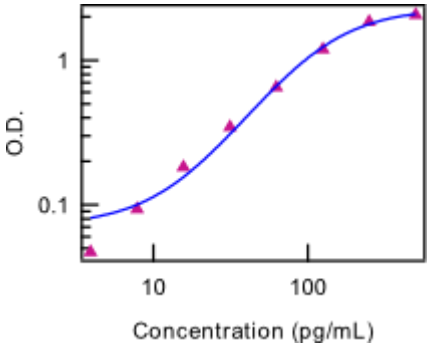
Human IFN gamma ELISA Ready-SET-Go!®

Catalog Number: 88-7316
Also known as: Interferon-gamma, IFN-g, IFNg
RUO: For Research Use Only. Not for use in diagnostic procedures.



Standard curve of Human IFN gamma ELISA Ready-SET-Go!®

Product Information

Contents: Human IFN gamma ELISA Ready-SET-Go!®
REF **Catalog Number:** 88-7316
Sensitivity: 4 pg/ml
Standard Curve Range: 4 - 500 pg/ml

Temperature Limitation: Store at 2-8°C except standard which should be stored at less than or equal to -70°C.
LOT **Batch Code:** Refer to vial
Use By: Refer to vial
Caution, contains Azide

Description

This Human IFN-γ ELISA Ready-SET-Go! reagent set (with or without high-affinity binding microwell plates) contains the necessary reagents, buffers and diluents for performing quantitative enzyme linked immunosorbent assays (ELISA). This ELISA reagent set is specifically engineered for accurate and precise measurement of human IFN-γ protein levels from samples including serum, plasma, and supernatants from cell cultures.

Special Note: To ensure optimal results from the Human IFNg ELISA Ready-SET-Go! Set, please only use the components included in the set. Exchanging of components is not recommended as a change in signal may occur.

Components

Capture Antibody. Pre-titrated, purified antibody
Detection Antibody. Pre-titrated, biotin-conjugated antibody
Standard. Recombinant cytokine for generating standard curve and calibrating samples
ELISA/ELISPOT Coating Buffer Powder. This Ready-Set-Go! ELISA Set may contain ELISA/ELISPOT Coating Buffer Powder (Reconstitute to 1L with dH20 and filter (0.22 uM)) or 10X PBS ELISA Coating Buffer (Dilute 1 part 10X Buffer into 9 parts dH20).
Assay Diluent. 5X concentrated
Detection enzyme. Pre-titrated Avidin-HRP
Substrate Solution. Tetramethylbenzidine (TMB) Substrate Solution
Certificate of Analysis. Lot-specific instructions for dilution of antibodies and standards
96 Well Plate. Corning Costar 9018 (included with product Cat. #'s ending in suffixes -22, -44, -76, -86)

References

Gupta AK, Holzgreve W, and Hahn W. 2005. Microparticle-free placentally derived soluble factors downmodulate the response of activated T cells. Hum Immunol. 66(9):977-84. (RSG ELISA kit, **TC supernatant**, PubMed)

Tao X, Li C, et al. 2007. Simultaneous detection of IFN-gamma and IL-4 in lesional tissues and whole unstimulated saliva from patients with oral lichen planus. J Oral Pathol Med. (RSG ELISA kit, Epub)

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Horita K, Yoshitomi H, et al. 2007. Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model. J Exp Med 204(12):2803-12. (RSG ELISA kit, **synovial fluid**, PubMed)

Gupta AK, Rusterholz C, et al. 2005. Constant IFNgamma mRNA to protein ratios in cord and adult blood T cells suggests regulation of IFNgamma expression in cord blood T cells occurs at the transcriptional level. Clin Exp Immunol 140(2):282-8. (RSG ELISA kit, **TC supernatant and protein lysate**, PubMed)

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full spectrum cell analysis
eBioscience™

TDS Protocol

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Other Materials Needed

- Buffers
  - Wash Buffer: 1 x PBS, 0.05% Tween-20 (or eBioscience ELISA Wash Buffer Powder, cat 00-0400)
  - Stop Solution: 1M H_3PO_4 or 2N H_2SO_4
- Pipettes and pipettors
- Refrigerator
- 96-well plate (Corning Costar 9018)

  NOTE: The use of ELISA plates which are not high affinity protein binding plates will result in suboptimal performance, e.g., low signal or inconsistent data. Do not use tissue culture plates or low protein absorption plates. Use only the Corning Costar 9018 or NUNC Maxisorp 96 well plates provided or suggested.
- 96-well ELISA plate reader (microplate spectrophotometer)
- ELISA plate washer

NOTE: To ensure optimal performance from this ELISA Ready-SET-Go! set, please only use the components included in the set. Exchanging of components is not recommended as a change in signal may occur.

Stability

This ELISA set is guaranteed to perform as specified at least 6 months from date of receipt if stored and handled as instructed according to this datasheet and the Certificate of Analysis, which is included with the reagents.

Storage Instructions for Cytokine Standards

The frozen cytokine standard is already aliquoted at 20 µl per vial. Upon receipt, frozen cytokine standard should be immediately stored at -80°C; stable for at least 6 months. After thawing, quick-spin vial prior to opening. Do not re-aliquot into smaller fractions. These are single use vials. Use one time and discard. For dilution of the standard, please see instructions on the Certificate of Analysis and follow these as written.

