ab229415
Human Interferon-gamma (IFNG) CatchPoint® SimpleStep ELISA® Kit

For the quantitative measurement of Interferon-gamma (IFNG) in human serum, plasma, and cell culture supernatant samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Interferon-gamma (IFNG) *in vitro* CatchPoint SimpleStep ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Interferon-gamma (IFNG) protein in human serum, plasma, and cell culture supernatant samples.

The CatchPoint SimpleStep ELISA employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. CatchPoint HRP Development Solution containing the Stoplight Red Substrate is added. During incubation, the substrate is catalyzed by HRP generating a fluorescent product. Signal is generated proportionally to the amount of bound analyte and the intensity is measured in a fluorescence plater reader at 530/570/590 nm Excitation/Cutoff/Emission.

IFN gamma 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.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

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Add 50 µL standard or sample to appropriate wells

⇩

Add 50 µL Antibody Cocktail to all wells

⇩

Incubate at room temperature for 1 hour

⇩

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

⇩

Add 100 µL of prepared CatchPoint HRP Development Solution to each well and incubate for 5-10 minutes

⇩

Read fluorescence at Ex/Cutoff/Em 530/570/590 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

– All kit components have been formulated and quality control tested to function successfully as a kit.

– We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.

– Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.

– Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.

– All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

− Assay kit intended for research use only. Not for use in diagnostic procedures.
− Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
− All data, except Typical Standard Curve and Sensitivity were collected using the colorimetric version of this kit (ab174443).

6. Materials Supplied

Item	Quantity	Storage Condition
Human Interferon-gamma (IFNG) Capture Antibody 10X	600 µL	+4°C
Human Interferon-gamma (IFNG) Detector Antibody 10X	600 µL	+4°C
Human Interferon-gamma (IFNG) Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI	6 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
Stoplight Red Substrate Buffer	12 mL	+4°C
100X Stoplight Red Substrate	120 µL	+4°C
500X Hydrogen Peroxide (H_2O_2, 3%)	50 µL	+4°C
Sample Diluent NS	50 mL	+4°C
Sample Diluent NBP	20 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescence microplate reader Ex/Cutoff/Em 530/570/590 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

- The incubation times provided in this protocol were optimized for fastest results with good signal. It is possible to increase the signal with longer incubation times, further optimization might be necessary.
- Keep in mind any RFU values shown are relative, NOT absolute. RFU from one plate reader are not comparable to another, especially if different make or model.
- **To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.**
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. Reagent Preparation

– Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

– Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

9.2 Antibody Cocktail:

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI. To make 3 mL of the Antibody Cocktail combine 300 μL 10X Capture Antibody and 300 μL 10X Detector Antibody with 2.4 mL Antibody Diluent CPI. Mix thoroughly and gently.

9.3 CatchPoint HRP Development Solution

Just prior to use prepare CatchPoint HRP Development Solution by diluting the 100X Stoplight Red Substrate and the 500X Hydrogen Peroxide in Stoplight Red Substrate Buffer.

For example, to make 6 mL of the CatchPoint HRP Development Solution combine 60 μL 100X Stoplight Red Substrate and 12 μL of 500X Hydrogen Peroxide with 5.928 mL Stoplight Red Substrate Buffer. Mix thoroughly and gently.

10. Standard Preparation

– Always prepare a fresh set of standards for every use.

– Discard working standard dilutions after use as they do not store well.

– The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the Interferon-gamma (IFNG) by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the Interferon-gamma (IFNG) standard by adding 500 µL Diluent. Hold at room temperature for 10 minutes and mix gently. This is the 48 ng/mL **Stock Standard** Solution.

For **serum and plasma samples**, reconstitute the Interferon-gamma (IFNG) protein standard sample by adding Sample Diluent NBP.
Note: Sample Diluent NBP should be warmed to 37 °C for 10 minutes and mixed thoroughly, then equilibrated to room temperature before use.
For **cell culture supernatant samples**, reconstitute the Interferon-gamma (IFNG) protein standard sample by adding Sample Diluent NS.

10.2 Label 11 tubes, Standards 1– 11.

10.3 For **serum and plasma samples**, Add 150 µL Sample Diluent NBP into tube number 1 and 150 µL of Sample Diluent NBP into numbers 2-11.
For **cell culture supernatant samples**, Add 150 µL Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-11.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #11 contains no protein and is the Blank control.

Standards will be added to the plate in step 13.3. If desired all 11 standards can be used for a full standard curve. Alternatively, to commit fewer wells to the standard curve, select a subset of at least 7 standards plus the blank control. If 7 standards are desired, we recommend standards #1-7.

