



## DELFLIA Eu-labeled Goat Anti-HRP Antibody Toolbox Kit

### *For ELISA Conversion*

**Product No.:** DFA500-96S-1, DFA500-96S-5, DFA500-HALF-1, DFA500-HALF-5

### Product Information

- Description:** DELFLIA® Eu-labeled Anti-HRP Kit contains all the necessary reagents to convert ELISA to DELFLIA (Dissociation-Enhanced Lanthanide Fluorescence ImmunoAssay) assays. DELFLIA Eu-labeled Anti-HRP Antibody included in the kit is used as a secondary detection antibody to detect HRP-conjugated primary detection antibodies or HRP-conjugated molecules. The existing ELISA kits that use HRP as enzyme can be easily converted the DELFLIA assay using DELFLIA® Eu-labeled Anti-HRP Kit.
- Application:** DELFLIA immunoassays are a superior performance alternative to ELISA and are similar in format and workflow. Hence, a seamless transition from ELISA to DELFLIA is possible. The DELFLIA assays can be build in all classical immunoassay formats such as direct or indirect, sandwich, and competition assay. The DELFLIA assays can be used to analyze the complex sample mattries such as blood, serum, plasma, and other samples. More details are provided in DELFLIA User Guide.
- DELFLIA:** Time-resolved fluorometry (TRF) is a well-established technique in drug discovery and basic research. Delivering high sensitivity and wide dynamic range, TRF is characterized by decreased background autofluorescence during measurement. TRF-based DELFLIA® technology provides a wash-based immunoassay technology that offers significant advantages over traditional ELISA:
- High Sensitivity:** Ideal for complex sample matrices; accurately detect femtogram quantities of analyte.
  - Wide Dynamic Range:** Save time and cost by eliminating extensive sample preparations, assay repeats, and dilutions
  - Superior Stability:** Read plates months later upon proper storage, with a stable fluorescent signal that is not time-sensitive
  - Proven Technology:** supported by thousands of peer-reviewed publications, studying disease diagnostics, neonatal screening, and drug discovery.
  - Formats:** In addition to using the DELFLIA Microtitration Plate (96-Well Clear Strip Plate), the assay can also be performed in a DELFLIA compatible ½ AreaPlate-96 HB (½ AreaPlate-96 High Binding) to save materials.
- Storage:** Store in the dark at 4 °C.
- Stability:** This toolbox kit is stable for at least 12 months from the manufacturing date when stored in its original packaging under recommended storage conditions.

## Kit Content: Reagents and Materials Provided

Components	DFA500-96S-1 (1 plate)	DFA500-96S-5 (5 plates)	DFA500-HALF-1 (1 half-area plate)	DFA500-HALF-5 (5 half-area plates)
DELFLIA Eu-labeled Anti-HRP Antibody*	20 µL @ 100 µg/mL (1 Clear tube, Clear cap)	100 µL @ 100 µg/mL (1 Clear tube, Clear cap)	20 µL @ 100 µg/mL (1 Clear tube, Clear cap)	50 µL @ 100 µg/mL (1 Clear tube, Clear cap)
DELFLIA Wash Concentrate	2X25 mL @ 25X (2 bottles)	250 mL @ 25X (1 bottle)	25 mL @ 25X (1 bottle)	125 mL @ 25X (1 bottle)
DELFLIA Assay Buffer	2X25 mL (2 bottles)	250 mL (1 bottle)	25 mL (1 bottle)	125 mL (1 bottle)
DELFLIA Enhancement Solution	25 mL (1 bottle)	125 mL (1 bottle)	15 mL (1 bottle)	75 mL (1 bottle)
DTPA-Purified BSA (7.5%)	2X2.5 mL (2 bottles)	2X12.5 mL (2 bottles)	2.5 mL (1 bottle)	12.5 mL (1 bottle)
DELFLIA Microplate	1 (DELFLIA Microtitration Plate)	5 (DELFLIA Microtitration Plate)	1 (½ AreaPlate-96, HB)	5 (½ AreaPlate-96, HB)

\* The amount is based on assay volume:

- 100 µL/well using a final concentration of 100 ng/mL in 96-well strip plate (DELFLIA Microtitration Plate) format, and
- 50 µL/well using a final concentration of 100 ng/mL in ½ AreaPlate-96, HB format.

