abcam

Product datasheet

Human IFN gamma ELISA Kit ab174443

SimpleStep ELISA

14 References 12 Images

Overview

Product name Human IFN gamma ELISA Kit

Detection methodColorimetric

Precision Intra-assay

Sample	n	Mean	SD	CV%
Overall	5			1.1%

Inter-assay

Sample	n	Mean	SD	CV%	
Overall	3			7.9%	

Sample type Cell culture supernatant, Serum, Plasma

Assay type Sandwich (quantitative)

Sensitivity 470 pg/ml

Range 0.468 ng/ml - 30 ng/ml

RecoverySample specific recovery

Sample type	Average %	Range
Serum	102	98% - 104%
Cell culture media	86	77% - 93%
Hep Plasma	244	197% - 298%
EDTA Plasma	122	117% - 124%
Cit plasma	107	96% - 115%

Assay time 1h 30m

Assay duration One step assay

1

Species reactivity

Product overview

Reacts with: Human

Human IFN gamma ELISA kit (ab174443) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of IFN-gamma protein in human cell culture supernatant, plasma and serum samples. It uses our proprietary SimpleStep ELISA® technology.

Quantitate human IFN gamma with 470 pg/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- -Single-wash protocol reduces assay time to 90 minutes or less
- -High sensitivity, specificity and reproducibility from superior antibodies
- -Fully validated in biological samples
- -96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (<u>ab203359</u>) is available to use as an alternative to the 96-well microplate provided with SimpeStep ELISA® kits.

ASSAY SPECIFICITY

This kit recognizes both native and recombinant human Interferon-gamma protein in serum, plasma, and cell culture supernatant samples only.

Urine, milk, and cell and tissue extract samples have not been tested with this kit.

ASSAY INTERFERENCE

This kit is incompatible with plasma (heparin) samples.

Notes

IFN gamma (IFNG) is produced by lymphocytes activated by specific antigens or mitogens. IFN gamma, in addition to having antiviral activity, has important immunoregulatory functions. It is a potent activator of macrophages, it has antiproliferative effects on transformed cells and it can potentiate the antiviral and antitumor effects of the type I interferons.

Platform

Microplate

Properties

Storage instructions

Store at +4°C. Please refer to protocols.

Components	1 x 384 tests	1 x 96 tests	1 x 96 tests
10X Human IFNG Capture Antibody	1 x 600µl	1 x 600µl	1 x 600µl
10X Human IFNG Detector Antibody	1 x 600µl	1 x 600µl	1 x 600µl

Components	1 x 384 tests	1 x 96 tests	1 x 96 tests
10X Wash Buffer PT (ab206977)	1 x 20ml	1 x 20ml	1 x 20ml
384 well CaptSure™ microplates	1 unit	0 x 0 unit	0 x 0 unit
Antibody Diluent CPI2	1 x 6ml	1 x 6ml	0 x 0ml
Human IFNG Lyophilized Recombinant Protein	2 vials	2 vials	2 vials
Plate Seals	1 unit	1 unit	1 unit
Sample Diluent NBP	1 x 20ml	1 x 20ml	1 x 20ml
Sample Diluent NS (ab193972)	1 x 100ml	1 x 12ml	1 x 12ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	0 x 0 unit	1 unit	1 unit
Stop Solution	2 x 12ml	1 x 12ml	1 x 12ml
TMB Development Solution	2 x 12ml	1 x 12ml	1 x 12ml

Function Produced by lymphocytes activated by specific antigens or mitogens. IFN-gamma, in addition to

having antiviral activity, has important immunoregulatory functions. It is a potent activator of

 $macrophages, it has \ antiproliferative \ effects \ on \ transformed \ cells \ and \ it \ can \ potentiate \ the \ antiviral$

and antitumor effects of the type I interferons.

Tissue specificity Released primarily from activated T lymphocytes.

