VeriKine-HS™ Human IL-15 ELISA Kit (Cat. No. 41702-1)
Technical Data Sheet

Assay Range: 0.51 – 32.8 pg/ml
Compatibility: Serum, Plasma, Tissue Culture Media (TCM)
Assay Length: 4 hr

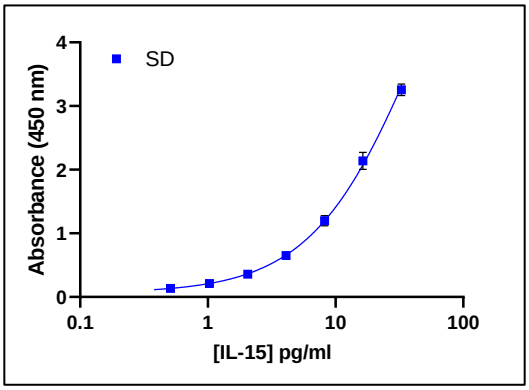
INTRODUCTION

Interleukin-15 (IL-15) is a member of the IL-2 family of cytokines which also includes IL-4, -7, -9 and -21[1,2,3]. It has similar and unique functions compared to IL-2. Signaling by IL-15 can be through the cytokine alone, in complex with soluble IL-15Rα and transpresented bound to IL-15Rα. It is produced largely by monocytic cells but also by dendritic cells, keratinocytes, fibroblasts, myocytes, and nerve cells. It seems to enhance the expansion of CD8 memory cells, NK and NK-T cells[4]. IL-15 plays a role in tumor biology and autoimmune diseases including psoriasis, rheumatoid arthritis, inflammatory bowel disease, celiac disease, alopecia areata and systemic lupus erythematosus[5,6].

This ELISA measures IL-15 in tissue culture media (TCM), serum and plasma. It measures both IL-15 alone and IL-15/IL-15Rα complexes and quantitates IL-15 in healthy donor matrices. This assay's standard is the IL-15 receptor heterodimer, but the standard curve is calibrated to the IL-15 portion of the complex since the antibodies used are IL-15 specific.

37 Healthy Donor Serum (HDS) and 43 Healthy Donor Plasma (HDP) samples were all quantifiable and as such this assay may allow for measurement of basal IL-15 levels.

Figure 1. Typical Standard Curve in Standard Diluent Human IL-15 standard curves were prepared in Standard Diluent (SD) and assayed in triplicate over 9 runs. The figure below shows dose response and absorbance values. Error bars indicate standard deviation.



Specifications This kit quantitates *free* human IL-15 and *bound* IL-15/IL-15R in serum, plasma and TCM using a sandwich immunoassay. The kit is based on an ELISA with biotinylated-detection antibody and streptavidin-conjugated horseradish peroxidase (HRP). Tetramethyl-benzidine (TMB) is the substrate.

Specificity Human IL-15. No cross-reactivity detected with human IL-2, IL-4, IL-7, IL-9, IL-21, IFN-α, IFN-β1a, IFN-γ, IFN-ω; cynomolgus IFN-α2; cynomolgus/rhesus IFN-α; mouse IL-15, IFN-αA, IFN-β, IFN-γ; rat IL-15, IFN-α1, IFN-α14, IFN-β, IFN-γ; bovine IFN-τ2; or pig IFN-α.

References

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2. Grabstein KH, *et al*. Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. Science. 1994 May;264(5161):965-8.
3. Burton, JD, *et al*. A lymphokine, provisionally designated interleukin T and produced by a human adult T-cell leukemia line, stimulates T-cell proliferation and the induction of lymphokine-activated killer cells. PNAS. 1994;91:4935-4939.
4. Ring, AM, *et al*. Mechanistic and structural insight into the functional dichotomy between IL-2 and IL-15. Nat Immunol. 2012;13(12):1187-95.
5. Waldmann, TA. The biology of IL-15: implications for cancer therapy and the treatment of autoimmune disorders. J Investig Dermatol Symp Proc. 2013 Dec;16(1):S28-30.
6. Clark, DN, *et al*. Cytokine inhibition as a strategy for treating systemic lupus erythematosus. Clin Immunol. 2013;148(3):335-43.

PRECISION

Table 1. Intra-Assay Precision To test precision within an assay, 26 replicates of six HDS samples with different endogenous levels were each tested on a single plate.