## Additional Reagents and Materials

The following items are required but not included in the toolbox:

Items	Suggested Source	Catalog #
PBS	GIBCO(ThermoFisher)	10010-023
Plate Lid	Revvity	6000027
TopSeal™-A Plus Adhesive Sealing Film	Revvity	6050185
Plate Reader with TRF Option	Revvity	EnVision™, Victor®, Victor Nivo™, EnSight™
DELFLIA plate shaker (optional)	Revvity	1296-003(For countries use 240 volt) 1296-004(For countries use 120 volt)
DELFLIA plate washer (optional)	Revvity / BioTek	1296-0010/ 405™TMS

## EnVision Plate Reader Instrument Setting for DELFIA

Excitation Source	Flash Lamp	TRF Laser Unit (337 nm)
Top Mirror	#402 (D400)	#445 (D400)
Excitation Filter	#101 (X340)	Not Applicable
Emission Filter	#203 (M615)	#203 (M615)
Measurement Height (mm)	6.5	6.5
Excitation Light (%)	100	100
Delay (µs)	400	400
Window time (µs)	400	400
Time between flashes (µs)	2000	2000
Number of flashes	100	100

## DELFIA General Manual

The manuals described below are the examples of building two DELFIA assays (human IL-18BPα and human IL-20) in DELFIA Microtitration Plate and ½ AreaPlate-96 HB (white).

- To build the human IL-20 DELFIA assay, analyte and antibodies in the IL-20 ELISA antibody pair set (Sino Biological, Cat # SEK13060) were bought separately. When building human IL-20 DELFIA assay, rabbit anti-human IL-20 antibody was coated to the assay plate and HRP-conjugated mouse anti-human IL-20 primary antibody was used as detection antibody. The HRP-conjugated detection antibodies in IL-20 DELFIA assays were then detected using DELFIA Eu-labeled Anti-HRP Antibody provided in the toolbox kit.
- To build human IL-18BPα DELFIA assay, analyte and antibodies in IL-18 BPα DuoSet ELISA kit (R&D System, Cat # DY119) were also bought separately. When building the IL-18BPα DELFIA assay, mouse anti-human IL-18BPα antibody was coated to the assay plate and biotinylated goat anti-human IL-18BPα antibody used as primary detection antibody. The biotinylated primary detection antibody was then bound to SA-HRP. SA-HRP was detected with DELFIA Eu-labeled Anti-HRP Antibody.
- To compare DELFIA assay performance to the corresponding ELISA, the ELISA assay was run in the same configuration as DELFIA except that the DELFIA Eu-labeled Anti-HRP antibody was replaced with alkaline phosphatase (AP) conjugated anti HRP antibody and the DELFIA Enhancement Solution in was replaced with ELISA AP substrate and stop solutions.
- Converting the existing ELISA kit to DELFIA: Human IL-20 ELISA Kit (Sino Biological, Cat # KIT13060) and human IL-18BPα Quantikine ELISA Kit (R&D System, Cat # DBP180) are converted to DELFIA kit by replacing ELISA HRP substrate and stop solution with DELFIA Eu-labeled Anti-HRP antibody and DELFIA enhancement solution. For this conversion, the assays are performed following procedures that are provided in the ELISA kit manual. These include using the pre-coated plate and all the reagents provided in the ELISA kit; except ELISA HRP substrate and stop solution are replaced with DELFIA Eu-labeled Anti-HRP antibody (200 ng/mL prepared in DELFIA Assay Buffer, 100 µL per well, incubate 1 hour, wash 6 times) and DELFIA Enhancement Solution (incubate 10 min and read the plate with Envision with TRF option).