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Stock	250	150	48	30
2	Standard#1	150	150	30	15
3	Standard#2	150	150	15	7.5
4	Standard#3	150	150	7.5	3.75
5	Standard#4	150	150	3.75	1.88
6	Standard#5	150	150	1.88	0.94
7	Standard#6	150	150	0.94	0.47
8	Standard#7	150	150	0.47	0.23
9	Standard#8	150	150	0.23	0.12
10	Standard#9	150	150	0.12	0.06
11	None	0	150	0	0

11. Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
48 hours PHA-stimulated PBMC supernatant	160 – 5X diluted

11.1 Plasma:

Collect plasma using citrate or EDTA. Centrifuge samples at 2,000 x g for 10 minutes. Neat plasma samples can be assayed without dilution. If needed, plasma samples should be diluted in the same negative matrix (negative plasma as appropriate) provided by a user. **Heparin-plasma samples are not compatible with this assay kit** because linearity of dilution cannot be guaranteed.

11.2 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Neat serum samples can be used without dilution. If needed, serum samples should be diluted in the same negative matrix (negative serum as appropriate) provided by a user.

11.3 Cell Culture Supernatants:

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants, dilute them at least two fold in Sample Diluent NS and assay.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well fluorescence or "edge effects" have not been observed with this assay.
- Ensure plate and all materials are equilibrated to room temperature during use.
- Cover the plate with a plate seal during incubation steps.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.

13.7 Add 100 µL of prepared CatchPoint HRP Development Solution to each well and incubate for 5-10 minutes in the dark on a plate shaker set to 400 rpm. Further optimization of incubation time vs signal strength can be performed if needed.

13.8 Record the fluorescence at Ex/Cutoff/Em 530/570/590 nm. If using a Molecular Devices' plate reader supported by SoftMax® Pro software, a preconfigured protocol for these CatchPoint SimpleStep ELISA Kits is available with all the protocol and analysis settings at www.softmaxpro.org

Mode:	Fluorescence
Instrument settings:	Endpoint
Excitation:	530 nm
Cutoff:	570 nm
Emission:	590 nm
Sensitivity:	6 flashes/read or 200ms
PMT:	Auto
Auto calibrate:	On
Read:	Top
Read Height:	1*

*For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

13.9   Analyze the data as described below.

14. Calculations

14.1 Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices

14.2 Calculate the average fluorescence value for the blank control (zero) standards. Subtract the average blank control standard fluorescence value from all other fluorescence values.

14.3 **Create a standard curve** by plotting the average blank control subtracted fluorescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

Δ **Note:** Most **fluorescence** reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.4 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted fluorescence **values against the standard curve.** Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.5 Samples generating fluorescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at fluorescence values less than that of the lowest standard should be retested in a less dilute form.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.
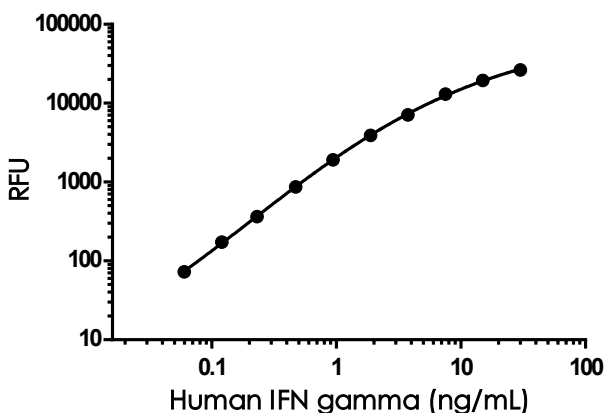
Standard Curve Measurements			
Concentration (ng/mL)	RFU		Mean RFU
	1	2	
0	41	37	39
0.06	109	116	112
0.12	221	205	213
0.23	400	409	404
0.47	913	898	906
0.94	1948	1965	1956
1.88	3925	3976	3951
3.75	7177	7162	7170
7.5	13079	13003	13041
15	19411	19410	19410
30	26352	26575	26464

Figure 1. Example of human Interferon-gamma (IFNG) standard curve in Sample Diluent NS. The Interferon-gamma (IFNG) standard curve was prepared as described in Section 10. Raw data generated on SpectraMax M4 Multi-Mode Microplate Reader is shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Typical Sample Values

SENSITIVITY –

The calculated minimal detectable dose (MDD) is 25 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=22) and adding 2 standard deviations then extrapolating the corresponding concentration.

RECOVERY –

Three concentrations of Interferon-gamma (IFNG) recombinant protein were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
50% Media (10F RPMI)	86	77-93
Neat Normal Human Serum	102	98-104
Neat Normal Human Plasma-EDTA	122	117-124
Neat Normal Human Plasma-citrate	107	96-115
Neat Normal Human Plasma-heparin	244	197-298

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 (IFNG) was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	5-fold diluted PHA-treated PBMC Supernatant
Undiluted	ng/mL	22.87
	% Expected value	100
2	ng/mL	11.74
	% Expected value	103
4	ng/mL	6.56
	% Expected value	115
8	ng/mL	3.33
	% Expected value	117
16	ng/mL	1.70
	% Expected value	119

Recombinant Interferon-gamma (IFNG) was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent NBP.

