ELISA Kit

Catalog Number KHC4021 (96 tests), KHC4022 (2 × 96 tests), KHC4021C (5 × 96 tests)

Pub. No. MAN0003973 **Rev.** 4.0 (31)

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 human serum, buffered solution, or cell culture medium. The assay recognizes both natural and recombinant human IFN-γ.

IFN-γ (type 2, immune IFN) is structurally and functionally distinct from type 1 (alpha/beta) interferons. The IFN-γ gene codes for a 146 amino acid protein that is post-translationally processed into two glycosylated species of 20 and 25 kDa. Native IFN-γ is highly basic, and can aggregate to form dimers that are biologically active. IFN-γ is produced by activated T (and NK) cells.

Contents and storage

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

Contents	Cat. No. KHC4021 (96 tests)
Hu IFN-γ Standard, lyophilized; contains 0.1% sodium azide	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide	25 mL
Antibody Coated Wells; 96-well plate	1 plate
Incubation Buffer; contains 8 mM sodium azide	12 mL
Hu IFN-γ Biotin Conjugate (Biotin-labeled anti-IFN-γ); contains 0.1% sodium azide	6 mL
Streptavidin-HRP (100X)	0.15 mL
Streptavidin-HRP Diluent; contains 3.3 mM thymol	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

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.

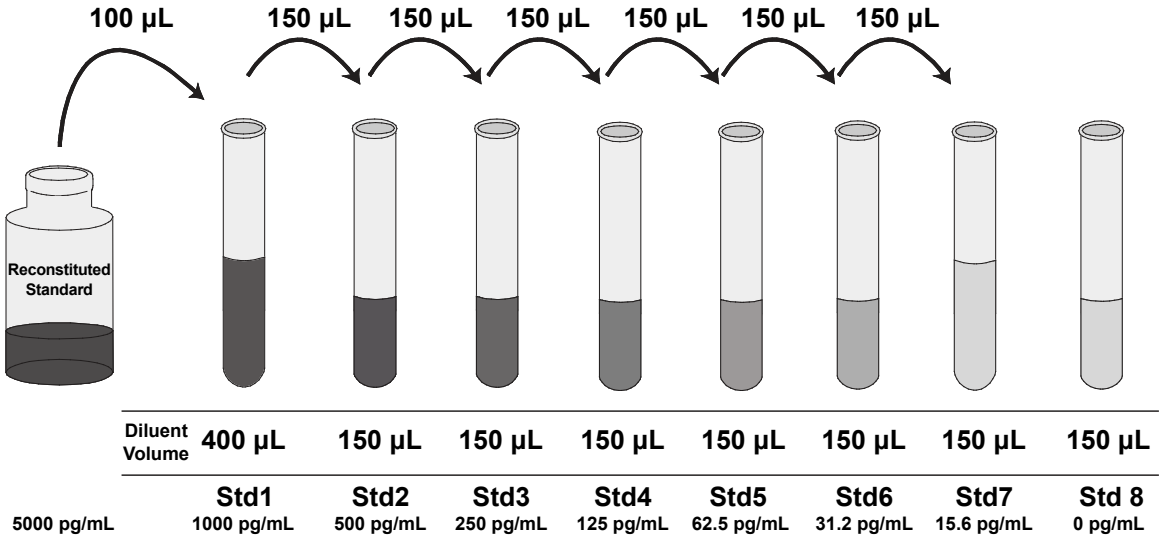
- Perform sample dilutions with Standard Diluent Buffer.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: This assay has been calibrated against the WHO reference preparation (87/586) for Hu IFN-γ (NIBSC, Hertfordshire, UK, EN6 3QG). One microgram equals 20,000 International Units.

1. Reconstitute human IFN-γ Standard to 5,000 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 5,000 pg/mL human IFN-γ. **Use the standard within 1 hour of reconstitution.**
2. Add 100 µL reconstituted standard to one tube containing 400 µL Standard Diluent Buffer and mix. Label as 1,000 pg/mL human IFN-γ.
3. Add 150 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 500, 250, 125, 62.5, 31.2, 15.6, and 0 pg/mL human 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.