## I. Manual for DELFIA Microtitration Plate (96-Well Clear Strip Plate):

### Assay Procedures

1. Dilute the capture antibody to the working concentration (2 µg/mL) in 1xPBS. Immediately coat a DELFIA Microtitration Plate with 100 µL per well (200 ng capture antibody per well) of the diluted capture antibody. Seal the plate cover slip and incubate overnight at 23 °C.
2. Wash the plate 3x with (300 µL in DELFIA wash buffer/well) using a plate washer. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Blocking is done by adding 300 µL per well 1% BSA prepared in 1xPBS (prepared from DELFIA BSA stabilizer 7.5%). Cover with a plate lid and incubate the plate at room temperature for 1 hour on a plate shaker ~300RPM.
4. Aspirate the blocking solution with a plate washer or discard the blocking solution from the plate by inverting the plate over the liquid waste container. Remove any remaining buffer by inverting the plate and blotting it against clean paper towels. The plates are now ready for standard or sample addition.
5. Prepare the standards and samples in DELFIA Assay Buffer during the 1 hour blocking step.
6. Add 100 µL of standards or samples to pre-designated wells. Cover with a plate lid and incubate for 1 hour at room temperature on a plate shaker at ~300 rpm.
7. Repeat plate washing as in step 2.
8. Add 100 µL (20 ng) of the 200 ng/mL biotinylated or HRP-conjugated primary detection antibody diluted in DELFIA Assay Buffer to each well. Cover with a plate lid and incubate for 1 hour at room temperature on a plate shaker at ~300 rpm.
9. Repeat plate washing as in step 2.  
  
**If the primary antibody is labeled with biotin, go to step 10.**  
**If the primary antibody is labeled with HRP, go directly to step 12.**
10. Add 100 µL of 1x HRP-labeled streptavidin (SA-HRP) prepared in DELFIA Assay Buffer to each well. Cover with a plate lid and incubate for 1 hour at room temperature on a plate shaker at ~300 rpm.
11. Repeat plate washing as in step 2.
12. Add 100 µL (20 ng) of 200 ng/mL DELFIA Eu-labeled anti-HRP antibody prepared in DELFIA Assay Buffer to each well. Cover with a plate lid and incubate for 1 hour at room temperature on a plate shaker at ~300 rpm.
13. Repeat plate washing as in step 2. However, wash **6** times at this step.
14. Add 200 µL of DELFIA Enhancement Solution to each well. Cover with a plate lid and incubate for 10 min at room temperature on a plate shaker at ~300 rpm.
15. Read plate using TRF settings (see the Table in Instrument Setting Section). The developed signal will be stable for at least 24 hours when stored properly by covering tightly with parafilm.

## II. Manual for ½ AreaPlate-96 HB:

### Assay Procedures

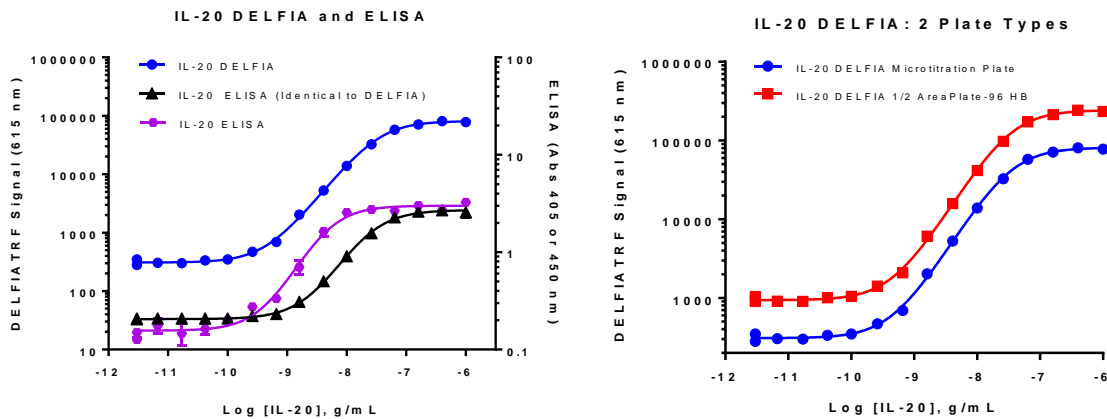
1. Dilute the capture antibody to the working concentration (2 µg/mL) in 1xPBS. Immediately coat a DELFIA Microtitration Plate with 50 µL per well (200 ng capture antibody per well) of the diluted capture antibody. Seal the plate cover slip and incubate overnight at 23 °C.
2. Wash the plate 3x with (150 µL in DELFIA wash buffer/well) using a plate washer. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Blocking is done by adding 150 µL per well 1% BSA prepared in 1xPBS (prepared from DELFIA BSA stabilizer 7.5%). Cover with a plate lid and incubate the plate at room temperature for 1 hour on a plate shaker ~300RPM.
4. Aspirate the blocking solution with a plate washer or discard the blocking solution from the plate by inverting the plate over the liquid waste container. Remove any remaining buffer by inverting the plate and blotting it against clean paper towels. The plates are now ready for standard or sample addition.
5. Prepare the standards and samples in DELFIA Assay Buffer during the 1 hour blocking step.
6. Add 50 µL of standards or samples to pre-designated wells. Cover with a plate lid and incubate for 1 hour at room temperature on a plate shaker at ~300 rpm.
7. Repeat plate washing as in step 2.
8. Add 50 µL (20 ng) of the 200 ng/mL biotinylated or HRP conjugated detection antibody diluted in DELFIA Assay Buffer to each well. Cover with a plate lid and incubate for 1 hour at room temperature on a plate shaker at ~300 rpm.
9. Repeat plate washing as in step 2.  
  
