TECHNICAL DATA SHEET

DELFIA® Research Reagents

Research Use Only. Not for use in diagnostic procedures.

DELFIA Eu-labeled Anti-Mouse IgG Antibody Toolbox Kit

For ELISA Conversion

Product No.: DFA300-96S-1, DFA300-96S-5, DFA300-HALF-1 and DFA300-HALF-5

Contents

Product Information

Description: DELFIA® Eu-labeled Anti-Mouse IgG toolbox Kit is provided to contain all the necessary reagents to build and perform DELFIA (Dissociation-Enhanced Lanthanide Fluorescence ImmunoAssay) assays using un-conjugated antibody pair and analytes. The un-conjugated antibodies can be come from existing ELISA kits, ELISA antibody pairs, or from research home-brew antibodies in developement for the ELISA kit. DELFIA® Eu-labeled Anti-Mouse IgG Antibody included in the kit is used as a secondary detection antibody to detect the target specific primary antibodies raised in **mice**.

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 96-Well Strip Plate, the assay can also be performed in a DELFIA compatible ½ Area OPTIPLATE-96 White High Binding (½ AreaPlate-96 HB) 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 Contents: Reagents and Materials Privided

Components	DFA300-96S-1 (1 plate)	DFA300-96S-5 (5 plates)	DFA300-HALF-1 (1 half area plate)	DFA300-HALF-5 (5 half area plates)
DELFIA Eu-labeled Anti-Mouse IgG Antibody *	2X30 µL@ 50 µg/mL (2 brown tubes, white cap)	2X150 µL@ 50 µg/mL (2 brown tubes, white cap)	30 µL@ 50 µg/mL (1 brown tube, white cap)	150 µL@ 50 µg/mL (1 brown tube, white 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 Microplates	1 (DELFIA Microtitration Plate)	5 (DELFIA Microtitration Plate)	1 (½ AreaPlate-96 HB)	5 (½ AreaPlate-96 HB)

* The amounts are based on adding 100 µL/well in 96-Well Strip Plate and 50 µL/well in ½ AreaPlate-96 HB by using 200 ng/mL DELFIA Eu-labeled Anti-Mouse IgG Antibody solution.

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 DELFIA assays using commercially available ELISA antibody pairs such as human IL-18BPa and human IL-20 and 96-Well Strip Plate and ½ AreaPlate-96 HB.

To build the human IL-20 DELFIA assay, analyte and unconjugated antibodies in the IL-20 ELISA antibody pair set (Sino Biological, Cat # SEK13060) were bought separately. Note that mouse anti-human IL-20 detection antibody in IL-20 ELISA antibody pair set is conjugated to HRP. When building human IL-20 DELFIA assay, unconjugated rabbit anti-human IL-20 antibody was used as capture antibody (coated to the assay plate) and unconjugated mouse anti-human IL-20 primary antibody was used as detection antibody.

To build human IL-18BPa DELFIA assay, analyte and unconjugated antibodies in IL-18 BPa DuoSet ELISA kit (R&D System, Cat # DY119) were also bought separately. When building the IL-18BPa DELFIA assay, non-biotinylated goat anti-human IL-18BPa antibody was used as capture (coated to the assay plate) and mouse anti-human IL-18BPa antibody was used as primary detection antibody. Note that in IL-18 BPa DuoSet ELISA kit, the goat anti-human IL-18BPa antibody supplied was biotinylated and was suggested to be used as detection antibody in IL-18 BPa DuoSet ELISA kit.

The un-conjugated primary detection antibodies in both IL-18BPa and IL-20 DELFIA assays were then detected using DELFIA Eu-labeled Anti-Mouse IgG Antibody (secondary detection antibody) provided in the toolbox kit.

To compare the DELFIA assay performance to the ELISA, HRP-conjugated Anti-Mouse-IgG secondary detection antibody was used in the ELISA assay instead of DELFIA Eu-labeled Anti-Mouse IgG Antibody. The ELISA assay protocol was the same as the DELFIA assay except the Enhancement Solution in DELFIA was replaced with ELISA substrate and stop solutions.

