

Kinase:	<b>PRKCB1</b>	P2291 (5 µg) P2281 (20 µg)
Antibody:	LanthaScreen™ Tb-pPKC Substrate Antibody	PV3560 (25 µg) PV3561 (1 mg)
Substrate:	Fluorescein-PKC Substrate	PV3506 (1 mg)
Kinase Dilution Buffer:	1X Kinase Buffer	PV3189 (4 mL of 5X)
Antibody Dilution Buffer:	TR-FRET Dilution Buffer	PV3574 (100 mL)

A two-fold serial dilution of kinase was incubated with 250 nM fluorescein-labeled substrate and 10 µM ATP in a total volume of 10 µL in a black Corning low-volume 384-well plate (Corning #3676). After a 90 minute incubation at room temperature, 10 µL TR-FRET dilution buffer containing EDTA and Tb-labeled phosphospecific antibody was added such that the final concentration of antibody was 0.5 nM and the final concentration of EDTA was 10 mM. After a 60 minute incubation at room temperature, the plate was read on a BMG LABTECH PHERAstar using the LanthaScreen™ filter module. Each datapoint represents the average of three wells.

The data generated under these conditions are shown in the graph below. We recommend these conditions as an unoptimized starting point for additional assay development. Assay performance may potentially be improved by using different assay buffers or buffer components, or by varying the concentrations of substrate, ATP, or antibody that are used.

### LanthaScreen™ PRKCB1 Kinase Titration Tb-pPKC Substrate Antibody Fluorescein-PKC Substrate

