TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

Research Use Only. Not for use in diagnostic procedures.

Mouse IFNβ AlphaLISA Detection Kit

Product No.: AL586HV/C/F

Contents

Product Information

Application: This kit is designed for the quantitative determination of mouse IFNβ (mIFNβ) in serum, plasma, mouse cell lysates and mouse cell culture supernatnats using a homogeneous AlphaLISA assay (no wash steps).

Sensitivity: Lower Detection Limit (LDL): 5.1 pg/mL

Lower Limit of Quantification (LLOQ): 18 pg/mL

EC_{50}: 9 ng/mL

Dynamic range: 5.1 – 100 000 pg/mL

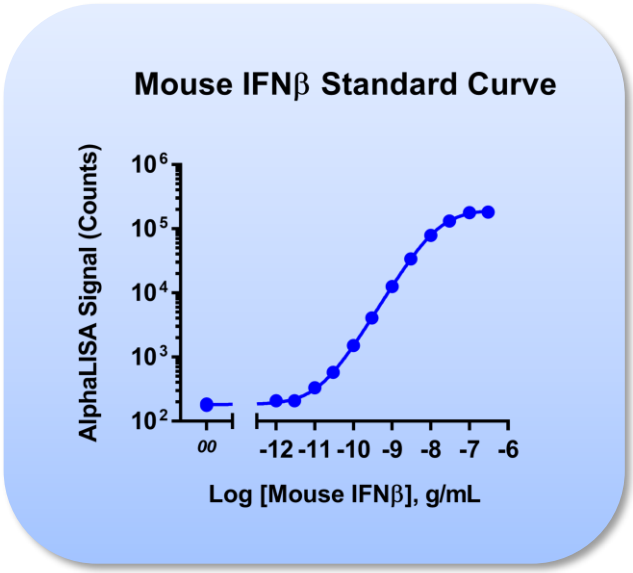

Figure 1. Typical sensitivity curve in AlphaLISA Immunoassay Buffer. The data was generated using a white Optiplate™-384 microplate and the EnVision® Multilabel Plate Reader 2102 with Alpha option.

Storage: Store kit in the dark at 4 ˚C. For reconstituted analyte aliquot and store at -20 °C. Avoid freeze-thaw cycles.

Stability: This kit is stable for at least 6 months from the manufacturing date when stored in its original packaging and the recommended storage conditions.

Quality Control

Lot to lot consistency is confirmed in an AlphaLISA assay. Maximum and minimum signals, EC_{50} and LDL were measured on the EnVision Multilabel Plate Reader with Alpha option using the protocol described in this technical data sheet. We certify that these results meet our quality release criteria. Maximum counts may vary between bead lots and the instrument used, with no impact on LDL measurement.

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Analyte of Interest

In mammals, interferon beta (IFNβ) is primarily produced by fibroblasts and it can also be produced by dendritic cells, macrophages, and endothelial cells in response to pathogen exposure. IFNβ is a member of type I interferon family. Mouse IFNβ (mIFNβ) with approximate molecular weight of 22kDa has 75% and 47% amino acid sequence identity to rat and human IFNβ proteins, respectively. Functionally, IFNβ protein involves in wide variety activities such as anti-bacterial and anti-viral infections, anti-tumor growth, and anti-inflammation. Furthermore, IFN-beta has been shown to suppress both multiple sclerosis (MS) and experimental autoimmune encephalomyelitis and has commonly been used as a treatment for MS to reduce the frequency of exacerbations and stabilize the course of the disease. Several types of interferon beta are on the market in the U.S for treatments of relapsing forms of MS.

Description of the AlphaLISA Assay

AlphaLISA technology allows the detection of molecules of interest in buffer, serum, plasma and Cerebrospinal Fluid (CSF) in a highly sensitive, quantitative, reproducible and user-friendly mode. In this AlphaLISA assay, a Biotinylated Anti-mouse IFNβ antibody binds to the Streptavidin-coated Alpha Donor beads, while another Anti-mouse IFNβ antibody is conjugated to AlphaLISA Acceptor beads. In the presence of the mouse IFNβ, the beads come into close proximity. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (Figure 2).