Involvement in disease In Caucasians, genetic variation in IFNG is associated with the risk of aplastic anemia (AA)

[MIM:609135]. AA is a rare disease in which the reduction of the circulating blood cells results from damage to the stem cell pool in bone marrow. In most patients, the stem cell lesion is caused by an autoimmune attack. T-lymphocytes, activated by an endogenous or exogenous, and most often unknown antigenic stimulus, secrete cytokines, including IFN-gamma, which would in turn be

able to suppress hematopoiesis.

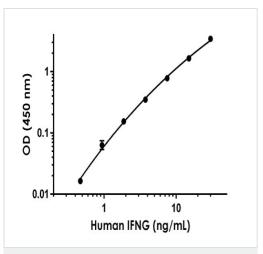
Sequence similarities Belongs to the type II (or gamma) interferon family.

Post-translational Proteolytic processing produces C-terminal heterogeneity, with proteins ending alternatively at

modifications Gly-150, Met-157 or Gly-161.

Cellular localization Secreted.

Images



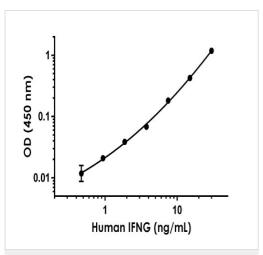
Example IFNG standard curve for cell culture supernatant samples measurements.

Background-subtracted data values (mean +/- SD) are graphed.

Standard Curve Measurements					
Concentration	O.D 4	Mean			
(ng/mL)	1	2	O.D		
0	0.146	0.125	0.136		
0.468	0.151	0.153	0.152		
0.937	0.206	0.192	0.199		
1.875	0.289	0.292	0.290		
3.75	0.499	0.471	0.485		
7.50	0.911	0.918	0.914		
15	1.791	1.785	1.788		
30	3.564	3.595	3.580		

Example of human Interferon-gamma standard curve in Sample Diluent NS (for supernatant samples)

The Interferon-gamma standard curve was prepared as described. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



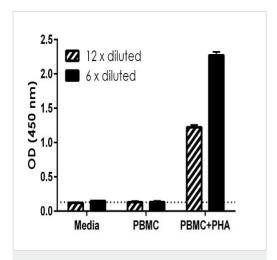
Example IFNG standard curve for serum/plasma samples measurements.

Background-subtracted data values (mean +/- SD) are graphed.

Standard Curve Measurements					
Concentration	O.D 4	Mean			
(ng/mL)	1	2	O.D		
0	0.051	0.053	0.052		
0.468	0.061	0.066	0.064		
0.937	0.074	0.072	0.073		
1.875	0.088	0.093	0.091		
3.750	0.120	0.120	0.121		
7.5	0.233	0.236	0.234		
15	0.488	0.466	0.477		
30	1.224	1.247	1.235		

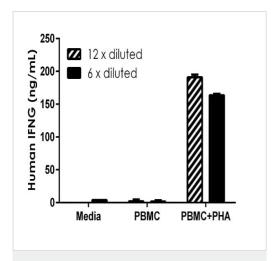
Example of human Interferon-gamma standard curve in Sample Diluent NBP (for serum/plasma samples)

The Interferon-gamma standard curve was prepared as described. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



PBMC were grown in the absence or presence of phytohemagglutinin (PHA) for 2 days. IFNG concentrations were measured in 12X and 6X diluted cell culture supernatants of the unstimulated PBMC and the stimulated PBMC, and media. Raw data values (mean +/-SD, n=3) are graphed. The dotted line represents zero sample background.

Comparison of secreted IFNG in unstimulated and PHA-stimulated Human PBMC.



The concentrations of IFNG were interpolated from data values shown in Figure 3 using IFNG standard curve and corrected for sample dilution. The mean IFNG concentration was determined to be 1.8 ng/mL in unstimulated PBMC supernatants and 177.2 ng/mL in stimulated PBMC supernatants.