**If the primary antibody is labeled with biotin, go to step 10.**  
**If the primary antibody is labeled with HRP, go directly to step 12.**
10. Add 50 µL of 1x HRP-labeled streptavidin (SA-HRP) prepared in DELFIA Assay Buffer to each well. Cover with a plate lid and incubate for 1 hour at room temperature on a plate shaker at ~300 rpm.
11. Repeat plate washing as in step 2.
12. Add 50 µL (20 ng) of 200 ng/mL DELFIA Eu-labeled anti-HRP antibody prepared in DELFIA Assay Buffer to each well. Cover with a plate lid and incubate for 1 hour at room temperature on a plate shaker at ~300 rpm.
13. Repeat plate washing as in step 2. However, wash **6** times at this step.
14. Add 10 µL of DELFIA Enhancement Solution to each well. Cover with a plate lid and incubate for 10 min at room temperature on a plate shaker at ~300 rpm.
15. Read plate using TRF settings (see the Table in Instrument Setting Section). The developed signal will be stable for at least 24 hours when stored properly by covering tightly with parafilm.

## Typical Standard Curve, Data Analysis, and Comparisons

Standard curve for DELFIA immunoassay was plotted in GraphPad Prism Version 7.0 and analyzed with nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) with  $1/Y^2$  weighting method. Lower limit of detection (LDL) were calculated using the following equations:  $LDL = \text{mean (blanks)} + 3 * SD$  (Standard Deviation). The signal of unknown samples should be interpolated to the standard curve prepared in same assay.

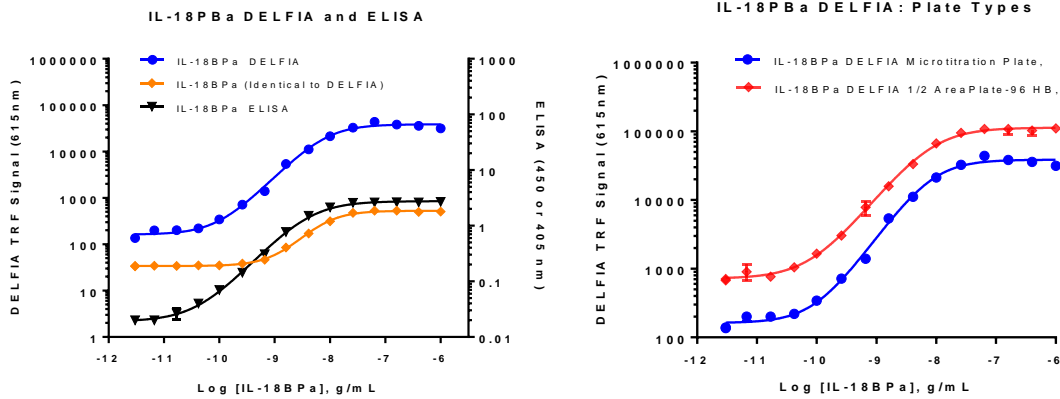
Typical results of DELFIA and ELISA assay by using human IL-20 Antibody Pair Set (Sino-Biological Cat # SEK13060) and human IL-18BPα DuoSet ELISA (R&D Systems Cat# DY119). The DELFIA plates were read on an EnVision-2105 multimode plate reader with TRF flash lamp option. The ELISA assay plates were read on an EnVision-2105 multimode plate reader equipped with absorbance options and the optical densities (OD) with read at 405, 450 nm and 540 nm. ELISA kit data was analyzed using two wavelength readings for background correction at 540 nm.