Storage Instructions for Other Set Reagents

Store at 4°C.

Time Requirements

- 1 overnight incubation
- 4½-hour incubations
- 1 hour washing and analyzing samples

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TDS Protocol

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Experimental Procedure

1. Coat Corning Costar 9018 ELISA plate with 100 µl/well of capture antibody in Coating Buffer (dilute as noted on Certificate of Analysis, which is included with the reagent set). Seal the plate and incubate overnight at 4°C.
2. Aspirate wells and wash 5 times with >250 µl/well Wash Buffer*. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes. Blot plate on absorbent paper to remove any residual buffer.
3. Dilute 1 part 5X concentrated Assay Diluent with 4 parts DI water.* Block wells with 200 µl/well of 1X Assay Diluent. Incubate at room temperature for 1 hour.
4. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
5. Using 1X Assay Diluent*, dilute standards as noted on the Certificate of Analysis (C of A). Add 100 µl/well of standard to the appropriate wells. Perform 2-fold serial dilutions of the top standards to make the standard curve. Add 100 µl/well of your samples to the appropriate wells. Cover or seal the plate and incubate at room temperature for 2 hours (or overnight at 4°C for maximal sensitivity).
6. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
7. Add 100 µl/well of detection antibody diluted in 1X Assay Diluent* (dilute as noted on C of A). Seal the plate and incubate at room temperature for 1 hour.
8. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
9. Add 100 µl/well of Avidin-HRP* diluted in 1X Assay Diluent (dilute as noted on C of A). Seal the plate and incubate at room temperature for 30 minutes.
10. Aspirate and wash as in step 2. In this wash step, soak wells in Wash Buffer* for 1 to 2 minutes prior to aspiration. Repeat for a total of 7 washes.
11. Add 100 µl/well of Substrate Solution to each well. Incubate plate at room temperature for 15 minutes.
12. Add 50 µl of Stop Solution to each well.
13. Read plate at 450 nm. If wavelength subtraction is available, subtract the values of 570 nm from those of 450 nm and analyze data.

***NOTE: Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.**

Ready-SET-Go Cytokine ELISA Set Buffers:

- Assay Diluent (5X concentrate): Dilute 1/5 in DI water.
- Substrate Solution: Ready to use (1X); 100 µl per well.
- ELISA/ELISPOT Coating Buffer Powder: Reconstitute in 1L dH20; filter (0.22 uM).

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TDS Protocol

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Standard Calibration

The standard of the Ready-SET-Go! is calibrated against NIBSC standards:

Table of Standard Calibration				
Cytokine	ng of eB standard	ng of NIBSC standard	U of NIBSC standard	NIBSC Lot #
hIL-2	1	1.1	14.6	86/564
hIL-4	1	2.2	22	88/656
hIL-5	1	2.2	22	90/586
hIL-6	1	1.7	170	89/548
hIL-10	1	0.8	4	93/722
hIL-12	1	0.8	8	95/544
hIFN-g	1	1.1	22	87/586
hTNF-a	1	0.9	36	87/650
mIL-2	1	3.1	310	93/566
mIL-4	1	3	30	91/656
mIL-6	1	8.5	850	93/730
mIFN-g*	1		4.5	Gg02-901-533
mTNF-a	1	1.7	340	88/532
* Mouse IFN-g is calibrated using NIH standard (Lot Gg02-901-533) and is measured in Units (U)				

ELISA Troubleshooting Guide		
Problem	Possibility	Solution
A. High Background	1. Improper and inefficient washing	1. Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as directed
	2. Cross contamination from other specimens or positive control	2. Repeat ELISA, be careful when washing and pipetting
	3. Contaminated substrate	3. Substrate should be colorless
	4. Incorrect dilutions, e.g., conjugate concentration was too high	4. Repeat test using correct dilutions; check with the recommendations of the antibody manufacturer
B. No signal	1. Improper, low protein binding capacity plates were used	1. Repeat ELISA, using recommended high binding capacity plates
	2. Wrong substrate was used	2. Repeat ELISA, use the correct substrate
	3. Enzyme inhibitor present in buffers; e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity	3. Repeat ELISA, make sure your system contains no enzyme inhibitor

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C. Very weak signal	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly
	2. Incorrect dilutions of standard	2. Follow recommendations of standard handling exactly as written on the certificate of analysis
	3. Insufficient incubation time	3. Repeat ELISA, follow the protocol carefully for each step□s incubation time
	4. Incorrect storage of reagents	4. Store reagents in the correct temperature, avoid freeze and thaw, avoid using the □frost free□ freezer
	5. Wrong filter in ELISA reader was used	5. Use the correct wavelength setting
	6. Wrong plate used	6. Use the recommended Corning Costar 9018 or NUNC Maxisorp flat bottom 96 well plates
D. Variation amongst replicates	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly; see certificate of analysis
	2. Poor mixing of samples	2. Mix samples and reagents gently and equilibrate to proper temperature
	3. Plates not clean	3. Plates should be wiped on bottom before measuring absorbance
	4. Improper, low binding capacity plates were used	4. Use recommended high binding capacity plates
	5. Reagents have expired	5. Do not use if past expiration date

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