I. Protocol for 96-Well Strip Plate:

Step 1: Preparing Microplates

- Add 100 µL of the capture antibody to each well. SEAL the plate with TopSeal and incubate overnight at 23°C to ensure the capture antibody binds to the plate.
  - Reconstitute and store antibody according to the data sheet.
  - Determine the amount of ng/well from the existing ELISA protocol or your optimized values.
- Wash each well 3 times with 1X DELFIA wash solution prepared from 25X Wash Concentrate.
  - We recommend using a plate washer for consistency. If being done by hand, it is simplest to dispense 300 µL of wash solution per well.
- Block the plates by adding 300 µL of PBS +1% BSA or other blocking buffer to each well.
- Cover the plate with a plate lid and incubate at room temperature on a plate shaker set to a slow speed (300 rpm) for a minimum of 1 hour.
- Remove and discard the blocking buffer.
- Remove remaining blocking buffer by inverting the plate and blotting it against clean paper towels.

Step 2: Performing the Assay

- Add 100 µL of standard analyte or sample to each well and cover the plate with a plate lid.
  - Reconstitute and store standard analyte according to the manufacture's data sheet
  - Prepare standards and any sample dilutions in DELFIA Assay Buffer
- Incubate plate for 2 hours at room temperature on a plate shaker set to a slow speed (300 rpm)
- Wash each well 3 times with 1X DELFIA wash solution
- Add 100 µL of primary detection antibody to each well and Cover the plate with a plate lid.
  - Reconstitute and store primary detection antibody according to the manufacture's data sheet
  - Determine the amount of ng/well from ELISA protocol or your optimized values
  - Prepare working primary detection antibody solution in DELFIA Assay Buffer
- Incubate 1 hour at room temperature on a plate shaker set to a slow speed (300 rpm)
- Wash 3 times with 1X DELFIA wash solution
- Add 100 µL (200 ng/mL) of DELFIA Eu-labeled Anti-Mouse IgG Antibody and cover the plate with a plate lid.
  - DELFIA Eu-labeled Anti-Mouse IgG Antibody solution stock concentration is 50 µg/mL
  - Prepare in DELFIA Assay Buffer to 200 ng/mL.
- Incubate 1 hour at room temperature on a plate shaker set to a slow speed (300 rpm)
- Wash 6 times with 1X DELFIA wash solution
  - The extra wash steps are necessary for removing any unbound DELFIA Eu-labeled Anti-Mouse IgG Antibody
- Add 200 µL of DELFIA Enhancement Solution and cover the plate with a plate lid
  - If the plate is to be stored prior to reading, it is recommended to cover the plate and add Enhancement Solution just prior needing to read the assay.
- Incubate at least 10 minutes at room temperature on a plate shaker set to a slow speed (300 rpm)
- 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.
  - Important Note: seals or tapes with adhesives should be avoided after DELFIA Enhancement Solution has been added to the plates.

II. Protocol for ½ AreaPlate-96, HB:

Step 1: Preparing Microplates

- Add 50 µL of capture antibody to each well.
  - Reconstitute and store antibody according to the data sheet.
  - Determine the amount of ng/well from the existing ELISA protocol or your optimized values.
- Seal the plate Top seal and incubate overnight at 23°C to ensure the capture antibody binds to the plate.
- Wash each well 3 times with 1X DELFIA wash solution.
  - We recommend using a plate washer for consistency. If being done by hand, it is simplest to dispense 150 µL of wash solution per well.
- Block the plates by adding 150 µL of PBS +1% BSA or other blocking buffer to each well. Incubate at room temperature on a plate shaker set to a slow speed (300 rpm) for a minimum of 1 hour.
- Remove and discard blocking buffer.
- Remove remaining blocking buffer by inverting the plate and blotting it against clean paper towels.

