Kinase:	**MAP4K2**	PV4211 (10 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 400 nM fluorescein-labeled substrate and 100 µM ATP in a total volume of 10 µL in a black Corning low-volume 384-well plate (Corning #3676). After a 60 minute incubation at room temperature, 10 µL of TR-FRET dilution buffer containing EDTA and Tb-labeled phosphospecific antibody was added and mixed such that the final volume per well was 20 µL, the final EDTA concentration was 10 mM, and the final antibody concentration was 2 nM. After a 60 minute incubation at room temperature, the plate was read on a TECAN Ultra 384 with a LanthaScreen™ filter set (Ex: 340/30; Em: 495/10, 520/25). Each data point represents the average of four 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.