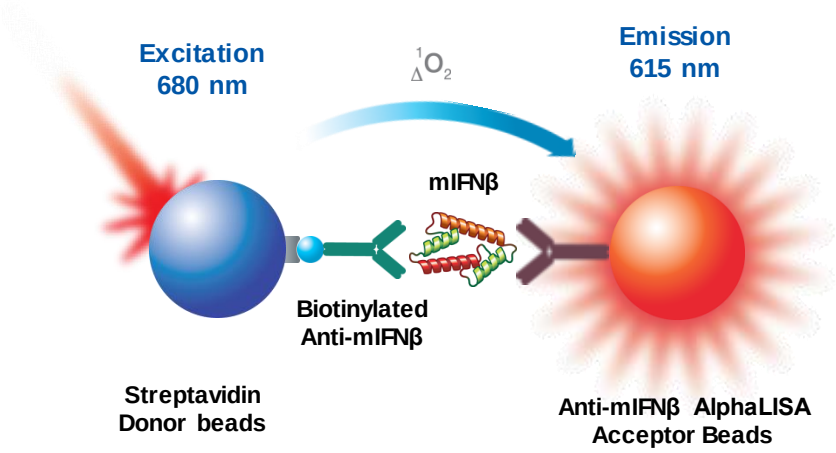


Figure 2. AlphaLISA mIFNβ Detection Assay Principle.

Precautions

- The Alpha Donor beads are light-sensitive. All the other assay reagents can be used under normal light conditions. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures.
- Take precautionary measures to avoid contamination of the reagent solutions.
- The Biotinylated anti-mIFNβ antibody contains sodium azide. Contact with skin or inhalation should be avoided.

Kit Content: Reagents and Materials

Kit components	AL586HV (100 assay points***)	AL586C (500 assay points***)	AL586F (5000 assay points***)
AlphaLISA Anti-mIFNβ Acceptor beads stored in PBS, 0.05% Kathon CG/ICP, pH 7.2	20 µL @ 5 mg/mL (1 brown tube, white cap)	50 µL @ 5 mg/mL (1 brown tube, white cap)	500 µL @ 5 mg/mL (1 brown tube, white cap)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Kathon CG/ICP, pH 7.4	80 µL @ 5 mg/mL (1 brown tube, black cap)	200 µL @ 5 mg/mL (1 brown tube, black cap)	2 X 1 mL @ 5 mg/mL (2 brown tubes, black caps)
Biotinylated Anti-mIFNβ Antibody stored in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	40 µL @ 500 nM (1 tube, black cap)	100 µL @ 500 nM (1 tube, black cap)	1 mL @ 500 nM (1 tube, black cap)
Lyophilized mIFNβ Analyte	0.3 µg (1 tube, clear cap)	0.3 µg (1 tube, clear cap)	0.3 µg (1 tube, clear cap)
AlphaLISA Immunoassay Buffer (10X)	2 mL, 1 small bottle	10 mL, 1 medium bottle	100 mL, 1 large bottle

* Reconstitute lyophilized analyte in 100 µL Milli-Q® grade H_2O. The reconstituted analyte should be used within 60 minutes or aliquoted into screw-capped 0.5 mL polypropylene vials and stored at -20 °C for future experiments. The aliquoted analyte at -20 °C is stable up to 90 days. Avoid freeze-thaw cycles. One vial contains an amount of analyte sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AL586S).

** Extra buffer can be ordered separately (cat # AL000C: 10 mL, cat # AL000F: 100 mL).

*** The number of assay points is based on an assay volume of 100 µL in 96-well plates or 50 µL in 384-well assay plates using the kit components at the recommended concentrations.

Sodium azide should **not** be added to the stock reagents. High concentrations of sodium azide (> 0.001% final in the assay) might decrease the AlphaLISA signal. Note that sodium azide from the Biotinylated anti-mIFNβ antibody stock solution will not interfere with the AlphaLISA signal (0.0001% final in the assay).