Step 2: Performing the Assay

- Remove adhesive film from the microplate if the plate has been covered.
- Add 50 µL of standard analyte or sample to each well and cover the plate with a plate lid.
  - Prepare standards and any sample dilutions in DELFIA Assay Buffer
  - Reconstitute and store standard analyte according to the manufacture's data sheet
- Incubate plate for 2 hours at room temperature on a plate shaker set to a slow speed (300 rpm)
- Wash each well 3 times with 1X DELFIA wash solution
- Add 50 µL of primary detection antibody to each well and cover the plate with a plate lid.
  - Reconstitute and store primary detection antibody according to the manufacture's data sheet
  - Determine the amount of ng/well from ELISA protocol or your optimized values
  - Prepare working primary detection antibody solution in DELFIA Assay Buffer
- Incubate 1 hour at room temperature on a plate shaker set to a slow speed (300 rpm)
- Wash 3 times with 1X DELFIA wash solution
- Add 50 µL (200 ng/mL) of DELFIA Eu-labeled Anti-Mouse IgG Antibody secondary detection antibody and cover the plate with a plate lid
  - DELFIA Eu-labeled Anti-Mouse IgG Antibody solution stock concentration is 50 µg/mL
  - Prepare in DELFIA Assay Buffer
- Incubate 1 hour at room temperature on a plate shaker set to a slow speed (300 rpm)
- Wash 6 times with 1X DELFIA wash solution
  - The extra wash steps are necessary for removing any unbound DELFIA Eu-labeled Anti-Mouse IgG Antibody
- Add 100 µL of DELFIA Enhancement Solution and cover the plate with a plate lid
  - If the plate is to be stored prior to reading, it is recommended to cover the plate and add Enhancement Solution just prior needing to read the assay.
- Incubate at least 10 minutes at room temperature on a plate shaker set to a slow speed (300 rpm)
- 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.
  - Important Note: seals or tapes with adhesives should be avoided after DELFIA Enhancement Solution has been added to the plates.

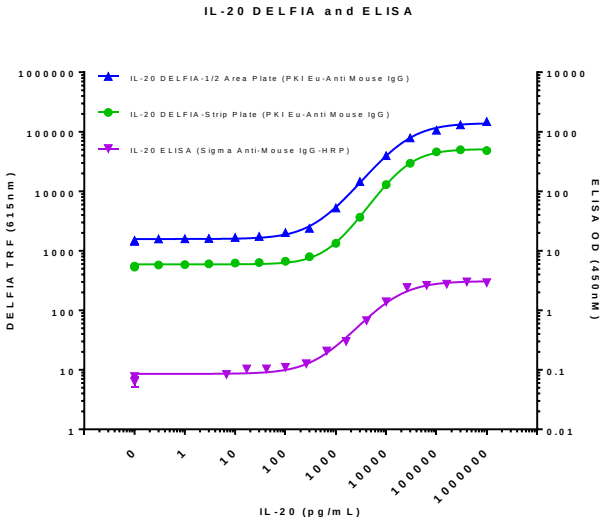
Standard Curve and Data Analysis

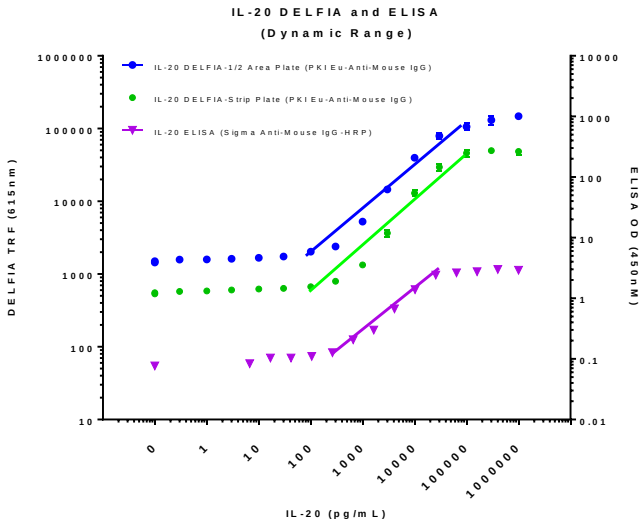
Standard curve for DELFIA immunoassay was plotted in GraphPad Prism Version 7.0 and analyzed with nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) with $1/Y^2$ weighting method. Lower limit of detection (LDL) and lower limit of quantitation (LLOQ) were calculated using the following equations:

LDL = mean (blanks) + 3 * SD (Standard Deviation).