### Comparisons of IL-20 DELFIA and ELISA Assays



IL-20 Assay Types	Plate Types	Manuals	Max Counts or Abs	Min Counts or Abs	Dynamic Range (pg/mL)	LDL (pg/mL)	EC <sub>50</sub> (ng/mL)	S/B Ratio
IL-20 DELFIA	DELFIA Microtitration Plate	DELFIA	80799	302	67-64000	67	34	267
IL-20 DELFIA	DELFIA 1/2 Area Plate	DELFIA	242396	976	107-64000	107	38	248
IL-20 ELISA	DELFIA Microtitration Plate	ELISA (Identical to DELFIA)	2.80	0.12	130-64000	130	22	23
IL-20 ELISA	DELFIA Microtitration Plate	ELISA Pair Set	2.69	0.17	155-10240	155	5	17

## Comparisons of IL-18BP<sub>a</sub> DELFIA and ELISA Assays



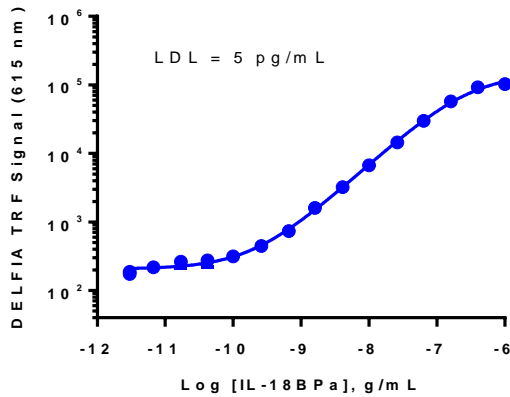
IL-18BP <sub>a</sub> Assay Types	Plate Types	Manuals	Max Counts or Abs	Min Counts or Abs	Dynamic Range (pg/mL)	LDL (pg/mL)	EC <sub>50</sub> (ng/mL)	S/B Ratio
IL-18BP <sub>a</sub> DELFIA	DELFIA Microtitration Plate	DELFIA	43878	138	5-160000	5	8	318
IL-18BP <sub>a</sub> DELFIA	1/2 AreaPlate-96 HB	DELFIA	108239	693	13-160000	13	8	166
IL-18BP <sub>a</sub> ELISA	DELFIA Microtitration Plate	ELISA (Identical to DELFIA)	1.87	0.14	34-25600	34	7	13
IL-18BP <sub>a</sub> ELISA	DELFIA Microtitration Plate	DuoSet ELISA	2.56	0.02	6-25600	6	4	142

These results indicate that DELFIA assays provide wider dynamic range, higher sensitivity, and greater signal to background ratio than traditional ELISA assays. Additionally, the DELFIA assays can be performed in DELFIA 1/2 AreaPlate-96 HB plate to save reagents.

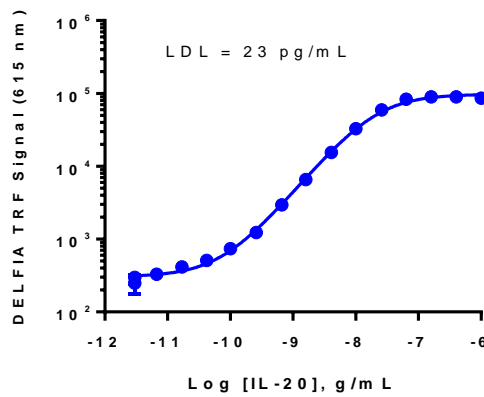
**The existing ELISA kits can be easily converted to DELFIA.**

Human IL-20 ELISA Kit (Sino Biological, Cat # KIT13060) and human IL-18BP a Quantikine ELISA Kit (R&D System, Cat # DBP180) are converted to DELFIA by replacing ELISA kit HRP substrate and stop solution with DELFIA Eu-labeled Anti HRP antibody and DELFIA enhancement solution. For this conversion, the assays are performed exact same procedures that is provided in the ELISA kit manual. These include using the pre-coated plate and all the reagents provided in the ELISA kit except ELISA HRP substrate and stop solution are replaced with DELFIA Eu-labeled Anti-HRP antibody (200 ng/mL prepared in DELFIA Assay Buffer, 100 µL per well, incubate 1 hour, wash 6 times) and DELFIA enhancement solution (200 µL per well, incubate 10 min, and read the plate on an EnVision-2105 multimode plate reader with TRF flash lamp option).