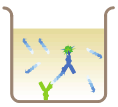
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, then dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 2 hours and 45 minutes)

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 the Incubation Buffer to all wells except the chromogen blanks.
b. Add 50 µL of standards, controls, or samples to the appropriate wells. Leave the wells for chromogen blanks empty.
c. Add 50 µL Hu IFN-γ Biotin Conjugate solution into each well except the chromogen blanks.
d. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate for 1 hour and 30 minutes at room temperature.
e. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Streptavidin-HRP

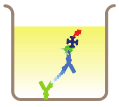
a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 45 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

3 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.

4 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 1,000 pg/mL human IFN-γ.

Standard Human IFN-γ (pg/mL)	Optical Density (450 nm)
1,000	2.94
500	1.57
250	0.82
125	0.43
62.5	0.24
31.2	0.17
15.6	0.12
0	0.08

High-dose hook effect

A sample spiked with human IFN-γ up to 8 ng/mL gives a response higher than that obtained for the last standard point.

Inter-assay precision

Samples were assayed 30 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	190.1	398.2	862.9
Standard Deviation	11.5	23.9	52.8
% Coefficient of Variation	6.0	6.0	6.1

Intra-assay precision

Samples of known human 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)	203.4	381.0	898.3
Standard Deviation	10.5	21.0	52.8
% Coefficient of Variation	5.2	5.5	5.9

Expected values

Ten serum and ten plasma (EDTA) samples from apparently normal individuals were evaluated using the Human IFN-γ ELISA Kit.

Sample	Range (pg/mL)	Average (pg/mL)
Serum	0–5.6	0.6
Plasma	0–15	2.5

Human PBMCs were cultured under stimulation with PMI and ionophore, LPS, or PHA. Cell culture supernatant was collected at 24, or 72 hours and assayed for released IFN-γ using the Human IFN-γ ELISA Kit.

Culture conditions	Concentration
Unstimulated, 24 hours	8 pg/mL
PMA (50 ng/mL) + ionophore (250 ng/mL), 24 hours	110,000 pg/mL
Unstimulated, 72 hours	22 pg/mL
PMA (50 ng/mL) + ionophore (250 ng/mL), 72 hours	190,000 pg/mL
LPS (25 µg/mL), 24 hours	190 pg/mL
PHA (10 µg/mL), 24 hours	490 pg/mL

Recovery

The recovery of human IFN-γ added to pooled human serum, and cell culture medium containing fetal bovine serum (FBS) was measured with the Human IFN-γ ELISA Kit.

Sample	Average % Recovery	Range %
Pooled human serum	98	89 – 106
Cell culture medium (containing 1% FBS)	105	—
Cell culture medium (containing 10% FBS)	111	—

Linearity of dilution

Human serum and cell culture medium containing 10% fetal bovine serum were spiked with human 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	Serum			Cell Culture		
	Measured (pg/mL)	Expected (pg/mL)	%	Measured (pg/mL)	Expected (pg/mL)	%
Neat	708	—	—	1,045	—	—
1/2	328	354	93	509	523	97
1/4	178	177	101	240	261	92
1/8	91	89	102	115	131	88
1/16	44	44	100	57	65	88
1/32	20	22	91	26	33	79

Sensitivity

The analytical sensitivity of the assay is <4 pg/mL human IFN-γ. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 35 times.

Specificity

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Hu IFN-γ Kit. The following substances were tested and found to have no cross-reactivity: **human** IL-4, IL-6, IL-10, TNF-α, SCF; **mouse** IL-2, IL-4, IL-6, IL-10, IFN-γ; **rat** IFN-γ.

Cross-reactivity

Significant cross-reactivity was not observed to any recombinant antigen tested except for rhesus monkey IFN-γ, which had 100% cross-reactivity in this assay.

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

Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
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.