



Invitrogen™ discovery screening

Redder is better

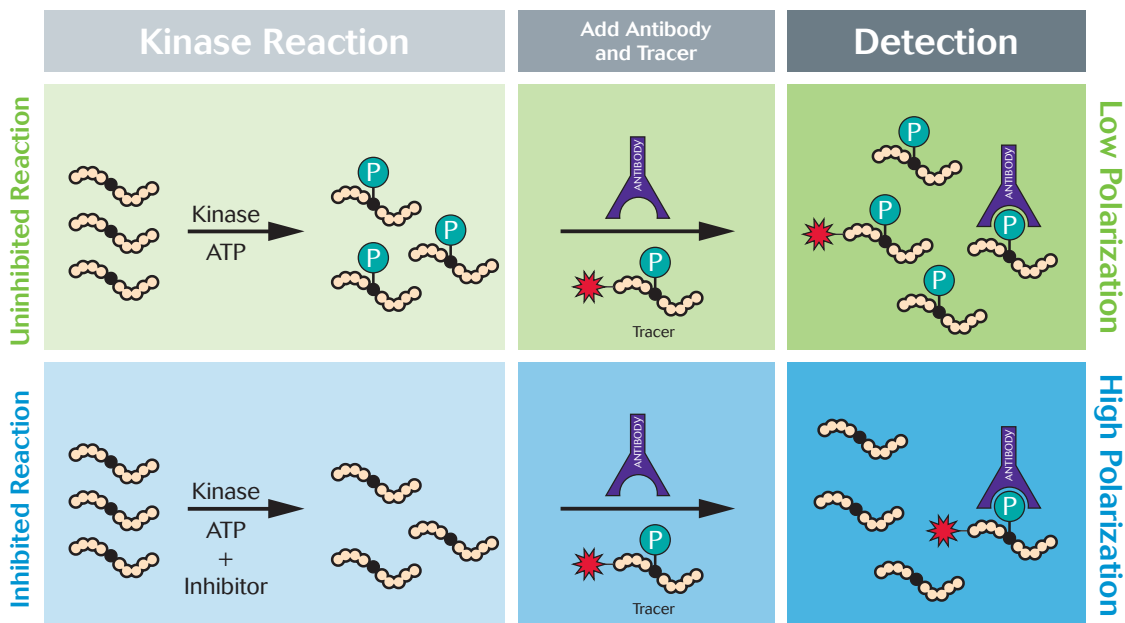
Far-Red PolarScreen™ FP Kinase Assays

Description

Far Red PolarScreen™ Fluorescence Polarization (FP) Kinase Assay Kits use a novel far-red fluorophore as a detection tool to reduce interference when screening compounds that have background fluorescent properties. In a far-red kinase assay, kinase, substrate, and ATP are allowed to react in the presence of library compounds. After the reaction, antibody and far-red-labeled tracer are added (Figure 1). The amount of antibody that binds to the tracer is inversely related to the amount of phosphorylated product present. Thus, library compounds that inhibit the reaction are identified as wells that have a high polarization value. The Far Red PolarScreen™ FP Kinase Assay Kits provide the following benefits:

- *Reduced compound interference*—a novel far-red fluorophore reduces effects of autofluorescent compounds, or from light scatter due to precipitation of compounds, leading to fewer false hits
- *Robust*— Z' -factor > 0.6 for all assays; high polarization shift
- *Easy to use*—Pipette, incubate, and measure
- *Accurate*—Measure true equilibrium binding
- *Miniaturizable*—Kits are easily adapted to use in 1536-well plates