**Direct Conversion of IL-18BP a ELISA kit to IL-18BP a DELFIA**



**Direct Conversion of IL-20 ELISA kit to IL-20 DELFIA**



IL-18BP a Assay Types	Plate Types	Manuals	Max Counts	Min Counts	Dynamic Range (pg/mL)	LDL (pg/mL)	EC <sub>50</sub> (ng/mL)	S/B Ratio
IL-18BP a DELFIA*	ELISA Pre-Coated Plate	ELISA Kit*	92585	182	5-400000	5	294	509
IL-20 DELFIA*	ELISA Pre-Coated Plate	ELISA Kit*	89922	278	23-64000	23	12	299

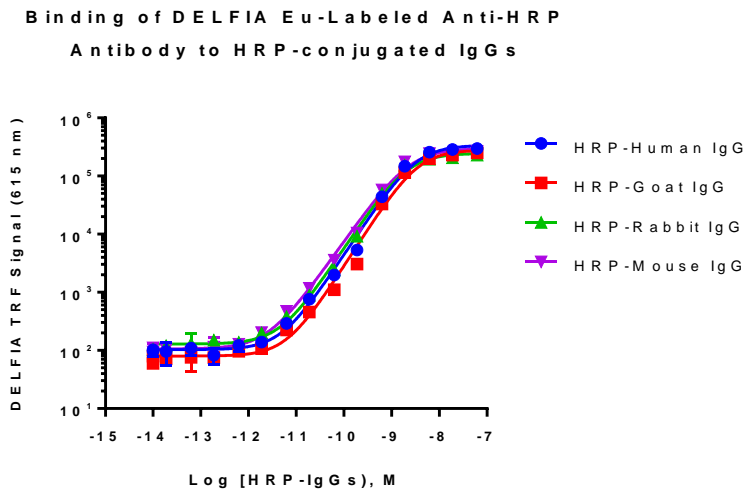
\*Replaced HRP substrate and stop solution in ELISA kit with DELFIA Eu-labeled Anti-HRP antibody and Enhancement solution. \*\* Standard curve analyte in ELISA kit was bought separately to extend the Dynamic range.

Results indicate that the existing traditional ELISA kit with HRP labeled detection antibodies can easily be converted to DELFIA assays in order to increase dynamic range, sensitivity, and signal to background ratio of the assay.



## The binding of Eu-labeled goat anti HRP antibody to HRP-conjugated mouse-, rabbit-, goat-, and human-IgGs

Increasing concentrations of HRP-IgGs were coated to DELFIA microtitration plate overnight at 23 °C. The plate wells were washed 3 times with 1X DELFIA wash solution. One-hour blocking was done by adding 300 µL per well 1%BSA prepared in 1xPBS (prepared from DELFIA BSA stabilizer 7.5%). Blocking solution was aspirated from each well and DELFIA Eu-labeled Anti-HRP antibody was added (100 µL/well, 200 ng/mL working solution) and incubated at room temperature for 1 hour. The plate wells were washed again 6 times with 1X DELFIA wash solution and added 200 µL/well DELFIA Enhancement Solution, incubated at room temperature for 10 minutes. The plate was read using TRF settings (see the Table in Instrument Setting Section).



Eu-Labeled Goat Anti-HRP Antibody	HRP-Human IgG	HRP-Goat IgG	HRP-Rabbit IgG	HRP-Mouse IgG
EC <sub>50</sub> (nM)	3.21	3.49	2.08	2.33
S/B Ratio	2937	3587	2217	2539

These results suggest that DELFIA Eu-labeled anti HRP antibody binds equally to HRP conjugated (widely used in ELISA) primary detection antibodies that are produced in goat, mouse, and rabbit.

