

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

Application note

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- Recombinant monoclonal antibody pair for consistent, sensitive and specific results
- Suitable for ELISA and ELISA -based assays in 96- or 384-well format
- Read on PHERAstar® FSX using high precision protocol

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, use recombinant antibodies and are highly sensitive reagents that cover a wide variety of targets.

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

In order to enhance assay performance, alternative measurement techniques and different secondary antibody 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 – Perkin Elmer) and read on the PHERAstar® FSX for the detection of IRAK4, a protein kinase involved in cell signalling as part of the immune response.

Assay principle

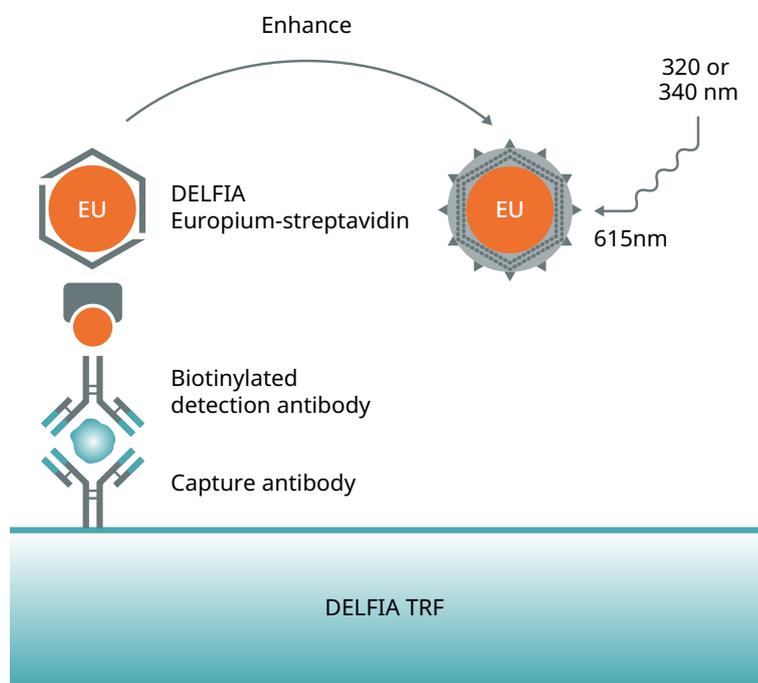


Figure 1. Schematic of DELFIA® TRF assay (adapted from Perkin-Elmer). In the DELFIA® TRF Assay, the MAP binds to the analyte in the sample and is then incubated with streptavidin-Europium in place of HRP. Bound streptavidin-Europium is detected using the DELFIA® enhancement solution, which liberates bound Europium and promotes the formation of a micellar chelate solution. The Europium chelate is excited at 340 nm and emits at 615 nm. Using the PHERAstar® FSX this can be read using either a Xenon flash lamp or laser. In this application note the laser, was used to maximize assay signal and sensitivity.

Materials and methods

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

Experimental procedure

Assay is 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.

Resolved fluorescence using 50 µL of DELFIA® enhancement solution.

Plate read on BMG LABTECH PHERAstar® FSX equipped with a TRF laser with both non-optimised and optimized settings.

Instrument settings

Optic settings	Time resolved fluorescence (TRF)	
	Filters	Excitation: 337 nm Emission: 615 nm
General settings	Number of flashes	5 with laser
	Settling time	0.0
	Integration start	400
	Integration time	400

Results and 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 is always achievable in any assay. The MARS data analysis package provided with the PHERAstar® FSX is capable of automatically generating a standard 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.

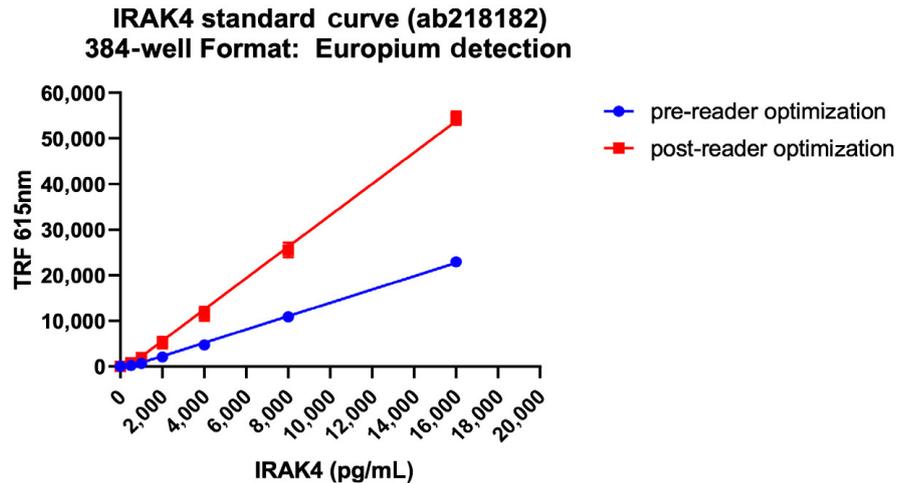


Figure 2. Calibration curve for Human IRAK4 MAP in 384-well format read on the PHERAstar® FSX. The optimal focal height for fluorescent measurements depends on the volume of sample used and dimensions of the microplate. The PHERAstar® FSX is capable of automatically selecting the optimal Z-height for 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 (blue) and after (red) optimization of the Z-height for the new microplate format. This adjustment improved both signal and signal to blank.

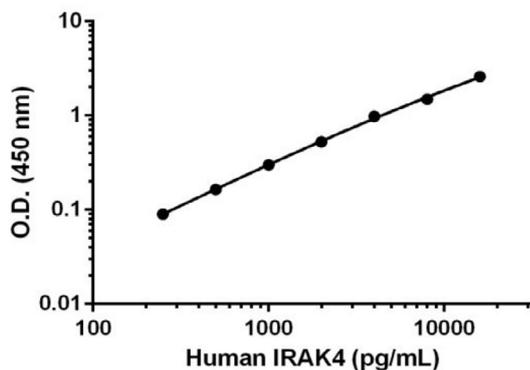


Figure 3. Standard calibration curve for the IRAK4 MAP kit used in the ELISA. Using a TRF-based assay (Fig. 2) with the Human IRAK4 MAP kit shows a similar, if not extended, working range when compared to the same pair used in an ELISA assay (Fig. 3) as saturation was not reached.

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.

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