Figure 1—Far-red kinase assay

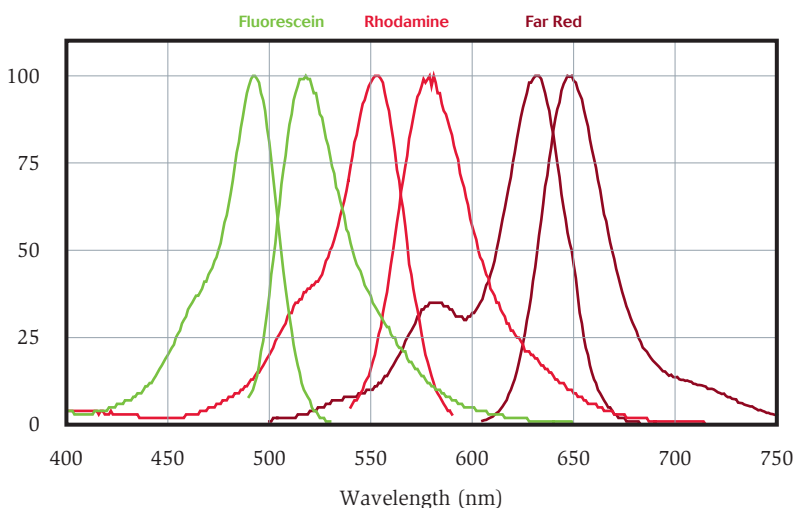


Optimized assays

Assays are read using an excitation wavelength of 610 nm (bandpass 20) and an emission wavelength of 670 nm (bandpass 40) as compared to Green FP Kinase Assay Kits using excitation wavelength of 485 nm and 530 nm (Figure 2). As a result, picogram to nanogram quantities of kinase will phosphorylate sufficient peptide substrate to affect an assay window of between 125 and 250 mp (Figure 3).

Figure 2—Assay interference decreases at longer wavelengths

A. Excitation/emission spectra of far-red tracers



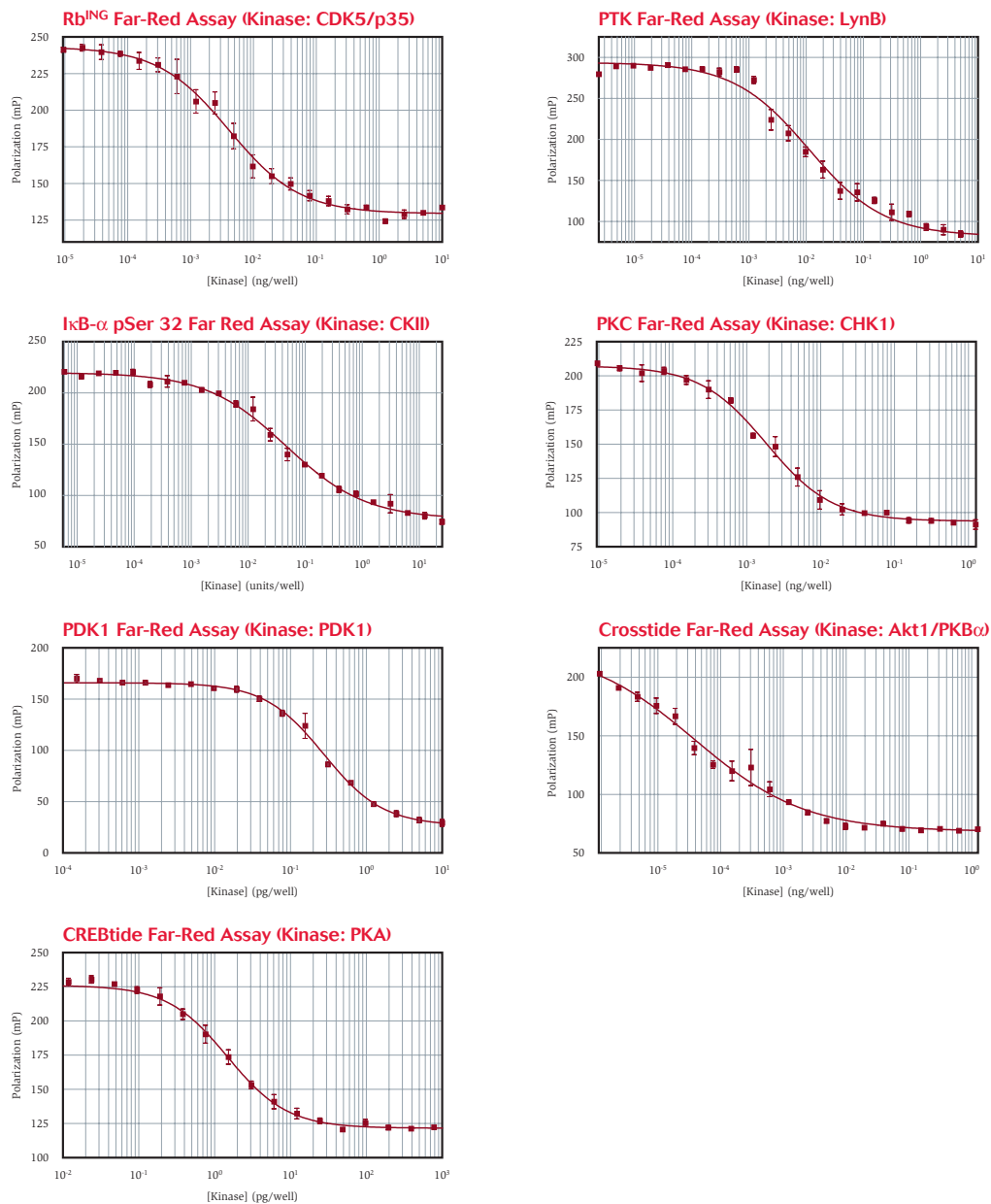
Excitation/Emission spectra of commonly-used fluorescein and rhodamine-based tracers compared to the spectra of Invitrogen's new far-red tracers. The excitation and emission maxima for the far-red tracer are red-shifted beyond 600 nm, allowing fluorescence polarization assays to be performed using a 610 nm excitation filter and 670 nm emission filters.