## Validation in Serum, Plasma, and Media

### The IL-18BP $\alpha$ DELFIA Assay was validated in serum, plasma, and cell culture media

- Spike Recoveries in Assay Buffer and Media

IL-18BP $\alpha$  spike recoveries in DELFIA Assay Buffer, DMEM+10%FBS and RPMI+10%FBS. Three known concentrations of analyte were spiked into DELFIA Assay Buffer, DMEM + 10% FBS, RPMI + 10% FBS. The spiked samples were assayed along the standard curve prepared DELFIA Assay Buffer. The concentrations in spiked samples were obtained by interpolating the signal of spiked samples to the standard curve. Then the percent recoveries were calculated using the spiked concentrations.

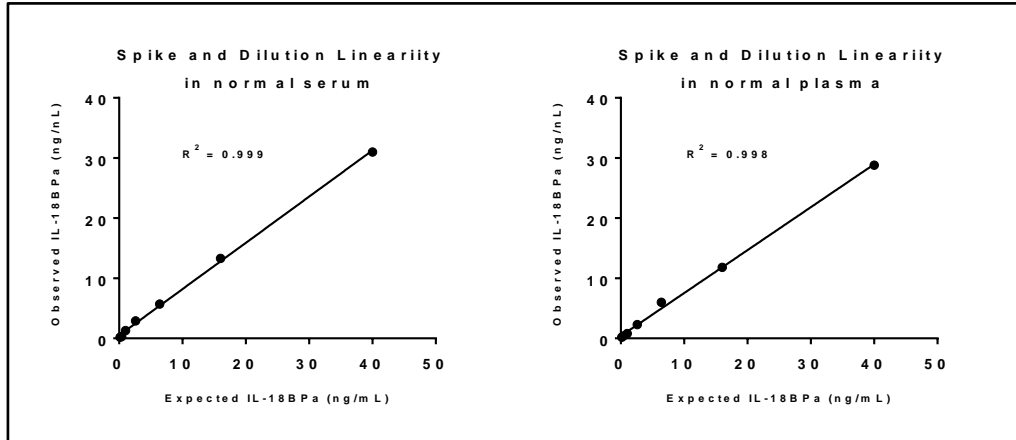
Spiked IL-18BP $\alpha$ (ng/mL)	% Recovery		
	DELFIA Assay Buffer	DMEM + 10% FBS	RPMI + 10% FBS
16	111	100	103
2.6	104	96	83
0.41	106	84	74

- Serum and Plasma Experiments

- Serum and plasma dilution linearity

Neat normal human serum and plasma were diluted with DELFIA Assay Buffer. IL-18BP-spiked (100 ng/mL) normal human serum and plasma samples were also diluted with DELFIA Assay Buffer. The diluted serum and plasma samples were assayed along the IL-18BP $\alpha$  standard curve prepared with DELFIA Assay Buffer. The concentrations of IL-18BP $\alpha$  in the serum and plasma samples were determined by interpolating signals of samples to the standard curve. In normal human serum and plasma, 13.3 ng/mL and 10.2 ng/mL IL-18BP $\alpha$  were detected, respectively, when the samples were diluted 2 to 128 fold. Excellent dilution linearity ( $R^2 > 0.998$ ) was achieved in the IL-18BP-spiked human serum and plasma samples that were diluted  $\geq 2$ -fold. The results are shown in the table and figure below.

Dilution Factor (x)	Expected IL-18BP $\alpha$ (ng/mL)	Observed IL-18BP $\alpha$ in Serum (ng/mL)	Observed IL-18BP $\alpha$ in Plasma (ng/mL)
2	40.0	31.0	28.8
4	16.0	13.3	11.8
8	6.4	5.7	6.0
16	2.6	2.9	2.3
32	1.00	1.3	0.8
64	0.41	0.3	0.4
128	0.16	0.2	0.18



○ Spike Recovery in Serum and Plasma

Three known amounts of IL-18BP a were spiked into normal human serum and plasma (100, 30, and 10 ng/mL IL-18BP a in spiked samples) then the samples were diluted 2-fold into DELFIA Assay Buffer. The samples were assayed along the standard prepared in DELFIA Assay Buffer. The spike recoveries of IL-18BP a were determined and the results are shown in table below.

