TECHNICAL DATA SHEET

DELFIA® Research Reagents

Research Use Only. Not for use in diagnostic procedures.

DELFIA 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

Contents

Product Information

Description: DELFIA® Eu-labeled Anti-HRP Kit contains all the necessary reagents to convert ELISA to DELFIA (Dissociation-Enhanced Lanthanide Fluorescence ImmunoAssay) assays. DELFIA 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 DELFIA assay using DELFIA® Eu-labeled Anti-HRP Kit.

Application: DELFIA immunoassays are a superior performance alternative to ELISA and are similar in format and workflow. Hence, a seamless transition from ELISA to DELFIA is possible. The DELFIA assays can be build in all classical immunoassay formats such as direct or indirect, sandwich, and competition assay. The DELFIA assays can be used to analyze the complex sample mattries such as blood, serum, plasma, and other samples. More details are provided in DELFIA User Guide.

DELFIA: 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 DELFIA® 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 DELFIA Microtitration Plate (96-Well Clear Strip Plate), the assay can also be performed in a DELFIA 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.

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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)
DELFIA 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)
DELFIA Wash Concentrate	2X25 mL @ 25X (2 bottles)	250 mL @ 25X (1 bottle)	25 mL @ 25X (1 bottle)	125 mL @ 25X (1 bottle)
DELFIA Assay Buffer	2X25 mL (2 bottles)	250 mL (1 bottle)	25 mL (1 bottle)	125 mL (1 bottle)
DELFIA 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)
DELFIA Microplate	1 (DELFIA Microtitration Plate)	5 (DELFIA Microtitration Plate)	1 (½ AreaPlate-96, HB)	5 (½ AreaPlate-96, HB)

* The amount is based on assay volume:

a) 100 µL/well using a final concentration of 100 ng/mL in 96-well strip plate (DELFIA Microtitration Plate) format, and

b) 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	PerkinElmer	6000027
TopSeal™-A Plus Adhesive Sealing Film	PerkinElmer	6050185
Plate Reader with TRF Option	PerkinElmer	EnVision™, Victor®, Victor Nivo™, EnSight™
DELFIA plate shaker (optional)	PerkinElmer	1296-003(For countries use 240 volt) 1296-004(For countries use120 volt)
DELFIA plate washer (optional)	PerkinElmer / BioTek	1296-0010/ 405™TS

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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 Protocol

The protocols described below are the examples of building two DELFIA assays (human IL-18BPa 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-18BPa DELFIA assay, analyte and antibodies in IL-18 BPa DuoSet ELISA kit (R&D System, Cat # DY119) were also bought separately. When building the IL-18BPa DELFIA assay, mouse anti-human IL-18BPa antibody was coated to the assay plate and biotinylated goat anti-human IL-18BPa 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-18BPa 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. Protocol 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 ºC.

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. Protocol 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 ℃.

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.

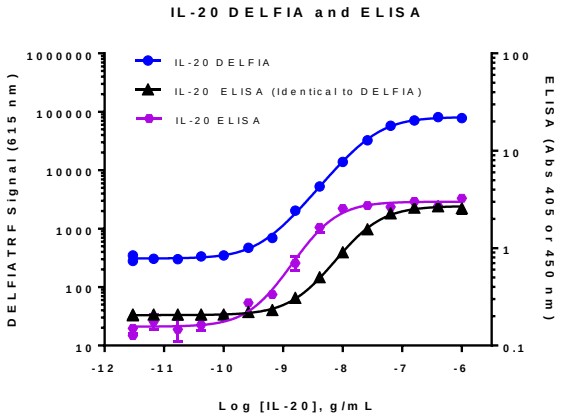
15. Read plate using TRF settings (see the Table in Instrument Setting Section). The developed signal will be stable for at least 24 hours when stored properly by covering tightly with parafilm.

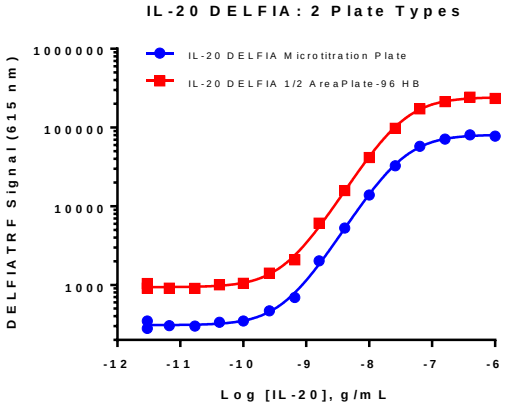
Typical Standard Curve, Data Analysis, and Comparisons