Specific additional required reagents and materials:

The following materials are recommended:

Item	Suggested source	Catalog #
TopSeal™-A Plus Adhesive Sealing Film	PerkinElmer Inc.	6050185
EnVision®-Alpha Reader	PerkinElmer Inc.	-

Recommendations

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube. To minimize loss when pipetting beads, it is preferable not to pre-wet the tip.
- Centrifuge all tubes (including lyophilized analyte) before use to improve recovery of content (2000g, 10-15 sec). Re-suspend all reagents by vortexing before use.
- Use Milli-Q® grade H_2O to dilute 10X AlphaLISA Immunoassay Buffer and to reconstitute the lyophilized analyte.
- When diluting the standard or samples, change tips between each standard or sample dilution. When loading reagents in the assay microplate, change tips between each standard or sample addition and after each set of reagents.
- When reagents are added to the microplate, make sure the liquids are at the bottom of the well.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal-A Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Film in place.
- The AlphaLISA signal is detected with an EnVision Multilabel Plate Reader equipped with the Alpha option using the AlphaScreen standard settings (e.g. Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).
- AlphaLISA signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.
- The standard curves shown in this technical data sheet are provided for information only. A standard curve must be generated for each experiment.

Assay Procedure

- The protocol described below is an example for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations). The protocols also include testing samples in 452 wells. If different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly, as shown in the table below. These calculations do not include excess reagent to account for losses during transfer of solutions or dead volumes.
- The standard dilution protocol is provided for information only. As needed, the number of replicates or the range of concentrations covered can be modified.
- Use of four background points in triplicate (12 wells) is recommended when LDL/LLOQ is calculated. One background point in triplicate (3 wells) can be used when LDL/LLOQ is not calculated.

		Volume					
Format	**# of data points**	**Final**	**Sample**	**AlphaLISA Acceptor Beads**	**Biotinylated Antibody**	**SA-Donor beads**	**Plate recommendation**
AL586HV	100	100 µL	10 µL	20 µL	20 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
AL586C	250	100 µL	10 µL	20 µL	20 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
	500	50 µL	5 µL	10 µL	10 µL	25 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate™-384 (cat # 6005350)
	1 250	20 µL	2 µL	4 µL	4 µL	10 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	2 500	10 µL	1 µL	2 µL	2 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)
AL586F	5 000	50 µL	5 µL	10 µL	10 µL	25 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350)
	12 500	20 µL	2 µL	4 µL	4 µL	10 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	25 000	10 µL	1 µL	2 µL	2 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)

3 Step Protocol described below is for 500 assay points including one standard curve (48 wells) and samples (452 wells). *If different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly.*

1) Preparation of 1X AlphaLISA Immunoassay Buffer:
Add 5 mL of 10X AlphaLISA Immunoassay Buffer to 45 mL Milli-Q® grade H_2O.

2) Preparation of mIFNβ analyte standard dilutions:

a. Reconstitute lyophilized mIFNβ (0.3 µg) in 100 µL Milli-Q® grade H_2O. The remaining reconstituted analyte should be aliquoted immediately and stored at -20 ºC for future assays (see page 4 for more details).
b. Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer (change tip between each standard dilution):

Tube	Vol. of mIFNβ (µL)	Vol. of diluent (µL) *	[mIFNβ] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	10 µL reconstituted mIFNβ	90	3.0E-07	300 000
B	60 µL of tube A	120	1.0E-07	100 000
C	60 µL of tube B	140	3.0E-08	30 000
D	60 µL of tube C	120	1.0E-08	10 000
E	60 µL of tube D	140	3.0E-09	3 000
F	60 µL of tube E	120	1.0E-09	1 000
G	60 µL of tube F	140	3.0E-10	300
H	60 µL of tube G	120	1.0E-10	100
I	60 µL of tube H	140	3.0E-11	30
J	60 µL of tube I	120	1.0E-11	10
K	60 µL of tube J	140	3.0E-12	3
L	60 µL of tube K	120	1.0E-12	1
M ** (background)	0	100	0	0
N ** (background)	0	100	0	0
O ** (background)	0	100	0	0
P ** (background)	0	100	0	0

* Dilute standards in diluent (e.g. 1X AlphaLISA Immunoassay Buffer).
At low concentrations of analyte, a significant amount of analyte can bind to the vial. Therefore, load the analyte standard dilutions in the assay microplate within 60 minutes of preparation.

