

## ab325737 – Human Interferon beta SimpleStep ELISA® Kit, Chemiluminescent

For the quantitative measurement of Interferon beta in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), and cell culture supernatant.

For research use only - not intended for diagnostic use.  
Patent pending.

For overview, typical data and additional information please visit: [www.abcam.com/ab325737](http://www.abcam.com/ab325737)

**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.

**Limitations:** All data, except typical standard curve, sensitivity, and serum from individual healthy male donors were collected using the colorimetric version of this kit (ab278127).

### Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Human Interferon beta Capture Antibody 10X	600 µL	+4°C
Human Interferon beta Detector Antibody 10X	600 µL	+4°C
Human Interferon beta Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BI	6 mL	+4°C
Sample Diluent 50BS	20 mL	+4°C
Sample Diluent NS	12 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
ChemiHRP Reagent A	3 mL	+4°C
ChemiHRP Reagent B	3 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 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:

Luminometer with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).

Deionized water.

Multi- and single-channel pipettes.

Tubes for standard dilution.

Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.

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 50BS 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 4BI. 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 4BI. Mix thoroughly and gently.

**Lumi HRP Development Solution:** Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

### 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).

#### For serum and plasma sample measurements:

1. Reconstitute the **Interferon beta** protein standard by adding the volume indicated on the protein vial label of Sample Diluent 50BS. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 11,200 pg/mL **Stock Standard** Solution.
2. Label nine tubes, Standards 1– 9.
3. Add 220 µL of Sample Diluent 50BS into tube number 1 and 150 µL of Sample Diluent 50BS into numbers 2-9.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #9 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	<b>Stock Standard</b>	60	220	11,200	2,400
2	Standard#1	75	150	2,400	800
3	Standard#2	75	150	800	266.7
4	Standard#3	75	150	266.7	88.9
5	Standard#4	75	150	88.9	29.6
6	Standard#5	75	150	29.6	9.88
7	Standard#6	75	150	9.88	3.29
8	Standard#7	75	150	3.29	1.10
9	Blank Control	0	150	0	0

**For cell culture supernatant sample measurements:**

1. Reconstitute the **Interferon beta** protein standard by adding the volume indicated on the protein vial label of Sample Diluent NS. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 11,200 pg/mL **Stock Standard** Solution.
2. Label nine tubes, Standards 1–9.
3. Add 222 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-9.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #9 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	<b>Stock Standard</b>	37	222	11,200	1,600
2	Standard#1	75	150	1,600	533.3
3	Standard#2	75	150	533.3	177.8
4	Standard#3	75	150	177.8	59.3
5	Standard#4	75	150	59.3	19.8
6	Standard#5	75	150	19.8	6.58
7	Standard#6	75	150	6.58	2.20
8	Standard#7	75	150	2.20	0.73
9	Blank Control	0	150	0	0

**Sample Preparation**

Typical Sample Dynamic Range	
Sample Type	Range
Serum	≤100%
Plasma – Citrate	≤100%
Plasma – EDTA	≤100%
Plasma – Heparin	≤100%
A549 Cell Culture Supernatant transfected with 400 ng/mL Poly I:C for 24 h	12.5 - 100%
A549 Cell Culture Supernatant transfected with 200 ng/mL Poly I:C for 24 h	50 - 100%

**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 50BS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

**Plasma** Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Assay, or dilute samples Sample Diluent 50BS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

**Cell Culture Supernatants** Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Assay, or dilute samples into Sample Diluent NS and assay. 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 30 minutes at room temperature on a plate shaker set to 750 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 30 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 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

<b>Mode:</b>	Luminescence
<b>Instrument settings:</b>	Endpoint
<b>Detection Mode:</b>	All wavelengths
<b>Read Time:</b>	0.5-1 sec
<b>Read:</b>	Top

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

**Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:**

<https://www.abcam.com/en-us/technical-resources/guides/elisa-guide>

### Technical Support

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## Additional information

### ASSAY SPECIFICITY

This kit is designed for the quantification of human Interferon beta. The standard protein in this kit is full length mature human Interferon beta.

Native signal was detected in cell culture supernatant sample types.

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

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

Neat pooled serum and plasma (EDTA, heparin, 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 male donors was measured in duplicate. All values were below the detectable range of the assay.

### SPECIES REACTIVITY

Other species reactivity not determined.

