Time Resolved Fluorescence (TRF) immunoassay in 384-well format using a matched antibody pair kit and the PHERAstar® *FSX*

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- Recombinant monoclonal antibody pair for consistent, sensitive and specific results
- Suitable for ELISA-based assays in 96- or 384-well microplate format
- Elevates sensitivity with optimized TRF detection on the PHERAstar *FSX*

Introduction

Most commercially available antibody pairs have been designed to be used in sandwich ELISA assays. They offer the flexibility of miniaturizing experiments to 384- and even 1536- well formats for higher throughput demands. Abcam's matched antibody pairs (MAPs) are well-characterized, recombinant antibodies with highly sensitive reagents that cover a wide variety of targets.

MAPs consist of an unlabelled capture antibody, a biotinylated detection antibody and a protein standard.

In order to enhance assay performance, alternative measurement techniques and different secondary anti-body labels can be used. As an example, Time-Resolved Fluorescence (TRF) detection uses long-lifetime fluorophores, known as lanthanides, that emit light over a longer period after excitation (microseconds as opposed to nanoseconds). This helps reduce background noise by delaying the start of the measurement until after the background signal has decayed. The measurement of assay signal using non-visible light spectra can increase dynamic range and is not limited to the same extent by saturation.

Here we describe how Abcam's MAP kits can be used in a 384-well TRF immuno-assay (DELFIA® TRF – PerkinElmer) and read on the PHERAstar® *FSX* for the detection of IRAK4, a protein kinase involved in cell signaling as part of the immune response.

Assay Principle

In the DELFIA® TRF Assay, the MAP binds to the analyte in the sample and is then incubated with streptavidin-Europium in place of a traditional label such as HRP. Bound streptavidin-Europium is detected using the DELFIA® enhancement solution which liberates bound Europium and promotes the formation of a micellar chelate solution. The Europium chelate is excited at 337 nm and emits at 615 nm (Fig. 1).

Using the PHERAstar *FSX* this can be read using either a Xenon flash lamp or laser for excitation. In this application note the laser was used to maximise assay signal and sensitivity.
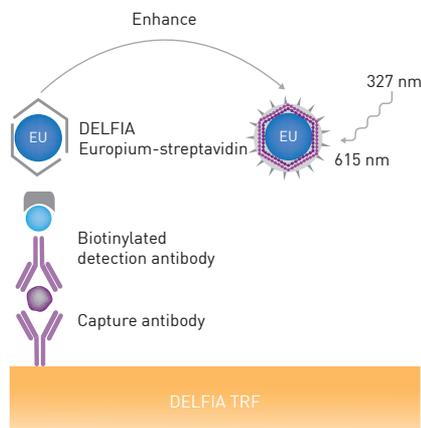

Fig. 1: Schematic of DELFIA® TRF assay (adapted from Perkin-Elmer).

Materials & Methods

- Black 384 NUNC Maxisorp™ plates
- Human IRAK4 Matched Antibody Pair kit (ab218182)
- DELFIA® Europium-labelled streptavidin
- DELFIA® assay buffer
- DELFIA® enhancement solution
- PHERAstar® *FSX* (BMG LABTECH)

Experimental procedure

Assay performed in total volume of 50 µl according to MAP kit protocol (1) using black 384-well NUNC Maxisorb™ plates. The plate was incubated for 30 mins with streptavidin-Europium diluted according to manufacturers' recommendations in DELFIA® assay buffer. After the addition of 50 µL of DELFIA® enhancement solution, time-resolved fluorescence was read on BMG LABTECH PHERAstar *FSX* equipped with a TRF laser with both non-optimized and optimized settings.

ABSORBANCE

FI & FRET

LUM & BRET

TRF & TR-FRET

FP

AlphaScreen®

NEPHELOMETRY

Optic settings	Time resolved fluorescence (TRF), top optic	
	Optic module	337 615 (Ex: 337 nm; Em: 615 nm)
	Excitation source	laser
	Number of flashes	5
	Integration start	400 µs
	Integration time	400 µs
General settings	Settling time	0 s

Fig. 3: Comparison of standard calibration curve for the IRAK4 MAP kit used in the classical ELISA (left) and TRF-based Eu detection (right).

Results & Discussion

The PHERAstar *FSX* is equipped with a laser excitation source, and photon counting detectors with an extended red detection range for TRF measurements. Combined with Z-height optimization for both top and bottom optics, this ensures the highest performance and sensitivity in any assay. The MARS data analysis package provided with the PHERAstar *FSX* is capable of automatically generating a calibration curve, from which the concentration of unknown samples can be determined. Calculation templates can also be saved to a protocol, meaning that future data analysis of test runs can be fully automated.
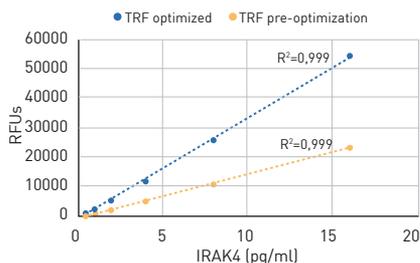

Fig. 2: Calibration curve for Human IRAK4 MAP in 384-well format read on the PHERAstar *FSX*.

The optimal focal height for fluorescent measurements is dependent on the volume of sample used and dimensions of the microplate. The PHERAstar *FSX* is capable of automatically selecting the optimal Z-height for the optic when reading the plate from top or bottom. The curve in Fig. 2 shows the samples read in a 384-well plate before (yellow) and after (blue) optimization of the Z-height for the new microplate format. This adjustment improved both signal and signal to blank.
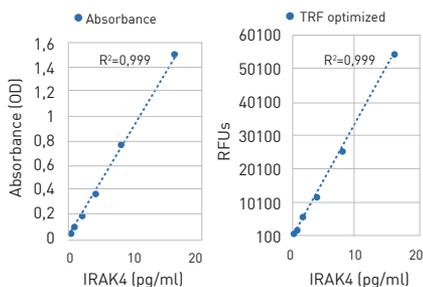
When compared to data previously obtained using the same MAP kit in a traditional absorbance based ELISA assay in a 96-well plate (Fig. 3), the TRF-based assay in a 384-well format with Human IRAK4 MAP kit shows a similar if not extended working range, as saturation was not reached. The linearity is given for both assays with an R^2 of 0.999.

Conclusion

Abcam's recombinant MAPs offer enhanced flexibility in the type of detection method used for plate-based immunoassays. This application note demonstrates that MAPs are suitable to use in miniaturized TRF immunoassays, providing sensitivity, specificity and reproducibility of results. This combined with the 384-well format make MAP kits particularly well suited for high-throughput screening. Additional detection methods can be achieved with Abcam's PBS-only formulations, which provide even greater flexibility. The PHERAstar *FSX* is capable of optimizing read conditions including focal height, and when coupled with a laser-based excitation source and photon-counting detection, maximizes sensitivity and signal window. These optimizations and features are particularly important when miniaturizing to 384-well microplates for high-throughput screening.

References

1. How to use Matched Antibody Pair kit – Protocol (abcam)

PHERAstar® *FSX*