Using the VeriKine™ Mouse Interferon Alpha ELISA Kit with Tissue Homogenates
Catalog No. 42120-1, -2

Summary:

PBL Assay Science VeriKine™ Mouse Interferon Alpha ELISA kit (Catalog No. 42120) was modified to accommodate various mouse homogenate matrices. Interference from complex matrices may result from any number of factors that can influence any component of an assay. To increase the utility of the Mouse Interferon Alpha ELISA kit, we assessed in excess of 30 additives using spike recovery to improve performance of the kit in complex mouse homogenate matrices. The addition of casein (0.33% final in each well) provided improved recovery while avoiding excessive dilution of the samples and buffers. Slight modifications to the existing protocol were required to include the casein additive.

For brain, liver, and kidney homogenates, samples may be run undiluted. For gut homogenates, a minimum 5-fold dilution is required for analyte recovery. For mesenchyme homogenates, a 5-fold dilution was tested with excellent recovery. Undiluted mesenchyme homogenate demonstrated poor recovery (~14%). Intermediate dilutions of homogenate sample were not evaluated. As with any ELISA, preparation of the standard curve in the matrix yields optimal results.

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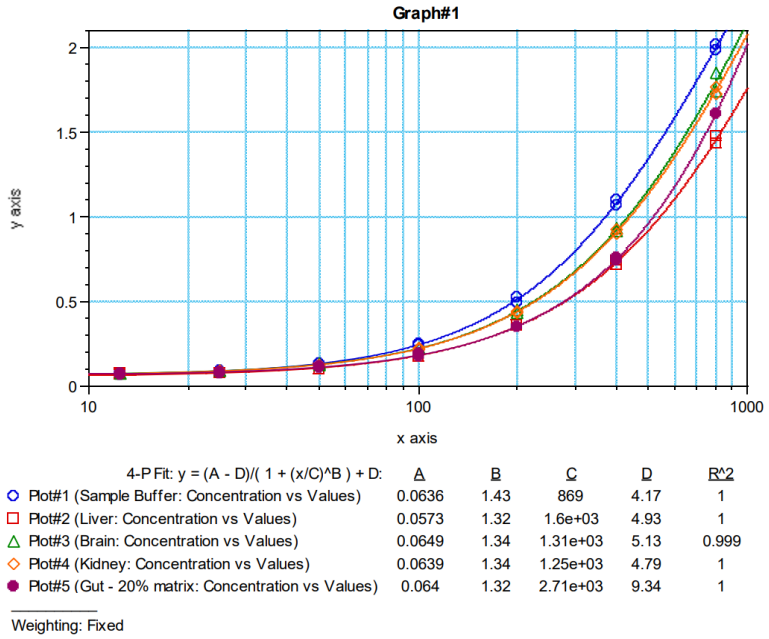
Performance of Matrices in Modified Assay using Sample Buffer to prepare standard curve:

Standard curves were prepared in sample buffer or matrix. ++**Please note** that *Gut homogenate and Mesenchyme homogenate were diluted 5-fold.* ++Percent recoveries were determined compared to sample buffer or matrix standard curves. When matrix is used to prepare the standard curve, percent recovery is ≥ 98% for all matrices (Table 2, next page). When matrix percent recoveries are compared to the standard curve prepared in sample buffer, percent recovery is ≥ 69% for all matrices (Table 1, next page).



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
Plot#1 (Sample Buffer: Concentration vs Values)	0.0636	1.43	869	4.17	1
Plot#2 (Liver: Concentration vs Values)	0.0573	1.32	1.6e+03	4.93	1
Plot#3 (Brain: Concentration vs Values)	0.0649	1.34	1.31e+03	5.13	0.999
Plot#4 (Kidney: Concentration vs Values)	0.0639	1.34	1.25e+03	4.79	1
Plot#5 (Gut - 20% matrix: Concentration vs Values)	0.064	1.32	2.71e+03	9.34	1

Weighting: Fixed

Graph #1

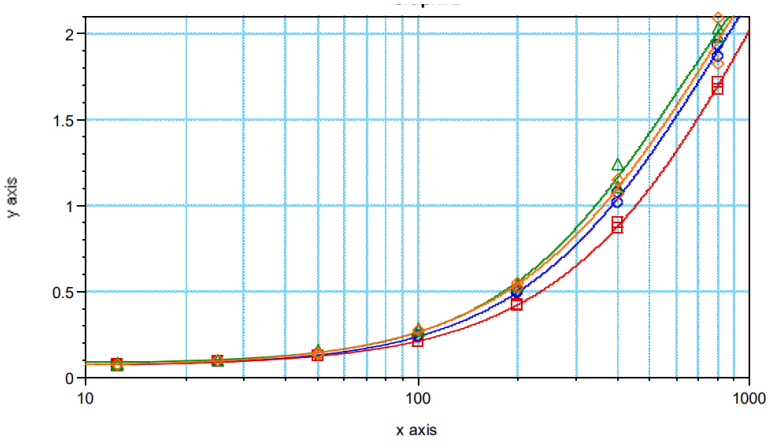
Blue: Sample Buffer

Red: Liver homogenate

Green: Brain homogenate

Orange: Kidney homogenate

Magenta: Gut homogenate (5-fold diluted)