### CALIBRATION

The 3rd WHO international standard for glycosylated human Interferon beta, NIBSC code: 00/572, was evaluated in this kit.

The dose response curve of NIBSC/WHO international standard Interferon beta 00/572 parallels the SimpleStep standard curve. To convert sample values obtained with the SimpleStep Human Interferon beta kit to approximate NIBSC units, use the equation below.

NIBSC (00/572) approximate value (IU/mL) = 0.5342 x SimpleStep Human Interferon beta value (pg/mL).

The Non-WHO reference material for glycosylated human Interferon beta, NIBSC code: 00/576, was evaluated in this kit.

The dose response curve of NIBSC reference material Interferon beta 00/576 parallels the SimpleStep standard curve. To convert sample values obtained with the SimpleStep Human Interferon beta kit to approximate NIBSC units, use the equation below.

NIBSC (00/576) approximate value (IU/mL) = 0.5389 x SimpleStep Human Interferon beta value (pg/mL).

### CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence 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.
- Note: Most chemiluminescence 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 chemiluminescence 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 chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence 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 (pg/mL)	RLU		Mean RLU
	1	2	
0	742	927	835
0.73	1,531	1,630	1,581
2.19	3,004	3,353	3,179
6.58	8,647	9,484	9,066
19.8	27,464	30,135	28,800
59.3	90,036	95,230	92,633
178	314,930	309,620	312,275
533	1,056,500	988,880	1,022,690
1,600	3,288,200	3,378,900	3,333,550

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

Standard Curve Measurements		
	RLU	

Concentration	1	2	Mean
0	1,016	1,024	1,020
1.10	1,978	1,880	1,929
3.29	4,062	3,959	4,011
9.88	14,336	8,640	11,488
29.6	39,999	37,518	38,759
88.9	136,850	141,620	139,235
267	430,610	436,490	433,550
800	1,322,100	1,356,300	1,339,200
2,400	4,091,000	4,006,800	4,048,900

Table 2. Example of human Interferon beta standard curve in Sample Diluent 50BS. The Interferon beta standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

### TYPICAL SAMPLE VALUES

#### Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	N=	Minimal Detectable Dose
Sample Diluent 50BS	16	0.52 pg/mL
Sample Diluent NS	16	0.34 pg/mL

#### Recovery

Three concentrations of Interferon beta 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% Serum	92	89 - 97
100% Plasma - Citrate	117	114 - 120
100% Plasma - EDTA	101	97 - 106
100% Plasma - Heparin	116	112 - 119
100% A549 Supernatant transfected with 400 ng/mL Poly I:C for 24 h	96	83 - 106
100% A549 Supernatant transfected with 200 ng/mL Poly I:C for 24 h	98	86 - 105

#### 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 beta 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	100% A549 Transfected Supernatant*	100% A549 Transfected Supernatant**
Undiluted	pg/mL	118	33.0
	% Expected value	100	100
2	pg/mL	64.9	17.2
	% Expected value	110	104
4	pg/mL	24.9	ND
	% Expected value	84	ND
8	pg/mL	12.3	ND
	% Expected value	83	ND

ND – Not Detected – below product dynamic range

\* Transfected with 400 ng/mL Poly I:C for 24 h

\*\* Transfected with 200 ng/mL Poly I:C for 24 h

Recombinant Interferon beta was spiked into the following biological samples and then diluted in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 50BS.

Dilution Factor	Interpolated value	100% Human Serum	100% Human Plasma (Citrate)	100% Human Plasma (EDTA)	100% Human Plasma (Heparin)
Undiluted	pg/mL	1,866	2,193	2,154	1,949
	% Expected value	100	100	100	100
2	pg/mL	819.2	1,075	888.1	922.8
	% Expected value	88	98	82	95
4	pg/mL	475.8	520.1	475.0	479.6
	% Expected value	102	95	88	98
8	pg/mL	237.5	275.0	241.6	254.1
	% Expected value	102	100	90	104
16	pg/mL	106.9	116.2	108.0	104.1
	% Expected value	92	85	80	85

## Precision

Mean coefficient of variations of interpolated values of Interferon beta from two concentrations of Interferon beta recombinant protein spiked into neat human serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	7	2

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

<https://www.abcam.com/en-us/technical-resources/guides/elisa-guide>

## Technical Support

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