** Four background points in triplicate (12 wells) are used when LDL is calculated. If LDL does not need to be calculated, one background point in triplicate can be used (3 wells).

3) Preparation of 5X AlphaLISA Anti-mIFNβ Acceptor beads (50 µg/mL):

a. Prepare just before use.
b. Add 50 µL of 5 mg/mL AlphaLISA Anti-mIFNβ Acceptor to 4950 µL of 1X AlphaLISA Immunoassay Buffer. Mix briefly.

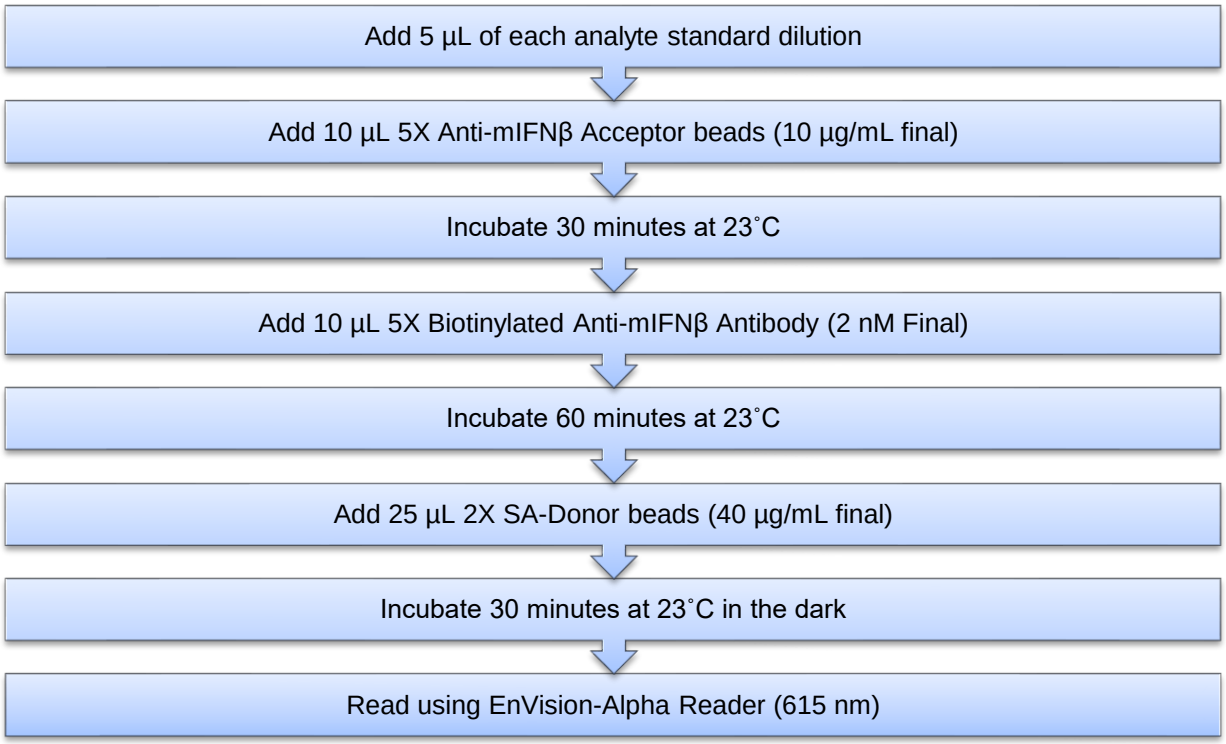
4) Preparation of 5X Biotinylated Anti-mIFNβ Antibody (10 nM):

a. Prepare just before use.
b. Add 100 µLof 500 nM Biotinylated Anti-mIFNβ Antibody to 4900 µL of 1X AlphaLISA Immunoassay Buffer. Mix briefly.