Intra-Assay Precision						
Sample	**1**	**2**	**3**	**4**	**5**	**6**
n	26	26	26	26	26	26
Mean (pg/ml)	4.17	4.42	4.20	2.81	3.85	3.87
Std. Dev.	0.18	0.09	0.18	0.10	0.13	0.13
CV (%)	4.4	1.9	4.3	3.6	3.3	3.3

Tables 2 – 4. Inter-Assay, Inter-Batch, and Intermediate Precision To test precision between assays, six individual HDS and one serum pool with different endogenous levels were tested over 27 assays by 7 operators using 3 different lots. Inter-assay precision measures sample concentration variance during five assays by the same operator within a single lot. Inter-batch precision measures 9 independent assays within each lot. Intermediate precision measures 27 assays run by 7 operators using 3 lots of components.

Inter-Assay Precision							
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Mean (pg/ml)	4.52	3.55	4.47	2.87	3.54	12.55	3.68
Std. Dev.	0.24	0.16	0.17	0.16	0.19	0.61	0.13
CV (%)	5.3	4.6	3.7	5.4	5.4	4.8	3.4

Inter-Batch Precision							
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Lot 1 (pg/ml)	5.26	3.97	5.12	3.24	4.02	13.94	4.18
Lot 2 (pg/ml)	5.16	3.94	5.09	3.20	3.97	13.58	4.26
Lot 3 (pg/ml)	4.80	3.62	4.71	2.85	3.66	12.77	3.86
Mean (pg/ml)	5.07	3.84	4.97	3.10	3.88	13.43	4.10
Std. Dev.	0.24	0.19	0.22	0.21	0.20	0.60	0.21
CV (%)	4.7	5.0	4.5	6.8	5.1	4.5	5.1

Intermediate Precision							
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
n	27	27	27	27	27	27	27
Mean (pg/ml)	5.07	3.84	4.97	3.10	3.88	13.43	4.05
Std. Dev.	0.63	0.49	0.57	0.38	0.45	1.30	0.40
CV (%)	12.5	12.9	11.5	12.4	11.6	9.7	9.9

SPIKE RECOVERY

Table 5. Spike Recovery Human IL-15 was spiked into three TCM, five Disodium-EDTA plasma and five HDS samples at three known concentrations. The range indicates the spread of the mean recovery among samples.

TCM			
Spike Sample	**1**	**2**	**3**
Target Conc. (pg/ml)	24.61	6.15	0.82
Mean Recovery (%)	92.8	97.0	94.0
Range (%)	91.5 – 95.1	93.3 – 99.0	93.5 – 94.5
Disodium-EDTA Plasma			
Spike Sample	**1**	**2**	**3**
Target Conc. (pg/ml)	24.61	6.15	0.82
Mean Recovery (%)	98.1	99.9	82.1
Range (%)	78.4 – 104.5	85.1 – 108.1	64.9 – 95.3
Serum			
Spike Sample	**1**	**2**	**3**
Target Conc. (pg/ml)	24.61	6.15	0.82
Mean Recovery (%)	99.2	94.7	75.6
Range (%)	93.1 – 103.4	90.0 – 99.6	57.1 – 97.4

PERFORMANCE CHARACTERIZATION

Figures 2 – 3. Matrix Compliance Human IL-15 standard curves were prepared in HDS, SD and TCM and assayed in triplicate over a single batch of kits. Figure 2 shows dose response curves and absorbance values in various matrices. The HDS pooled sample contains endogenous human IL-15 quantified separately at 4.05 pg/ml (see Sample 7, Table 4), and this endogenous IL-15 in the HDS pool results in the vertical OD displacement throughout the HDS curve. In addition, whereas the top standard point on the SD and TCM curves was 32.8 pg/ml, the top standard on the HDS curve was prepared by spiking in 24.6 pg/ml of PBL's IL-15 ELISA standard into the HDS pool so that the additional 4.05 pg/ml of endogenous HDS IL-15 would not contribute to an excessive OD that would be off scale. This standard adjustment from 32.8 to 24.6 pg/ml of IL-15 accounts for the apparent horizontal shift in the HDS standard curve vs. the SD and TCM curves. Figure 3 shows percent recoveries of the human IL-15 standard at different concentrations. The SD curve was used to calculate percent recovery for both curves. Endogenous levels were subtracted in the HDS curve to calculate and plot corrected backfit concentrations of the standard. Both Figure 2 and 3 display the mean of 9 runs, and error bars indicate the standard deviation.