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 = 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-18BPa 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	Protocols	Max Counts or Abs	Min Counts or Abs	Dynamic Range (pg/mL)	LDL (pg/mL)	EC_{50} (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

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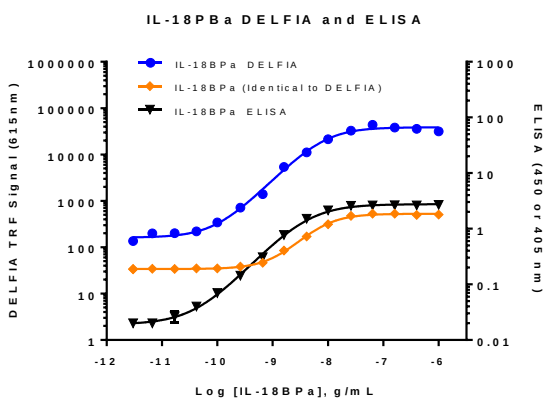
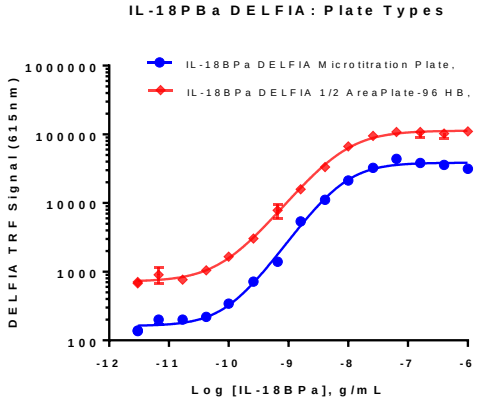
Comparisons of IL-18BPa DELFIA and ELISA Assays





IL-18BPa Assay Types	Plate Types	Protocols	Max Counts or Abs	Min Counts or Abs	Dynamic Range (pg/mL)	LDL (pg/mL)	EC_{50} (ng/mL)	S/B Ratio
IL-18PBa DELFIA	DELFIA Microtitration Plate	DELFIA	43878	138	5-160000	5	8	318
IL-18PBa DELFIA	1/2 AreaPlate-96 HB	DELFIA	108239	693	13-160000	13	8	166
IL-18BPa ELISA	DELFIA Microtitration Plate	ELISA (Identical to DELFIA)	1.87	0.14	34-25600	34	7	13
IL-18BPa 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 ½ AreaPlate-96 HB plate to save reagents.

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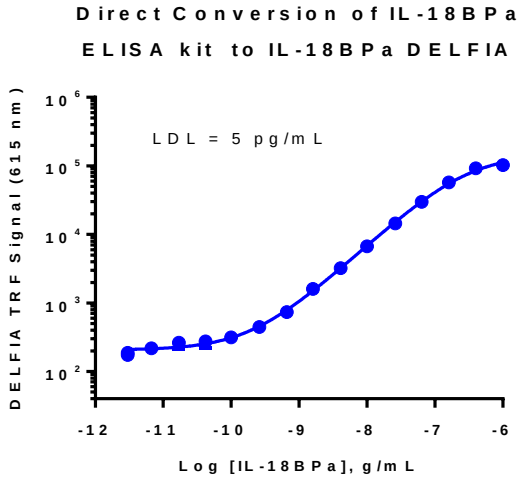
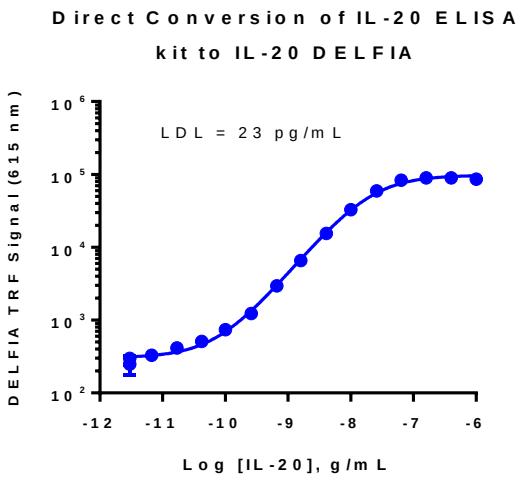
The existing ELISA kits can be easily converted to DELFIA.

Human IL-20 ELISA Kit (Sino Biological, Cat # KIT13060) and human IL-18BPa 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).





IL-18BPa Assay Types	Plate Types	Protocols	Max Counts	Min Counts	Dynamic Range (pg/mL)	LDL (pg/mL)	EC_{50} (ng/mL)	S/B Ratio
IL-18BPa 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.