Interpolated concentrations of secreted IFNG in unstimulated and PHA-stimulated Human PBMC.

Dilution Factor	Interpolated value	20% PHA PBMC Supernatant	100% Human Serum	100% Human Plasma (Citrate)	100% Human Plasma (EDTA)
Undiluted	ng/mL	22.9	27.2	28.1	31.3
orialiorea	% Expected value	100	100	100	100
2	ng/mL	11.7	13.8	13.7	14.6
2	% Expected value	103	101	98	94
4	ng/mL	6.56	6.51	7.20	7.13
4	% Expected value	115	96	103	91
8	ng/mL	3.33	3.47	3.87	3.44
O	% Expected value	117	102	110	88
16	ng/mL	1.70	2.37	2.01	1.53
10	% Expected value	119	139	115	78

Linearity of dilution.

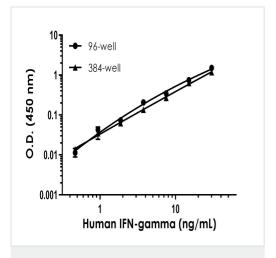
Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native Interferon-gamma was measured in PHA-stimulated PBMC cell culture supernatant samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Recombinant Interferon-gamma was spiked into human serum and plasma (citrate and EDTA) samples and diluted in a 2-fold dilution series in Sample Diluent NBP.

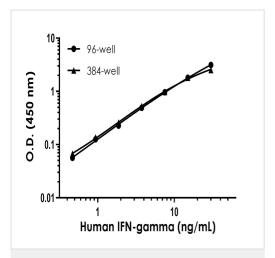
Neat pooled serum and plasma (EDTA and Citrate) samples from healthy donors was measured in duplicate. All values were below the detectable range of the assay.

Neat serum from ten individual healthy human female/male donors was measured in duplicate. All values were below the detectable range of the assay.



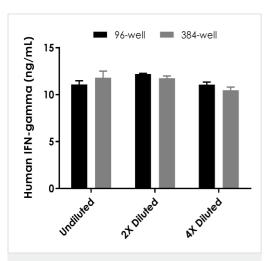
Example of human IFN-gamma standard curve in Sample Diluent NBP in 96-well vs. 384-well plate.

Example of human IFN-gamma standard curve in 96-well vs. 384-well plate. Background-subtracted data values (mean +/- SD) are graphed.



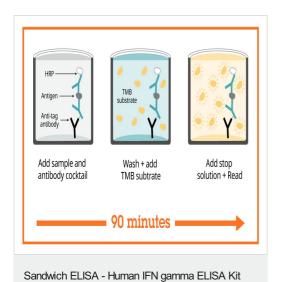
Example of human IFN-gamma standard curve in 96-well vs. 384-well plate. Background-subtracted data values (mean +/- SD) are graphed.

Example of human IFN-gamma standard curve in Sample Diluent NS in 96-well vs. 384-well plate.



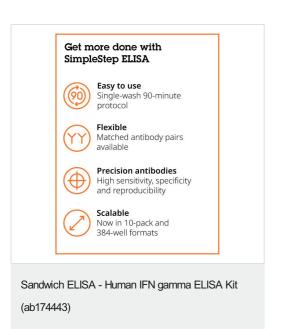
Interpolated concentrations of human IFN-gamma in stimulated PBMC supernatant in 96-well vs. 384-well plates.

Interpolated concentration of native IFN-gamma was measured in duplicate at different sample concentrations in 96-well vs. 384-well plates. Undiluted samples are 20% treated human PBMC supernatant (5% PHA-M for 46hr). The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). Sample dilutions are made in Sample Diluent NS.



(ab174443)

SimpleStep ELISA technology allows the formation of the antibodyantigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.



To learn more about the advantages of SimpleStep $\mathsf{ELISA}^{\circledR}$ kits see $\underline{\mathsf{here}}$.

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