Figure 2. Representative Standard Curves in Various Matrices
(Note: SD and TCM curves overlap)

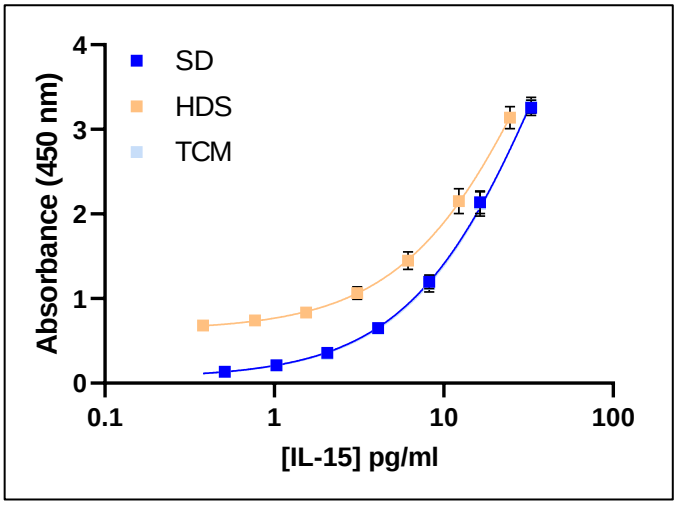

Figure 3. Percent Recovery of IL-15 in Various Matrices

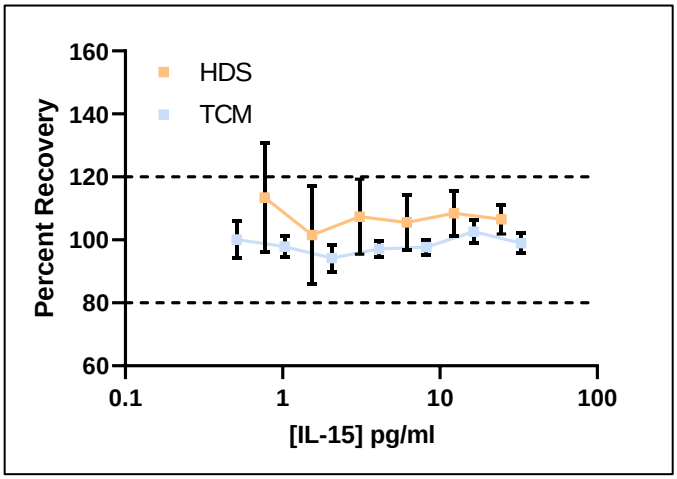

Table 6. Assay Sensitivity Three runs were evaluated to measure minimal detectable dose (MDD) and limit of quantitation (LOQ). MDD and LOQ are determined by adding 3 or 10 standard deviations, respectively, to the mean optical density value of eighty zero standard replicates and calculating corresponding concentrations. The standard curve was prepared using 2-fold dilutions to assess an assay range of 0.26–32.8 pg/ml.

MDD had a mean value of 0.151 pg/ml (range: 0.104-0.175 pg/ml). LOQ had a mean value of 0.496 pg/ml (range: 0.341-0.575 pg/ml).

MDD/LOQ			
Assay	**1**	**2**	**3**
n	80	80	80
Mean Blank (A_{450})	0.055	0.052	0.057
Std. Dev.	0.004	0.007	0.008
MDD (A_{450})	0.067	0.072	0.079
MDD (pg/ml)	0.104	0.173	0.175
LOQ (Abs_{450})	0.096	0.121	0.132
LOQ (pg/ml)	0.341	0.571	0.575

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Figures 4 – 5. **Linearity of Dilution in HDS & TCM** Pooled HDS with known IL-15 endogenous levels and an IL-15-free TCM were spiked with a known concentration of IL-15. Samples were diluted two-fold in their respective matrix to assess reliable quantification after dilution within the standard curve. Pooled serum was diluted to a 32-fold dilution and endogenous levels were subtracted from dilution-corrected backfit concentrations; TCM to a 64-fold dilution. Linearity was performed among 3 kit batches with 9 independent operator runs per batch. Error bars indicate the standard deviation of the percent recovery against the top spiked value.

