TECHNICAL DATA SHEET

DELFIA® Research Reagents

Research Use Only. Not for use in diagnostic procedures.

DELFIA® Eu-Labeled Streptavidin Toolbox Kit

For ELISA Conversion

Product No.: DFA100-96S-1, DFA-96S-5, DFA-HALF-1 and DFA100-HALF-5

Contents

PerkinElmer
For the Better

Product Information

Description: DELFIA® Eu-labeled streptavidin toolbox kit is provided to contain all the necessary reagents to build and perform a DELFIA (Dissociation-Enhanced Lanthanide Fluorescence ImmunoAssay) immunoassay using either established ELISA antibody pairs or other research antibodies. DELFIA® Eu-labeled streptavidin included in the kit is used for binding detection antibody conjugates such as those labeled with biotin. The kit also includes a companion microplate relevant for the assay.

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 toolbox kit can be used to perform DELFIA assays using antibodies either from ELISA kits, ELISA antibody pairs sold commercially, or other research antibodies. DELFIA immunoassays can be performed in all classical immunoassay formats such as direct or indirect, sandwich or competitive assay. DELFIA assays can be used to analyze complex biological sample matrices such as blood, serum, plasma, and other samples. Additional details are available in the DELFIA User Guide.

DELFIA Assay: 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 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 preparation, assay repeats and additional dilutions;

Superior Stability: Read plates months post-assay 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 AreaPlate-96 High Binding (HB) to save materials.

Format:

Components	DFA100-96S-1 (1 plate)	DFA100-96S-5 (5 plates)	DFA100-HALF-1 (1 half-area plate)	DFA100-HALF-5 (5 half-area plates)
DELFIA Eu-labeled Streptavidin*	20 µL @ 100 µg/mL (1 tube, clear cap)	60 µL @ 100 µg/mL (1 brown tube, clear cap)	20 µL @ 100 µg/mL (1 tube, clear cap)	30 µL @ 100 µg/mL (1 tube, clear cap)
DELFIA Wash Concentrate	2 x 25 mL @ 25X (2 bottles)	250 mL @ 25X (1 bottle)	25 mL @ 25X (1 bottle)	125 mL @ 25X (1 bottle)
DELFIA Assay Buffer	2 x 25 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%)	2 x 2.5 mL (2 bottles)	2 x 12.5 mL (2 bottles)	2.5 mL (1 bottle)	12.5 mL (1 bottle)
DELFIA Microplate	1 x 96-Well Strip Plate	5 x 96-Well Strip Plate	1 x ½ AreaPlate-96, HB	5 x 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 format, and

b) 50 µL/well using a final concentration of 100 ng/mL in half-area plate format.

Storage: Store in the dark at 4 °C.

Stability: This product is stable for at least 12 months from the manufacturing date when stored in its original packaging under recommended storage conditions.

Additional Reagents and Materials

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

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 (240 volt for Europe use) 1296-004 (120 volt for US use)
DELFIA plate washer (optional)	PerkinElmer / BioTek	1296-0010/ 405™TS

EnVision Instrument Setting

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

I. Protocol for 96-Well Strip Plate:

Step 1: Preparing the plate

- Add 100 µL of the 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.
- Incubate the plate overnight at 23 °C to ensure the capture antibody binds to the plate.
- Wash each well 3 times with 1X DELFIA wash solution prepared from 25X DELFIA 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 plate by adding 300 µL of PBS +1% BSA or other blocking buffer to each well. Incubate the plate 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 plate if the plate has been covered.
- Add 100 µL of standard analyte or sample to each well.
    - Prepare standards and any sample dilutions in DELFIA Assay Buffer
    - Reconstitute and store standard analyte according to the manufacture's data sheet
- Incubate the 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 biotinylated detection antibody to each well
    - Prepare working detection antibody solution in DELFIA Assay Buffer
    - Determine the amount of ng/well from ELISA protocol or your optimized values
    - Reconstitute and store detection antibody according to the manufacture's data sheet
- Incubate the plate for 1 hour 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 DELFIA Eu-labeled Streptavidin (100 ng/mL) to each well
    - DELFIA Eu-labeled Streptavidin solution stock concentration is 100 µg/mL
    - Prepare in DELFIA Assay Buffer
- Incubate the plate for 20 minutes at room temperature on a plate shaker set to a slow speed (300 rpm)
    - Cover the plate with a plate lid.
    - Do not cover the plate with an adhesive film from this point forward
- Wash each well 6 times with 1X DELFIA wash solution
    - The extra wash steps are necessary for removing any unbound DELFIA Eu-labeled Streptavidin
- Add 200 µL of DELFIA Enhancement Solution to each well 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 DELFIA Enhancement Solution just prior needing to read the plate.
- Incubate the plate at least 5 minutes at room temperature on a plate shaker set to a slow speed (300 rpm)
- Read the 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 the plate

