DELFIA™

Eu-labeled Anti-goat IgG Antibody Toolbox Kit

Product number: DFA400-96S-1, DFA400-96S-5
DFA400-HALF-1, DFA400-HALI

Product Information

Description: DELFIA Eu-labeled anti-goat IgG toolbox kit is provided to contain all the necessary reagents to build and perform a DELFIA (Dissociation-Enhanced Lanthanide Fluorescence ImmunoAssay) immunoassay using either the established ELISA antibody pairs or other research antibodies. DELFIA Eu-labeled anti-goat IgG included in the kit is used for binding to detection antibody conjugates such as those originating from goat. 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. The DELFIA assays can be used to analyze the complex biological sample matrices such as blood, serum, plasma, and other samples. More 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 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 preparation, assay repeats and additional 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 product is stable for at least 12 months from the manufacturing date when stored in its original packaging under recommended storage conditions.

Kit contents: Reagents and Materials

Components	DFA400-96S-1 (1 plate)	DFA400-96S-5 (5 plates)	DFA400-HALF-1 (1 half area plate)	DFA400-HALF-5 (5 half area plates)
DELFIA Eu-N1 Anti-Goat-IgG Antibody*	10 µL @ 1 mg/mL (1 clear tube, clear cap)	25 µL @ 1 mg/mL (1 clear tube, clear cap)	10 µL @ 1 mg/mL (1 clear tube, clear cap)	15 µL @ 1 mg/mL (1 clear tube, clear cap)
DELFIA Wash Concentrate	25 mL @ 25X (2 bottles)	250 mL @ 25X (1 bottle)	25 mL @ 25X (1 bottle)	125 mL @ 25X (1 bottle)
DELFIA Assay buffer	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%)	2.5 mL (2 bottles)	12.5 mL (2 bottles)	2.5 mL (1 bottle)	12.5 mL (1 bottle)
DELFIA Microplate	1 (96 well Clear Strip Plate)	5 (96 well Clear Strip Plate)	1 (96 Well 1/2 Area HB White Plate)	5 (96 Well 1/2 Area HB White Plate)

* The amount is based on assay volume:

a) 100 µL/well using a final concentration of 500 ng/mL in 96-well strip plate format, and

b) 50 µL/well using a final concentration of 500 ng/mL in half-area plate format.

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	Revvity	6000027
TopSeal™-A Plus Adhesive Sealing Film	Revvity	6050185
Plate Reader with TRF Option	Revvity	EnVision™, Victor™, Victor Nivo™, EnSight™
DELFIA plate shaker (optional)	Revvity	1296-003 (240 volt for Europe use) 1296-004 (120 volt for US use)
DELFIA plate washer (optional)	Revvity / BioTek	1296-0010/ 405™TS

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

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 detection antibody of goat origin 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 Anti-goat IgG (500 ng/mL) to each well
    - DELFIA Eu-labeled Anti-goat IgG solution stock concentration is 1 000 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-Anti-goat IgG
- 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 area plate-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 detection antibody of goat origin 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 Anti-goat IgG (500 ng/mL) to each well
    - DELFIA Eu-labeled Anti-goat IgG solution stock concentration is 1 000 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 Anti-goat IgG
- 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

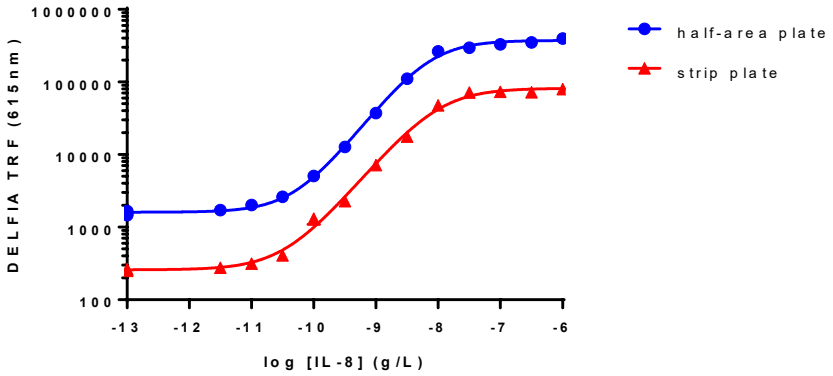
Standard curve for DELFIA immunoassay was plotted in GraphPad Prism Version 7.0 and analyzed with nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) with $1/Y^2$ weighting method. Lower limit of detection (LDL) was calculated using the following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

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

The unknowns can be interpolated by using the standard curve.

Typical results of DELFIA strip plates and half-area plates using human IL-8 ELISA (R&D Systems Cat# D8000C):

The DELFIA assay was made using the capture antibody and analyte from the kit, the detection antibody AF210-NA and the Eu-labeled anti-Goat IgG and enhancement solution as described above. One assay was performed in a 96 wells Clear Strip Plate and the other in a 96 wells White HB Half-Area Plate

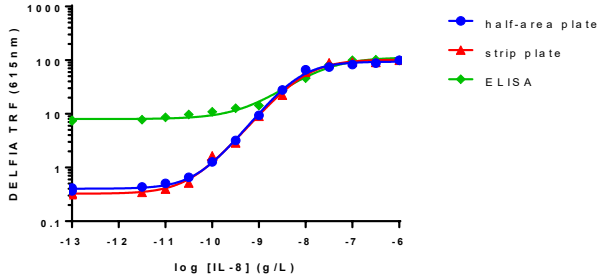


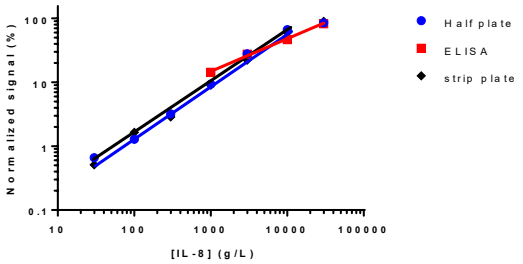
Typical results of DELFIA and ELISA assay using human IL-8 ELISA (R&D Systems Cat# D8000C):

The ELISA assay was made using the capture antibody, analyte, HRP substrate and stop solution from the kit, the detection antibody AF210-NA and anti-goat IgG-HRP labeled from Jackson ImmunoResearch.

The DELFIA assays were made using the capture antibody and analyte from the kit, the detection antibody AF210-NA and the Eu-labeled anti-Goat IgG and enhancement solution as described above.

All assays used coating buffer, wash buffer, blocking solution and assay buffers from Revvity as described above.





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. Results were normalized to the maximum signal for clarity.

Troubleshooting Guide

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

https://www.revvity.com/ask/delfia-time-resolved-fluorescence-assays

DFA400-R Rev01

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