ab174443 – Human IFN-gamma SimpleStep ELISA® Kit

For the quantitative measurement of IFN-gamma in human serum, plasma (citrate), plasma (EDTA), and cell culture supernatant.
For research use only - not intended for diagnostic use.

This kit is available in a 384-well plate format. This plate utilises smaller volumes of standards and samples per well. Directions for using this format can be found on pg 8.

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity	Storage Condition
Human IFN-gamma Capture Antibody 10X	600 µL	+4°C
Human IFN-gamma Detector Antibody 10X	600 µL	+4°C
Human IFN-gamma Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI2	6 mL	+4°C
Sample Diluent NBP	20 mL	+4°C
Sample Diluent NS	12 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 wells	+4°C
Plate Seal	1	+4°C

Note: Antibody Diluent CPI2 – This buffer has been reformulated to enhance stability after freeze-thaw cycles while producing data equivalent to the original formulation of antibody diluent CPI previously used in this kit. While we run stock down, you may receive kits containing antibody diluent CPI. This does not affect the way you should use the kit. If you have any questions, please contact Abcam Scientific Support.

Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- 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).

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.

Sample Diluent NBP may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

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.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI2. 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 CPI2. Mix thoroughly and gently.

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).

1. Reconstitute the IFN-gamma standard sample by adding the volume indicated on the protein vial label. For **serum and plasma samples measurements**, use Sample Diluent NBP. For **cell culture supernatant samples measurements**, use Sample Diluent NS. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 48 ng/mL **Stock Standard** Solution.
1. Label eight tubes, Standards 1– 8.
2. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 150 µL of Sample Diluent into tube numbers 1-8.
3. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	**Stock Standard**	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	1.875
6	Standard#5	150	150	1.875	0.937
7	Standard#6	150	150	0.937	0.468
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum*	≤100%
Plasma – Citrate*	≤100%
Plasma – EDTA*	≤100%
48 hours PHA-Stimulated PBMC Supernatant	0.625 – 20%

*Based on spiked sample

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. Assay, or dilute samples into Sample Diluent NBP and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate or EDTA. Centrifuge samples at 2,000 x g for 10 minutes. Assay, or dilute samples into Sample Diluent NBP and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Note: This kit is incompatible with plasma (heparin) samples.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

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 absorbance or "edge effects" have not been observed with this assay.

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.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
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.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
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.
7. Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9. Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

For technical support contact information, visit:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)

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Additional information

ASSAY SPECIFICITY

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

Native signal was detected in cell culture supernatant sample types.

Spiked protein experiments were used to validate serum, plasma (citrate), and plasma (EDTA) sample types.

Neat, pooled serum and plasma (Citrate and EDTA) samples from healthy donors were 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.

This kit is incompatible with plasma (heparin) samples.

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

SPECIES REACTIVITY

Other species reactivity not determined.

CALCULATION

- Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- Create a standard curve by plotting the average blank control subtracted absorbance 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 microplate 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.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance 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.
- Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at absorbance values less than that of the lowest standard should be retested in a less dilute form.

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)	O.D 450 nm		Mean O.D
	1	2	
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

Table 1. Example of human IFN-gamma standard curve in Sample Diluent NS. The IFN-gamma standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (ng/mL)	O.D 450 nm		Mean O.D
	1	2	
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

Table 2. Example of human IFN-gamma standard curve in Sample Diluent NBP. The IFN-gamma standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 0.47 ng/mL. The MDD was determined by calculating the mean of zero standard replicates (n=25) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of IFN-gamma 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 (%)
100% Human Serum	102	98 - 104
100% Human Plasma – Citrate	107	96 - 115
100% Human Plasma – EDTA	122	117 - 124
50% Cell Culture Media*	86	77 - 93

*Media is RPMI 1640 containing 10% fetal bovine serum.

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.

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

Precision

Mean coefficient of variations of interpolated values of IFN-gamma from three concentrations of PHA-stimulated PBMC cell culture supernatant within the working range of the assay.

	Intra-assay	Inter-assay
N=	5	3
CV (%)	1.1	7.9

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For technical support contact information, visit:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)

DIRECTIONS FOR 384-WELL PLATE FORMAT:

Materials Supplied for 384-well Format

Item	Quantity	Storage Condition
Human IFN-gamma Capture Antibody 10X	600 µL	+4°C
Human IFN-gamma Detector Antibody 10X	600 µL	+4°C
Human IFN-gamma Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI2	6 mL	+4°C
Sample Diluent NBP	20 mL	+4°C
Sample Diluent NS	100 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	2 x 12 mL	+4°C
Stop Solution	2 x 12 mL	+4°C
SimpleStep Pre-Coated 384-Well Microplate	384 wells	+4°C
Plate Seal	1	+4°C

Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 or 600 nm in a 384-well plate.
- 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).
- Optional: Automated liquid handler.

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for one full plate. The sample volumes below are sufficient for running all 384 wells; adjust volumes as needed for the number of samples and dilution scheme for 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.

Sample Diluent NBP may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

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.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI2. To make 6 mL of the Antibody Cocktail combine 600 µL 10X Capture Antibody and 600 µL 10X Detector Antibody with 4.8 mL Antibody Diluent CPI2. Mix thoroughly and gently.

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).

1. Reconstitute the IFN-gamma standard sample by adding the volume indicated on the protein vial label. For **serum and plasma samples measurements**, use Sample Diluent NBP. For **cell culture supernatant samples measurements**, use Sample Diluent NS. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 48 ng/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 75 µL of Sample Diluent into tube numbers 1-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	**Stock Standard**	125	75	48	30
2	Standard#1	75	75	30	15
3	Standard#2	75	75	15	7.5
4	Standard#3	75	75	7.5	3.75
5	Standard#4	75	75	3.75	1.875
6	Standard#5	75	75	1.875	0.937
7	Standard#6	75	75	0.937	0.468
8	Blank Control	0	75	0	0

Plate Preparation

The 384-well plate included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
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 absorbance or "edge effects" have not been observed with this assay.

Assay Procedure for 384-well Plate Format

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Add 12.5 µL of all sample or standard to appropriate wells.
3. Add 12.5 µL of the Antibody Cocktail to each well.
4. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 700 rpm.
5. Wash each well with 3 x 100 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 100 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.
6. Add 25 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 700 rpm.
   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
7. Add 25 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading. Proper mixing of the Stop Solution is required for proper measurement.
8. Alternative to 6 – 7: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec – 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 25 µL Stop Solution to each well and recording the OD at 450 nm.

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