- 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.
- Incubate the plate overnight at 23 °C to ensure the capture antibody binds to the plate.
- Wash each well 3 times with 1X DELFIA wash solution prepared from 25X DELFIA Wash Concentrate.
    - 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 plate 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 wash buffer by inverting the plate and blotting it against clean paper towels.

Step 2: Performing the assay

- Remove adhesive film from the plate if the plate has been covered.
- Add 50 µL of standard analyte or sample to each well.
    - Prepare standards and any sample dilutions in DELFIA Assay Buffer
    - Reconstitute and store standard analyte according to the manufacture's data sheet
- Incubate the 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 biotinylated detection antibody to each well
    - Prepare working detection antibody solution in DELFIA Assay Buffer
    - Determine the amount of ng/well from ELISA protocol or your optimized values
    - Reconstitute and store detection antibody according to the manufacture's data sheet
- Incubate the plate for 1 hour 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 DELFIA Eu-labeled Streptavidin (100 ng/mL) to each well
    - DELFIA Eu-labeled Streptavidin solution stock concentration is 100 µg/mL
    - Prepare in DELFIA Assay Buffer
- Incubate the plate for 20 minutes at room temperature on a plate shaker set to a slow speed (300 rpm)
    - Cover the plate with a plate lid.
    - Do <u>not</u> cover the plate with an adhesive film from this point forward
- Wash each well 6 times with 1X DELFIA wash solution
    - The extra wash steps are necessary for removing any unbound DELFIA Eu-labeled Streptavidin
- Add 100 µL of Enhancement Solution to each well 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 plate.
- Incubate the plate at least 5 minutes at room temperature on a plate shaker set to a slow speed (300 rpm)
- Read the 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 enhancement solution has been added to the plates.

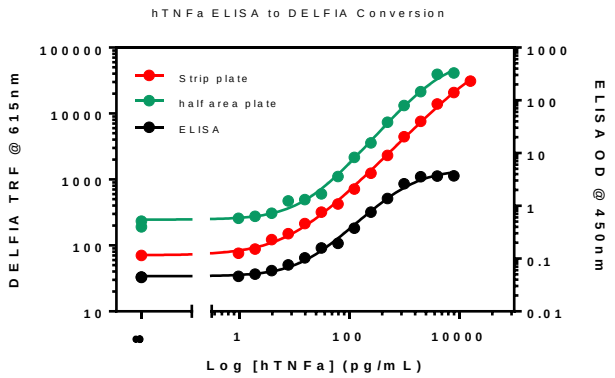
Standard Curve and Data Analysis

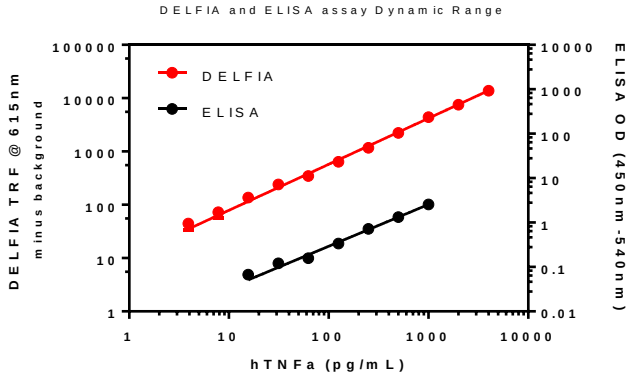
Standard curve for DELFIA immunoassay was plotted in GraphPad Prism Version 7.0 and analyzed with nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) with $1/Y^2$ weighting method. Lower limit of detection (LDL) was calculated using the following 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 human TNFα DuoSet ELISA (R&D Systems Cat# DY210):





The dynamic ranges were generated using a 96-well strip plate. ELISA assay and DELFIA assay were run side by side in the separate plates. The DELFIA plate was read by an EnVision-2105 multimode plate reader with TRF flash lamp option. The ELISA plate was read on a plate reader equipped with absorbance option and the optical densities (OD) were read at 450 nm and 540 nm, respectively. ELISA kit recommended to use two wavelength readings for background correction.

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

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