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
Plot#1 (Sample Buffer P2: Concentration vs Values)	0.0648	1.44	821	3.81	0.999
Plot#2 (Spleen: Concentration vs Values)	0.0663	1.37	1.2e+03	4.53	1
Plot#3 (Spleen - 20% matrix: Concentration vs Val...	0.0805	1.54	625	3.33	0.998
Plot#4 (Mesenchyme - 20% matrix: Concentration ...	0.0654	1.38	829	3.95	0.994

Weighting: Fixed

Graph #2

Blue: Sample Buffer

Red: Spleen homogenate

Green: Spleen homogenate (5-fold diluted)

Orange: Mesenchyme homogenate (5-fold diluted)

Table 1. Percent recoveries obtained using modified protocol: *Compared to Standard Curve Prepared in Standard Diluent*

[mulFNa] pg/ml	Sample Buffer*	Liver	Brain	Kidney	Gut (20%)	Mesen-chyme (20%)	Spleen	Spleen (20%)
800	100	67.8	86.9	84	76.1	104.5	86	108.3
400	100.15	68.8	85.7	84.4	70.6	106	84.7	112.8
200	99.25	72.2	87.5	87	70.8	107.2	85.9	107.3
100	101.05	71.2	91	90.2	74.5	115.5	88.9	117.6
50	104.05	67.7	95.5	92.8	79.2	105.9	95.4	126.3
25	97.85	68.9	104.6	106.8	74.9	127.2	100.9	128.5
12.5	77.75	57.8	102.9	92.9	92.2	122.8	93.6	131.2
Average	100.4	69.4	93.4	91.2	76.9	112.7	90.8	116.8
Blanks	0.054	0.054	0.068	0.058	0.063	0.066	0.065	0.059

*Average of two plates. Shaded Values are excluded from analysis.

Table 2. Percent recoveries obtained using modified protocol: *Compared to Standard Curve Prepared in Homogenate Matrix*

[mulFNa] pg/ml	Sample Buffer*	LIver	Brain	Kidney	Gut (20%)	Mesen-chyme (20%)	Spleen	Spleen (20%)
800	100	100	100	100	100	100	100	99.9
400	100.15	99.7	100	100	100.1	100.2	100.1	100.8
200	99.25	101.5	99.9	100.1	99.5	99	99.7	96.7
100	101.05	97.5	100	99.8	101	104	99.9	106.4
50	104.05	93.9	100.2	98.3	102.5	91.8	102.9	110.2
25	97.85	105.1	103.5	108.7	90.4	107.2	102.4	89.5
12.5	77.75	123.8	91	87.9	105.2	97.7	82.2	ND
Average	100.4	99.6	99.2	99.3	99.8	100.0	98.2	100.6
Blanks	0.054	0.054	0.068	0.058	0.063	0.066	0.065	0.059

*Average of two plates. Shaded Values are excluded from analysis. ND = not detected

Table 3. Spike Recovery using 100% Mesenchyme Matrix *(Final Assay Conditions)*

Condition	OD (450 nm)	Percent Recovery
Mesenchyme - neat	0.059	---
Mesenchyme - 100 pg/ml spke	0.076	14.5%
Sample Buffer - neat	0.057	---
Sample Buffer - 10 pg/ml spike	0.218	92.2%

For the mesenchyme matrix, spike recovery using the final modified assay conditions yields low recovery in neat matrix.

Modified Protocol for Improved Results using Mouse Homogenate Matrices:

Reagents:

PBL Assay Science VeriKine™ Mouse Interferon Alpha ELISA Kit (Catalog No. 42120)
Casein Buffer 20X-4X, 5.5%, biotin-free (SDT Reagents, Germany. #CBC1, Lot# 361748, or equivalent)

Procotol:

1. Prepare Modified standard curve in Sample Buffer (no casein) or matrix:

Mouse Interferon Alpha stock concentration: 10,000 pg/ml

Mouse IFN-α concentration	Tube	Amount of Sample Buffer added to tube	Preparation
800 pg/ml	S7	920 ul	Add 80 ul of Mouse IFN-α stock. Mix**
400 pg/ml	S6	500 ul	Add 500 ul of S7. Mixx**
200 pg/ml	S5	500 ul	Add 500 ul of S6. Mix**
100 pg/ml	S4	500 ul	Add 500 ul of S5. Mix**
50 pg/ml	S3	500 ul	Add 500 ul of S4. Mix**
25 pg/ml	S2	500 ul	Add 500 ul of S3. Mix**
12.5 pg/ml	S1	500 ul	Add 500 ul of S2. Mix**
Blank (0 pg/ml)	B1	500 ul	No additions (sample buffer only)

** Mix each gently by repeated pipetting of ~500 ul volume without introducing air bubbles. Discard tips in between dilutions.

Procotol (continued):

2. Prepare “Sample Buffer + 1% Casein”. For a single ELISA plate, 6 ml would be required.
   4.9 ml sample buffer
   1.1 ml 5.5% casein solution

3. Plate preparation (0.33% Casein final):
   a) Add 50 ul of ”Sample Buffer + 1% Casein” to all wells.
   b) Add 50 ul of standard curve or matrix.

   ++*Please note that gut and mesenchyme homogenate must be diluted prior to step b.*++
   c) Add 50 ul of antibody solution (prepared as described in the original protocol).
   d) Remainder of assay is executed identically to the published protocol.