5) Preparation of 2X Streptavidin (SA) Donor beads (80 µg/mL):

a. Prepare just before use.
b. Keep the beads under subdued laboratory lighting.
c. Add 200 µL of 5 mg/mL SA-Donor beads to 12 300 µL of 1X AlphaLISA Immunoassay Buffer.

6) In a white OptiPlate (384 wells):



Data Analysis

- Calculate the average count value for the background wells.
- Generate a standard curve by plotting the AlphaLISA counts versus the concentration of analyte. A log scale can be used for either or both axes. No additional data transformation is required.
- Analyze data according to a nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) and a $1/Y^2$ data weighting (the values at maximal concentrations of analyte after the hook point should be removed for correct analysis).
- The LDL is calculated by interpolating the average background counts (12 wells without analyte) + 3 x standard deviation value (average background counts + (3xSD)) on the standard curve.
- The LLOQ as measured here is calculated by interpolating the average background counts (12 wells without analyte) + 10 x standard deviation value (average background counts + (10xSD)) on the standard curve. Alternatively, the true LLOQ can be determined by spiking known concentrations of analyte in the matrix and measuring the percent recovery, and then determining the minimal amount of spiked analyte that can be quantified within a given limit (usually +/- 20% or 30% of the real concentration).
- Read from the standard curve the concentration of analyte contained in the samples.
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Assay Performance Characteristics

AlphaLISA assay performance described below was determined a 3-step protocol using AlphaLISA Immunoassay Buffer (IAB) as assay buffer. The analytes (standards) were prepared in IAB, DMEM + 10% FBS, RPMI + 10% FBS, 100%FBS and AlphaLISA Lysis Buffer, and all other components were prepared in IAB.

- Assay Sensitivity:

The LDL was calculated as described above. The values correspond to the lowest concentration of analyte that can be detected in a volume of 5 µL sample using the recommended assay conditions.

LDL (pg/mL)	(Analyte diluent)	# of experiments
5.1	IAB	6
5.1	DMEM + 10% FBS	6
1.6	RPMI + 10% FBS	6
3.3	100%FBS	6
7.2	AlphaLISA Lysis Buffer	6

- Assay Precision:

The following assay precision data were calculated from the three independent assays using two different kit lots. In each lot, the analytes were prepared in IAB, DMEM + 10% FBS, RPMI + 10% FBS, 100%FBS and AlphaLISA Lysis Buffer. All other components were prepared in IAB. Each assay consisted of one standard curve comprising 12 data points (each in triplicate) and 12 background wells (no analytes). The assays were performed in 384-well format.

- Intra-assay precision:

The intra-assay precision was determined using a total of 16 independent determinations in triplicate. Shown as CV%.

mIFNβ	IAB	DMEM + 10% FBS	RPMI + 10% FBS	100%FBS	AlphaLISA Lysis Buffer
CV (%)	4.5	6	5.2	4.9	4.8

- Inter-assay precision:

The inter-assay precision was determined using a total of 3 independent determinations with 9 measurements for 30 ng/mL sample. Shown as CV%.

mIFNβ	IAB	DMEM + 10% FBS	RPMI + 10% FBS	100%FBS	AlphaLISA Lysis Buffer
CV (%)	6.5	6.8	7	6.6	7.2

- Spike Recovery:

Three known concentrations of analyte were spiked into IAB, DMEM + 10% FBS, RPMI + 10% FBS, 100%FBS and AlphaLISA Lysis Buffer. All samples, including non-spiked diluents were measured in the assay. Note that the analytes for the respective standard curves were prepared in IAB, DMEM + 10% FBS, RPMI + 10% FBS, 100%FBS and AlphaLISA Lysis Buffer. All other assay components were diluted in IAB.