Figure 4. Linearity in Pooled Serum (HDS)

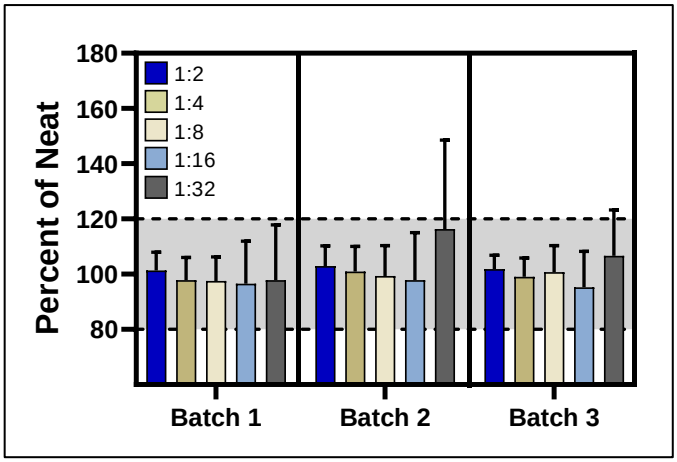
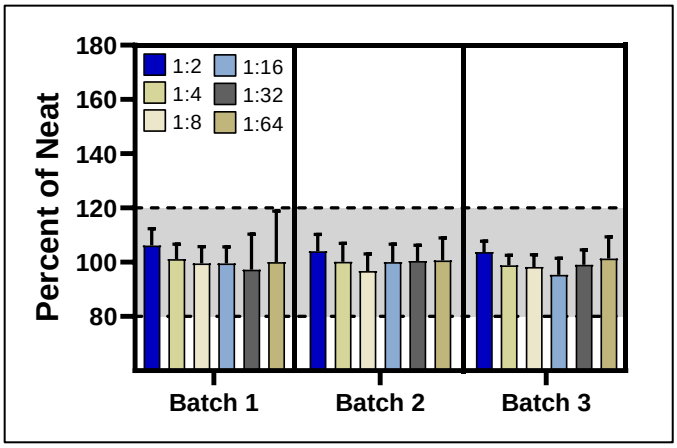


Figure 5. Linearity in Tissue Culture Media



Figures 6 – 7. Parallelism in Serum and Plasma Four HDS and three HDP samples with high levels of endogenous IL-15 were assayed in duplicate to assess reliable quantification. The standard curve was prepared using 2-fold dilutions to assess an assay range of 0.26–32.8 pg/ml. Endogenous samples were diluted in SD to a 16-fold dilution. Mean sample parallelism fell within 100±20% of the neat value. Error bars indicate standard deviation. Bars with the hash fill indicate the calculated sample concentration fell below the LOQ, but read at values higher than the 0.26 pg/ml calibration level.

Figure 6. Parallelism in Individual Sera (HDS)

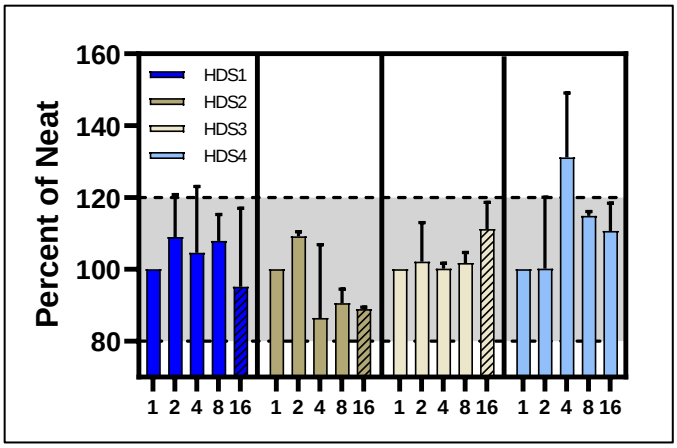


Figure 7. Parallelism in Plasma (HDP)