Dilution Factor	Interpolated value	Neat Human Serum	Neat Human Plasma (Citrate)	Neat Human Plasma (EDTA)	Neat Human Plasma (Heparin)
Undiluted	ng/mL	27.17	28.05	31.27	33.63
	% Expected value	100	100	100	100
2	ng/mL	13.76	13.70	14.64	16.76
	% Expected value	101	98	94	100
4	ng/mL	6.51	7.20	7.13	7.36
	% Expected value	96	103	91	88
8	ng/mL	3.47	3.87	3.44	2.44
	% Expected value	102	110	88	58
16	ng/mL	2.37	2.01	1.53	0.61
	% Expected value	139	115	78	29
32	ng/mL	0.89	0.85	NL	NL
	% Expected value	105	96	NL	NL

NL – Non-Linear

PRECISION –

Mean coefficient of variations of interpolated values of Interferon-gamma (IFNG) from three concentrations of PHA-stimulated PBMC supernatant within the working range of the assay.

	Intra-Assay	Inter-Assay
n =	5	3
CV(%)	1.1	7.9

17. Assay Specificity

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

IFNG concentrations in ten individual donor normal human serum samples measured less than the MDD, 0.7 ng/mL.
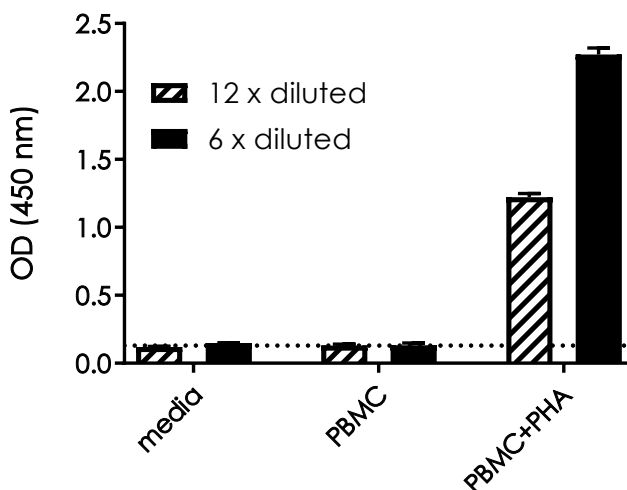

Figure 2. Comparison of secreted IFNG in unstimulated and PHA-stimulated Human PBMC. PBMC were grown in the absence or presence of phytohemagglutinin (PHA) for 2 days. IFNG concentrations were measured in 12X and 6X diluted cell culture supernatants of the unstimulated PBMC and the stimulated PBMC, and media. Raw data values (mean +/-SD, n=3) are graphed. The dotted line represents zero sample background.
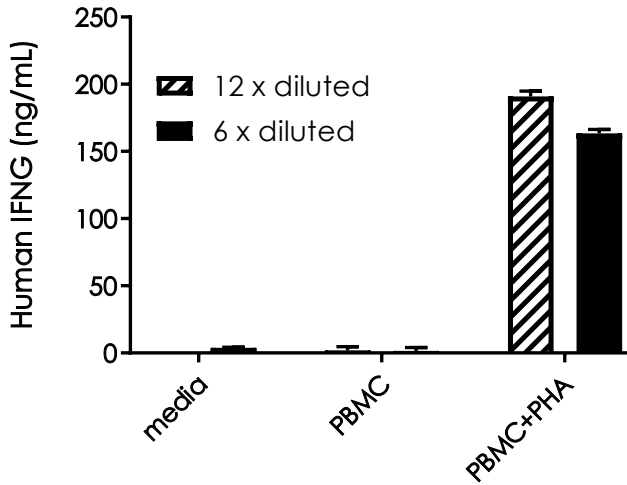
Figure 3. Interpolated concentrations of secreted IFNG in unstimulated and PHA-stimulated human PBMC. The concentrations of IFNG were interpolated from data values shown in Figure 3 using IFNG standard curve and corrected for sample dilution. The mean IFNG concentration was determined to be 1.8 ng/mL in unstimulated PBMC supernatants and 177.2 ng/mL in stimulated PBMC supernatants.

18. Species Reactivity

This kit recognizes human Interferon-gamma (IFNG) protein.

Heparin-plasma samples are not compatible with this assay kit because linearity of dilution cannot be guaranteed.

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with CatchPoint HRP Development Solution too brief	Read plate again after longer incubation time
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep Stoplight Red Substrate protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

20. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)