

PolarScreen™ Far Red Tyrosine Kinase Assay

Tecan Infinite™ F500, Fluorescence Polarization



Introduction

Kinases are probably some of the most important molecular keys in biological systems. They transfer phosphate groups from donor molecules like adenosine triphosphate (ATP) to specific target molecules (kinase-substrates), a process which is called phosphorylation. Phosphorylation of proteins is critical to a majority of biological mechanisms, including cell growth, cell death (apoptosis) and cell differentiation.

One of the most important types of kinases are the so called protein tyrosine kinases (PTKs), which seem to play a leading part in various diseases (e.g. cancer) (1). Invitrogen has developed the PolarScreen™ Tyrosine Kinase Far Red Assay Kit, an assay based on fluorescence polarization (FP) to analyze PTK-activity via tyrosine phosphorylation.

Assay Principle

Fluorescence polarization is a useful tool to study binding events (ligand-receptor, antibody-antigen, etc.) in molecular biology. It measures the rotational freedom of a fluorophore during its fluorescence lifetime. If a small fluorescent

molecule is excited with linear polarized light, it is able to tumble during its FL, resulting in the emission of depolarized light. If a small molecule is immobilized (e.g. by binding to a big molecule), it is limited in its rotational freedom, thus the emission of polarized light is the consequence.

The principle of a typical FP-kinase assay is shown in Figure 1. First of all the kinase reaction is allowed to proceed under normal conditions, usually in the presence of an inhibitor (or a compound which may act as an inhibitor). Subsequently, a labeled tracer and an antibody are added, whereas the antibody can either bind to the tracer or to the phosphorylated kinase substrate. In the case of high kinase activity, the substrate will be phosphorylated, the competition between the tracer and the substrate will be high, with the consequence of low FP values. If the kinase is somehow inhibited, the substrate would not be phosphorylated and the antibody will mostly bound to the tracer, resulting in a high FP value. The amount of antibody that is bound to the tracer is inversely proportional to the amount of phosphorylated substrate present, and in this manner kinase activity can be detected and measured by a decrease in FP value (1).

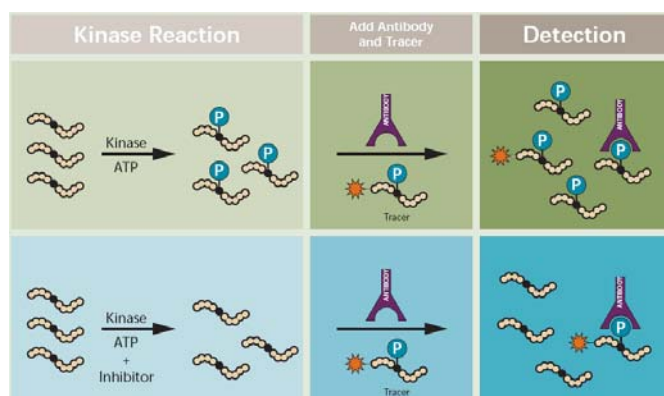


Figure 1: Schematic illustration of an FP-based kinase assay.

In this study the PTK Far Red Assay Kit was used together with three different kinases (JAK3, EGFR, KDR) in order to validate the new Infinite™ F500 for Far Red FP assays. A dilution series of three kinases were incubated (in triplicate) with substrate (E4Y, 20 ng/ml) and ATP (100 μ M). After 1 hour, EDTA was added to quench the reaction, along with phospho-tyrosine specific antibody and Far-Red labeled phosphopeptide tracer. After addition of tracer, antibody and EDTA the plate was incubated at room temperature for 1 hour prior fluorescence polarization readout (Figure 3). Kinase activity results in formation of the phosphopeptide product, which competes with antibody for binding to the tracer, resulting in a low polarization value. In the absence of kinase activity, no product is formed, and the tracer binds to the antibody, resulting in a high polarization value.

To combat interference due to compound autofluorescence or light scatter from precipitated library compounds, Invitrogen's PolarScreen Far-Red assays incorporate a proprietary dye with excitation and emission maxima above 600 nm (see Figure 2). Unlike other far-red fluorophores such as Cy5, Invitrogen's PolarScreen Far-Red tracer has a fluorescent lifetime suitable to competitive FP binding assays.