IL-18BP a	Diluent: DELFIA Assay Buffer	
	Spiked sample (Normal Human Serum)	
Spike (ng/mL)	Concentration (ng/mL)	Recovery (%)
No spike	13.3*	N/A
100	83	83
30	33	110
10	8.4	84

IL-18BP a	Diluent: DELFIA Assay Buffer	
	Spiked sample (Normal Human Plasma)	
Spike (ng/mL)	Concentration (ng/mL)	Recovery (%)
No spike	10.2*	N/A
100	82	82
30	26	87
10	9.6	96

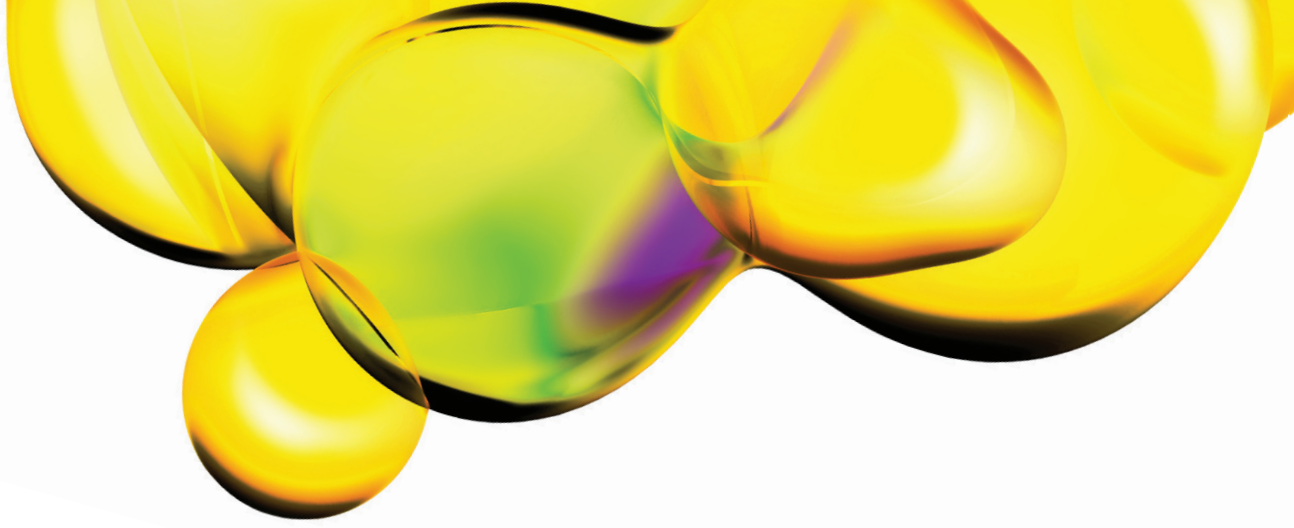
\*Recoveries were calculated after the no spike IL-18BP a level was subtracted (in this case, 13.3 ng/mL and 10.2 ng/mL in serum and plasma, respectively). Excellent recoveries were achieved for all three spikes tested.

## Troubleshooting Guide

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

[www.revvity.com](http://www.revvity.com)

**FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**



The information provided in this document is for reference purposes only and may not be all-inclusive. Revvity, Inc., its subsidiaries, and/or affiliates (collectively, "Revvity") do not assume liability for the accuracy or completeness of the information contained herein. Users should exercise caution when handling materials as they may present unknown hazards. Revvity shall not be liable for any damages or losses resulting from handling or contact with the product, as Revvity cannot control actual methods, volumes, or conditions of use. Users are responsible for ensuring the product's suitability for their specific application. REVVITY EXPRESSLY DISCLAIMS ALL WARRANTIES, INCLUDING WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, REGARDLESS OF WHETHER ORAL OR WRITTEN, EXPRESS OR IMPLIED, ALLEGEDLY ARISING FROM ANY USAGE OF ANY TRADE OR ANY COURSE OF DEALING, IN CONNECTION WITH THE USE OF INFORMATION CONTAINED HEREIN OR THE PRODUCT ITSELF

[www.revvity.com](http://www.revvity.com)

revvity

**Revvity, Inc.**  
940 Winter Street  
Waltham, MA 02451 USA  
[www.revvity.com](http://www.revvity.com)

For a complete listing of our global offices, visit [www.revvity.com](http://www.revvity.com)  
Copyright ©2023, Revvity, Inc. All rights reserved.