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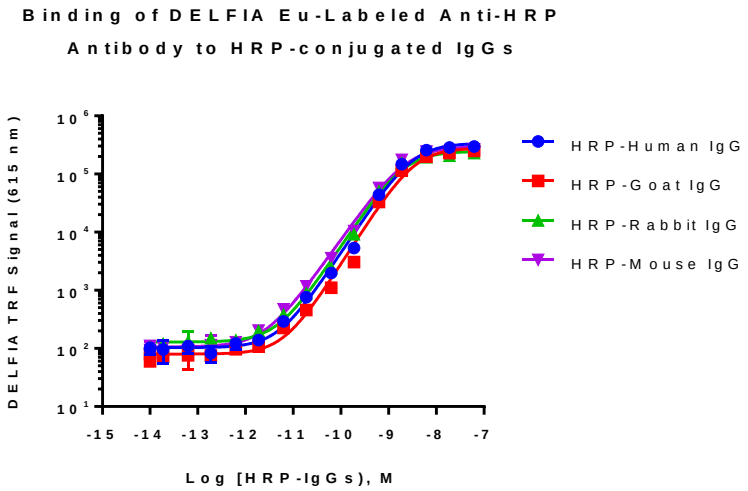
The binding of Eu-labeled goat anti HRP antibody to HRP-conjugated mouse-, rabbit-, goat-, and human-IgGs

Increasing concentrations of HRP-IgGs were coated to DELFIA microtitration plate overnight at 23 ºC. 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_{50} (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.

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Validation in Serum, Plasma, and Media

The IL-18BPa DELIFA Assay was validated in serum, plasma, and cell culture media

- Spike Recoveries in Assay Buffer and Media

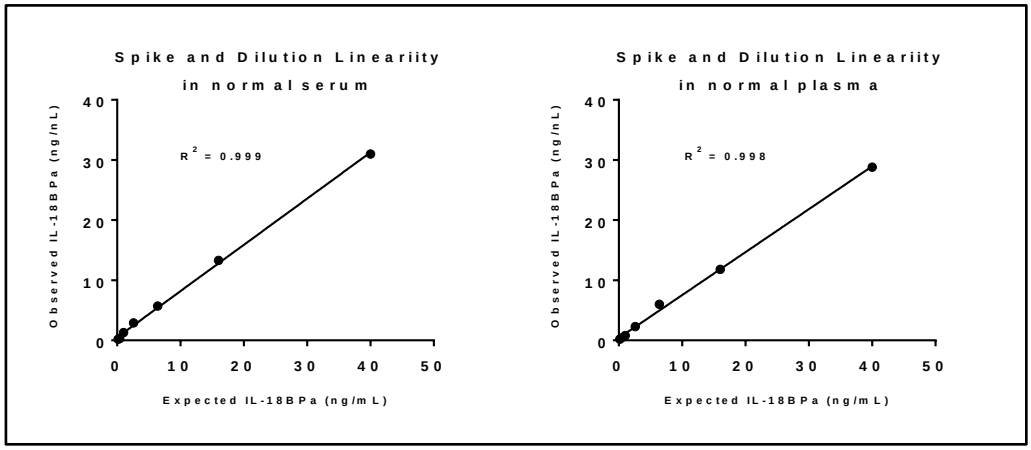
IL-18BPa spike recoveries in DELFIA Assay Buffer, DMEM+10%FBS and RPMI+10%FBS. Three known concentrations of analyte were spiked into DELFIA Assay Buffer, DMEM + 10% FBS, RPMI + 10% FBS. The spiked samples were assayed along the standard curve prepared DELFIA Assay Buffer. The concentrations in spiked 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	% Recovery		
IL-18BPa (ng/mL)	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-18BPa standard curve prepared with DELFIA Assay Buffer. The concentrations of IL-18BPa 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-18BPa 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 ≥ 2-fold. The results are shown in the table and figure below.

Dilution Factor (x)	Expected IL-18BPa (ng/mL)	Observed IL-18BPa in Serum (ng/mL)	Observed IL-18BPa 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-18BPa were spiked into normal human serum and plasma (100, 30, and 10 ng/mL IL-18BP 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-18BPa were determined and the results are shown in table below.

IL-18BPa	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-18BPa	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-18BPa 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:

http://www.perkinelmer.com/lab-solutions/resources/docs/APP_DELFIA_Miniaturization.pdf

http://www.perkinelmer.com/lab-solutions/resources/docs/APP_DELFIA_ELISA_DuoSet_Conversion.pdf

https://www.perkinelmer.com/lab-solutions/resources/docs/MAN_DELFIA_ELISA_Conversion.pdf

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