The unknowns can be interpolated by using the standard curve.

Typical results of DELFIA and ELISA assay by using un-conjugated antibody pair in human IL-20 Antibody Pair Set (Sino-Biological Cat # SEK13060) and in 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 plates were also read on an EnVision-2105 multimode plate reader equipped with absorbance options and the optical densities (OD) were read at 450 nm and 540 nm, respectively. ELISA kit data was analyzed using two wavelength readings for background correction.





IL-20 Assay Types	Protocols	Max Counts or Abs	Min Counts or Abs	Dynamic Range (pg/mL)	LDL (pg/mL)	EC_{50} (ng/mL)	S/B Ratio
DELFIA ½ AreaPlate-96 HB	DELFIA Assay Protocol	148058	1498	50-100000	50	24.1	99
DELFIA 96-Well Strip Plate	DELFIA Assay Protocol	49822	554	64-100000	64	23.9	90
IL-20 ELISA 96 Well-Strip Plate	ELISA (Identical to DELFIA)	2.99	0.07	108-25600	108	13.8	43

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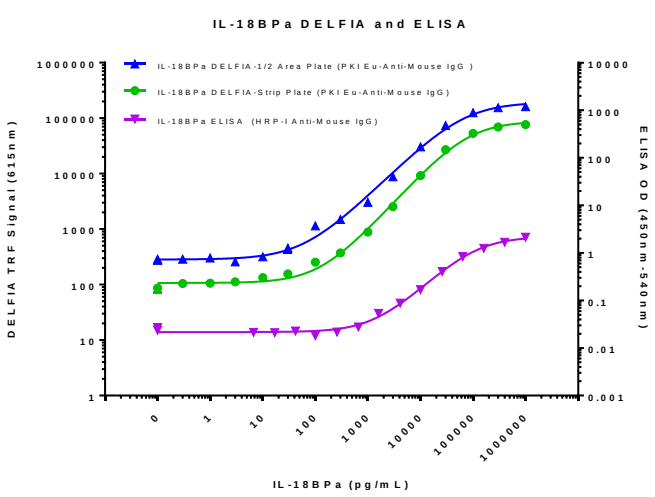

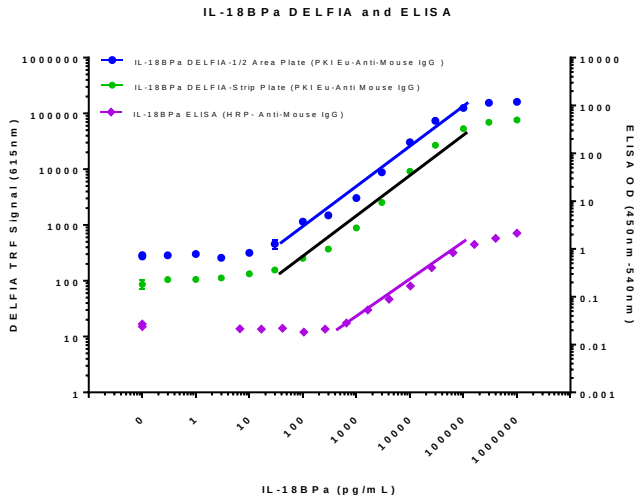

IL-18BPa Assay Types	Protocols	Max Counts or Abs	Min Counts or Abs	Dynamic Range (pg/mL)	LDL (pg/mL)	EC_{50} (ng/mL)	S/B Ratio
DELFIA ½ AreaPlate-96 HB	DELFIA Assay Protocol	125294	281	22-100000	22	64	447
DELFIA 96-Well Strip Plate	DELFIA Assay Protocol	53111	87	11-100000	11	78	610
IL-18BPa ELISA 96-Well Strip Plate	ELISA (Identical to DELFIA)	2.13	0.03	595 -160000	595	109	84

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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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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