B. Compounds in LOPAC^{12807M} with greater than 50% signal intensity of 1 nM corresponding tracer

| Tracer | λ (Ex / Em) | # of Compounds |
|---------|---------------------|----------------|
| Green | 485 / 535 | 19 |
| Red | 535 / 590 | 9 |
| Far-Red | 590 / 650 | 3 |

To determine an approximation of the amount of fluorescent interference that can be expected in a typical compound library at green, red, and far-red wavelengths, the LOPAC^{12807M} library was prepared at 10 μ M in buffer and read in fluorescence intensity mode using filters typical to a green, red, or far-red tracer. Wells containing 1 nM of each tracer were placed on the assay plate so that signal intensity could be referenced to a typical tracer concentration.

Figure 3—PolarScreen™ Far-Red FP Kinase Kits address a range of kinase targets



Seven far-red kinase assays are available to address a wide variety of kinase targets. The plots in above figure show the results from kinase titrations in 10 μ l, 90 minute kinase reactions under conditions of non-limiting substrate and ATP. In general, picogram to nanogram quantities of kinase will phosphorylate sufficient peptide substrate to effect an assay window of between 125 and 250 mP.

Assays

| Assay description | Assay size | Corresponding kinases | Cat. no. | Price |
|---|-------------|--|----------|-------|
| PolarScreen™ Protein Kinase C Assay Kit, Far-Red | 800 × 20 µl | PKA, PKB α /Akt1, all PKC isoforms, CHK1 | PV3337 | \$610 |
| PolarScreen™ Tyrosine Kinase Assay Kit, Far-Red | 800 × 20 µl | All tyrosine kinases | PV3327 | \$610 |
| PolarScreen™ Ser/Thr Kinase Assay Kit, Crosstide, Far-Red | 800 × 20 µl | PKA, PKB α /Akt1, PKC, PKG1 α , MSK1, MAPKAP-K1 α | PV3331 | \$610 |
| PolarScreen™ Ser Kinase Assay Kit, I κ B- α pSer32, Far-Red | 800 × 20 µl | IKK-like kinases | PV3334 | \$610 |
| PolarScreen™ Ser/Thr Kinase Assay Kit, CREBtide, Far-Red | 800 × 20 µl | PKA, CAMKII, MSK1, p90RSK | PV3340 | \$610 |
| PolarScreen™ CDK Assay Kit, Rb ^{INC} , Far-Red | 800 × 20 µl | CDK5/p35 | PV3343 | \$610 |
| PolarScreen™ PDK1 Threonine Kinase Assay Kit, Far-Red | 800 × 20 µl | PDK1 | PV3346 | \$610 |

Substrates

| Substrate | Assay size | Cat. no. | Price |
|--|------------|----------|-------|
| PKC Substrate, 100 µM | 1 ml | P2760 | \$150 |
| Crosstide Substrate Peptide, 1 mM | 200 µl | P3107 | \$295 |
| I κ B- α Substrate Peptide, 100 µM | 1 ml | P2826 | \$150 |
| CREBtide Substrate, 100 µM | 1 ml | PV3188 | \$135 |
| Rb ^{INC} Substrate Peptide, 100 µM | 1 ml | P2939 | \$150 |
| PDK1 Substrate Peptide, 100 µM | 1 ml | P2925 | \$295 |



These products may be covered by one or more Limited Use Label Licenses (see the Invitrogen catalog or our web site, www.invitrogen.com). By the use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses.

For research use only. Not intended for any animal or human therapeutic or diagnostic use. Printed in the U.S.A.
©2004 Invitrogen Corporation. All rights reserved. Reproduction forbidden without permission.

Corporate headquarters:

1600 Faraday Avenue • Carlsbad, CA 92008 USA • Tel: 760 603 7200 • Fax: 760 602 6500 • Toll Free Tel: 800 955 6288 • E-mail: tech_service@invitrogen.com • www.invitrogen.com

European headquarters:

Invitrogen Ltd • Inchinnan Business Park • 3 Fountain Drive • Paisley PA4 9RF, UK • Tel: +44 (0) 141 814 6100 • Fax: +44 (0) 141 814 6260 • E-mail: eurotech@invitrogen.com