Spiked mIFNβ (ng/mL)	% Recovery				
	IAB	DMEM + 10% FBS	RPMI + 10% FBS	100%FBS	AlphaLISA Lysis Buffer
10	88	95	88	96	105
1	91	92	100	99	89
0.1	86	92	102	103	87

- Specificity:

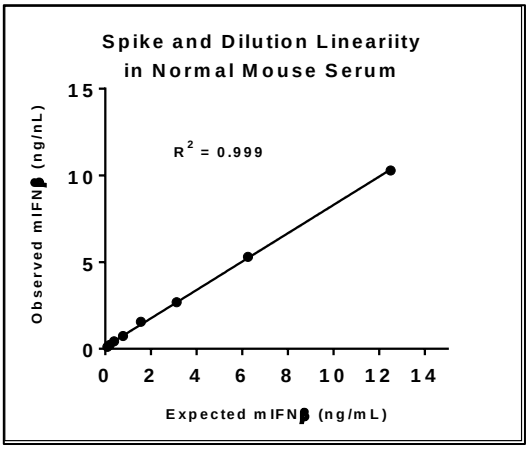
Cross-reactivity of the mIFNβ AlphaLISA Detection Kit was tested using the following proteins at 100 ng/mL in IAB. The cross reactivities were calculated using the signals of 100 ng/mL mIFNβ as 100%.

Proteins	**% Cross Reactivity**
Mouse IFNα	0.09
Mouse IFNγ	0.10
Human IFN	0.10
Human IFNβ	0.09
Human IFNγ	0.09
Rat IFNα	0.09
Rat IFNβ	0.09
Rat IFNγ	0.10

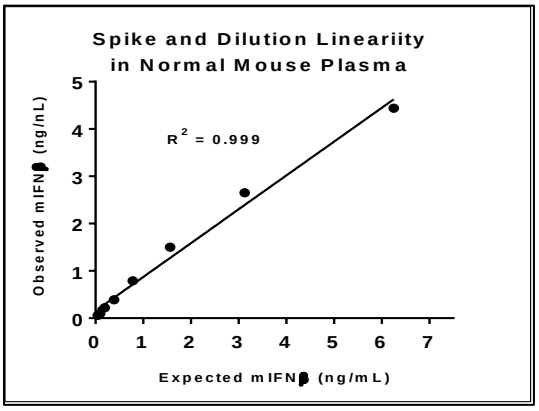
- Mouse Serum and Plasma Experiments:
    - Dilution Linearity

Normal mouse serum, normal mouse plasma, mIFNβ-spiked (100 ng/mL) normal mouse serum, and mIFNβ-spiked (100 ng/mL) normal mouse plasma samples were diluted with 100% FBS and the assay was performed along with a standard curve prepared in 100% FBS. Concentrations of IFNβ in diluted samples were determined by interpolating to the standard curve. The other components (anti mouse IFNβ acceptor beads, biotinylated anti mouse IFN antibody, and SA-Donor beads) of the assays were prepared in AlphaLISA Immunoassay Buffer. mIFNβ was not detected in normal mouse serum or in normal mouse plasma. Excellent dilution linearity ($R^2 > 0.999$) was achieved in the mIFNβ-spiked mouse serum (8- to 1024-fold dilutions) or plasma (16- to 1024-fold dilutions) samples. The results are shown in table and figure below.

Serum Dilution Factor (x)	Expected mIFNβ (ng/mL)	Observed mIFNβ (ng/mL)
8	12.50	10.29
16	6.25	5.30
32	3.13	2.69
64	1.56	1.56
128	0.78	0.74
256	0.39	0.43
512	0.20	0.22
1024	0.10	0.11



Plasma Dilution Factor (x)	Expected mIFNβ (ng/mL)	Observed mIFNβ (ng/mL)
16	6.25	4.44
32	3.13	2.65
64	1.56	1.50
128	0.78	0.79
256	0.39	0.39
512	0.20	0.22
1024	0.10	0.10