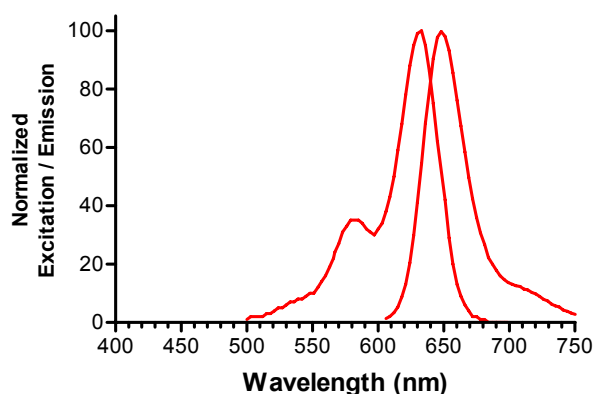


Figure 2. Spectra of Invitrogen's PolarScreen Far-red Tracer

Material and methods

Instrument

Tecan Infinite™ F500 filter-based microplate reader

Microplates

384 Flat bottom Black Polystyrol microplates (Corning)

Reagents

PolarScreen™ Tyrosine Kinase Assay Kit, Far Red (Invitrogen, PV3327)
 JAK3 Kinase (Invitrogen, PV3855)
 EGFR Kinase (Invitrogen, PV3872)
 KDR Kinase (Invitrogen, PV3660)

Measurements

Measurement	
Plate	[COS384fb.pdf]
Mode	Fluorescence Polarization
Excitation Wavelength	610 [20] nm
Emission Wavelength	670 [40] nm
Gain	102 (optimal)
Number of Reads	10
Integration Time	20 μ s
Lag Time	0 μ s
Settle Time	0 ms
Mirror (Automatic)	Dichroic 630 (e.g. Cy5)

Table 1: Infinite™ F500 measurement parameters for PolarScreen™ Tyrosine Kinase Assay Kit, Far Red.

Results and discussion

Fluorescence polarization values measured with the Infinite™ F500 were plotted against kinase concentrations (Figure 3) for determination of EC50 values. Table 2 summarizes the obtained results for the three kinases JAK3, EGFR, and KDR.

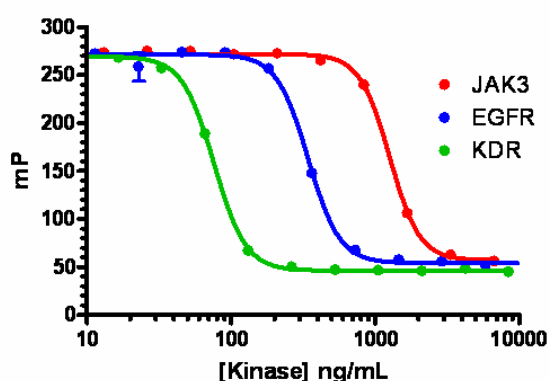


Figure 3: JAK3, EGFR, and KDR Kinase Assay on Infinite™ F500. Measured mP values were plotted against JAK3, EGFR, and KDR Kinase dilution series.

	JAK3	EGFR	KDR
mP High	272	272	270
mP Low	57	54	46
Δ mP	215	218	224
EC50 (ng/mL)	1264	343	76

Table 2: Summary of data measured with Tecan Infinite™ F500.

Figure 3 and Table 2 clearly demonstrate a very good performance of Infinite F 500 in combination with the PolarScreen™ Far-Red Kinase Assay. Additionally, all measurements were performed on Tecan Safire² and Tecan Ultra 384. All three instruments show similar Δ mP and EC50 values for all three kinases (data not shown).

Conclusion

This application note describes the successful performance of Invitrogen's PolarScreen™ Far-Red Kinase Assay on the Tecan Infinite™ F500 filter based multimode detection system.

In regard to the obtained data, the instrument is perfectly performing according to the given assay requirements.

Acknowledgements

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Literature

- [1] PolarScreen™ Tyrosine Kinase Assay Kit, Far Red Protocol (Invitrogen, V3327)
- [2] Overcoming compound interference in fluorescence polarization-based kinase assays using far-red tracers. Vedvik KL, Eliason HC, Hoffman RL, Gibson JR, Kupcho KR, Somberg RL, Vogel KW. *Assay Drug Dev Technol.* 2004 Apr;2(2):193-203

List of abbreviations

ATP	adenosine triphosphate
EDTA	Ethylenediaminetetraacetic acid
FL	Fluorescence Lifetime
FP	Fluorescence Polarization
PTK	Protein Tyrosine Kinase

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