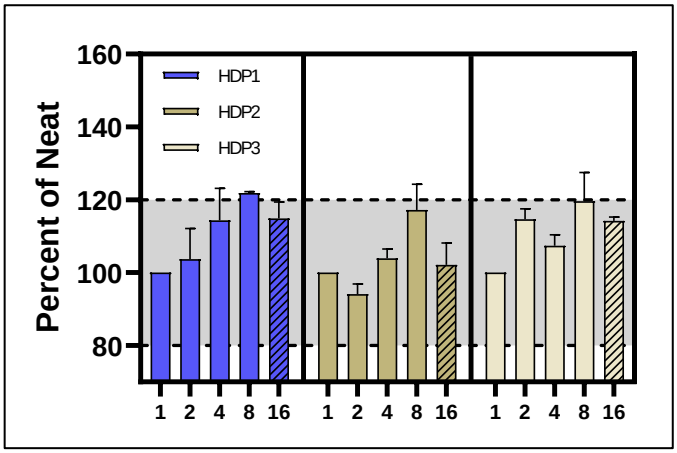
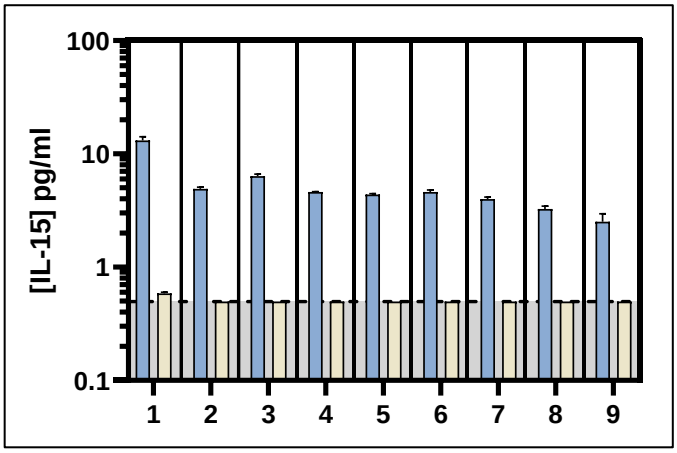


Figure 8 and Table 7. Knockout of IL-15 in HDS and HDP Samples under Control (Blue) and Specific (Beige) Knockout Conditions Nine samples (7 sera and plasma previously tested within assay parallelism, one serum, and one pooled serum) were pre-incubated with 100x excess of the PBL IL-15 capture antibody and a non-specific isotype-matched antibody. Sample concentration recoveries were compared against IL-15 control conditions where the sample was not treated with a blocking antibody and assayed following kit protocol.

In figure 8, samples treated with PBL's IL-15 specific capture antibody (beige) displayed knockdown of the observed sample concentration when compared to untreated control samples (blue). Samples treated with the specific IL-15 capture antibody recorded IL-15 levels at or below the assay LOQ.

Figure 8. IL-15 Knockout under Control (Blue) and Specific (Beige) Conditions



In table 7, incubation with PBL's IL-15 reagent resulted in knockout efficiencies of 95-100%. Under isotype incubation conditions, most samples displayed concordance with the observed control IL-15 levels.

Table 7. IL-15 Concentrations in under Knockout Conditions in HDS and HDP Samples

Sample	[IL-15] pg/ml Control	[IL-15] pg/ml Specific	[IL-15] pg/ml Isotype
1	13.12	0.59	6.99
2	4.92	<LOQ	4.4
3	6.38	<LOQ	3.87
4	4.61	<LOQ	4.66
5	4.38	<LOQ	4.55
6	4.61	<LOQ	4.66
7	3.98	<LOQ	3.51
8	3.27	<LOQ	2.95
9	2.52	<LOQ	2.46

PERFORMANCE COMPARISON

Summary PBL's IL-15 ELISA was internally characterized against three commercial IL-15 ELISAs. PBL exhibited higher sensitivity and greater normal sample precision. Note that PBL's standard is human cell-expressed (HEK293) IL-15/IL-15R, whereas competitor standards are *E.coli*-expressed free IL-15 protein.

Figure 9. Representative Standard Curves on PBL's IL-15 ELISA (41702) PBL's and three competitor's standards were assayed on their respective ELISAs. Dose response curves and absorbance values of all IL-15 standards are shown below. PBL's standard exhibited greater sensitivity than competitive IL-15 standards; PBL's standard curve demonstrated an improvement of 1 – 2 log units.