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- Spike Recovery

Three known amounts of mIFNβ were spiked into normal mouse serum (100 ng/mL, 12.5 ng/mL, and 1.6 ng/mL) or into normal mouse plasma (100 ng/mL, 12.5 ng/mL, and 1.6 ng/mL). The spiked serum and plasma samples were diluted to 8-fold and 16-fold, respectively, using 100% FBS, resulting in 12.5, 1.56, and 0.2 ng/mL in 8x diluted serum samples and 6.25, 0.78, and 0.1 ng/mL in 16x diluted plasma samples. The samples were assayed along the standard prepared in 100% FBS. Other components (anti mouse IFNβ acceptor beads, biotinylated anti mouse IFN antibody, and SA-Donor beads) of the assays were prepared in AlphaLISA Immunoassay Buffer. The spike recoveries of mIFNβ were determined. The results shown in table below indicating that excellent recoveries were achieved for all three spikes tested.

	Diluent: 100% FBS	
	Spiked sample (Normal Mouse Serum)	
Spike (ng/mL)	Concentration (ng/mL)	Recovery (%)
No spike	0	N/A
12.50	11.7	93
1.56	1.7	107
0.20	0.2	116

	Diluent: 100% FBS	
	Spiked sample (Normal Mouse Plasma)	
Spike (ng/mL)	Concentration (ng/mL)	Recovery (%)
No spike	0	N/A
6.25	5.1	82
0.78	0.8	107
0.10	0.1	113

- Mouse Raw 264.7 Cell Lysate and Cell Supernatant Experiments

The RAW 264.7 mouse cell line was obtained from ATCC (ATCC® TIB-71™). RAW 264.7 cells were cultured in DMEM supplemented with 10% FBS, 100 units/mL penicillin, and 100 µg/mL streptomycin and maintained in a humidified incubator at 37 ºC in 5% carbon dioxide (CO_2). The cells were plated and cultured in a 6-well plate at a density of 1.0 x 10^5 cells/mL for 24 h. After 24 h incubation, the cells were treated with or without LPS (1 µg/mL) for 4 h. Cell culture supernatants were then harvested and stored at -80 ºC until assayed. The cells in the plate wells were then lysed with 500 µL 1X AlphaLISA Lysis Buffer and the cell lysate samples were also stored at -80 ºC until assayed. The levels of IFNβ in cell culture supernatant samples and in cell lysate samples were measured using AlphaLISA mIFN-β detection kit. To measure mouse IFNβ in cell culture supernatant samples, the standard curve was prepared in DMEM supplemented with 10% FBS and the supernatant samples were diluted with in DMEM supplemented with 10% FBS. To measure mouse IFNβ in cell lysate samples, the standard curve was prepared 1X Alpha Lysis Buffer and the lysate samples were diluted with 1X AlphaLISA Lysis Buffer. All other components (anti mouse IFNβ acceptor beads, biotinylated anti mouse IFNβ antibody, and SA-Donor beads) of the assays were prepared in AlphaLISA Immunoassay Buffer. Table below showed that when RAW 264.7 cells were stimulated with LPS, the synthesis and secretion of mouse IFNβ from RAW 264.7 cells were increased (> 10 folds) comparing to non-stimulated controls. These results suggest that AlphaLISA mouse IFNβ Detection Kit can be used to measure the mouse IFNβ in cell lysate and cell culture supernatant samples.

Raw 264.7 Cell	mIFNβ levels in cell culture supernatant samples (pg/mL)	mIFNβ levels in cell lysate samples (pg/mL)
Un-Treated Control	4.2 ± 1.4*	3.1 ± 0.7*
LPS-Treated	59.2 ± 8.4*	42.0 ± 6.5**

*Average of samples diluted up to 8x; **Average of samples diluted up to 32x.

Troubleshooting Guide

You will find detailed recommendations for common situations you might encounter with your AlphaLISA Assay kit at:

http://www.perkinelmer.com/lab-products-and-services/application-support-knowledgebase/alphalisa-alphascreen-no-wash-assays/alpha-troubleshooting.html

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