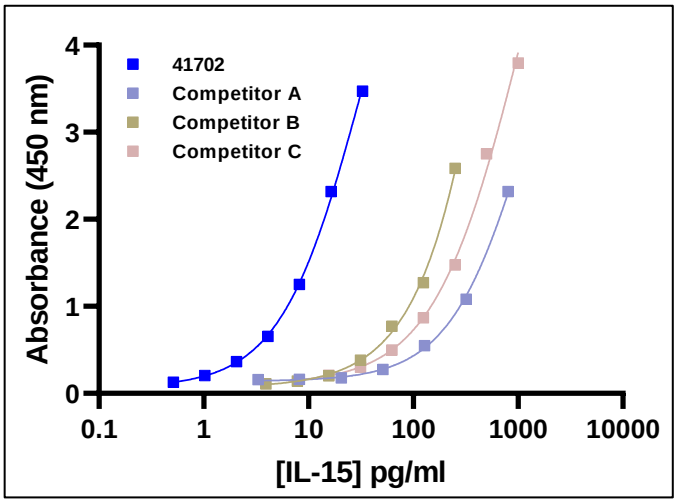


Figure 10. Comparison of PBL's HEK Standard on Commercial ELISAs PBL's HEK293 IL-15/IL-15R standard was assayed on three commercial IL-15 kits based on the suggested kit calibration range in order to investigate selectivity towards the glycosylated IL-15/IL-15R complex. Human cell-expressed IL-15 contains multiple glycosylation patterns and may be predominately expressed as a complex, as opposed to the free protein. Accurate and precise measurements may require recognition of this form of IL-15[5]. Competitor A and C's IL-15 ELISAs were not able to detect the glycosylated IL-15/IL-15R complex. Competitor B's ELISA displayed an approximately 50% decrease in calibration dose response. The 41702 assay standard curve is presented for reference purposes.

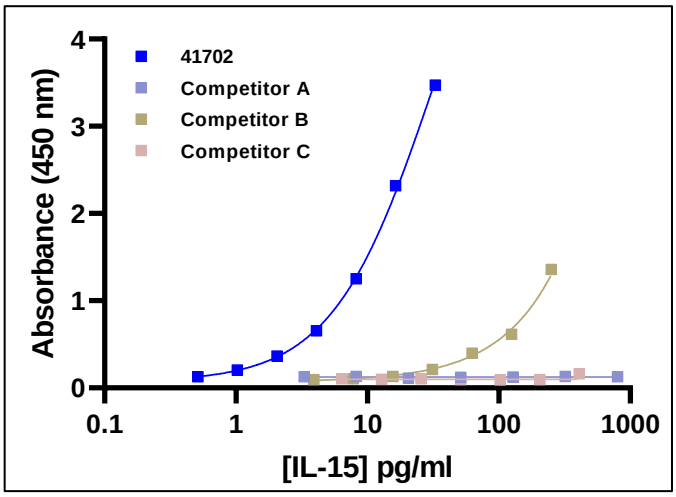


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Table 8. Healthy Donor Readability Comparison 37 HDS and 43 HDP samples were assayed on PBL and Competitor IL-15 ELISAs. Relative serum and plasma were assayed in duplicate on each respective kit. The PBL assay exhibited the ability to detect low endogenous levels in healthy samples. Competitors A-C quantified between 0-30% of HDS and 0-50% of HDP; PBL's 41702 quantitated 100% of HDS and HDP samples.

Sample Type	Quantifiable Mean (pg/ml)	Range (pg/ml)	% above LOQ	% below LOQ	%CV
PBL's ELISA					
Serum	4.21	2.64– 13.75	100 (30/30)	0 (0/30)	3.0
EDTA Plasma	3.01	1.75– 4.17	100 (17/17)	0 (0/17)	2.4
Citrate Plasma	2.42	1.41– 3.99	100 (13/13)	0 (0/13)	3.1
Heparin Plasma	2.63	1.62– 3.87	100 (13/13)	0 (0/13)	2.9
Competitor A's ELISA					
Serum	91.51	17.84– 244.51	30 (9/30)	70 (21/30)	30.1
EDTA Plasma	35.13	8.89– 126.25	47.1 (8/17)	52.9 (9/17)	10.7
Citrate Plasma	22.00	10.56– 38.40	46 (6/13)	54 (7/13)	14.7
Heparin Plasma	58.48	40.31– 76.66	20 (2/10)	80 (8/10)	5.0
Competitor B's ELISA					
Serum	5.78	4.13– 8.11	10 (3/30)	90 (27/30)	6.1
EDTA Plasma	4.74	N/A	5.9 (1/17)	94.1 (16/17)	N/A
Citrate Plasma	N/A	N/A	0 (0/13)	100 (13/13)	N/A
Heparin Plasma	N/A	N/A	0 (0/10)	100 (10/10)	N/A
Competitor C's ELISA					
Serum	N/A	N/A	0 (0/30)	100 (30/30)	N/A
EDTA Plasma	N/A	N/A	0 (0/18)	100 (18/18)	N/A
Citrate Plasma	N/A	N/A	0 (0/12)	100 (12/12)	N/A
Heparin Plasma	N/A	N/A	0 (0/10)	100 (10/10)	N/A

Visit the product page on PBL's website
(https://pblassaysci.com)
to view the one-page CoA/Protocol and
further information on this product (including references).