Kinase:	**MAPKAPK3**	PV3299 (10 µg)
Antibody:	LanthaScreen™ Tb-pCREB pSer133 Antibody	PV3566 (25 µg) inquire (1 mg)
Substrate:	Fluorescein-CREBtide Substrate	PV3508 (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 350 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, 5 µL ofTR-FRET dilution buffer containing EDTA was added followed by 5 µL of TR-FRET dilution buffer containing Tb-labeled phosphospecific antibody. The final volume per well was 20 µL, the final concentration of EDTA was 10 mM, and the final concentration of antibody was 3 nM. After a 60 minute incubation at room temperature, the plate was read on a BMG LABTECH PHERAstar using the LanthaScreen filter module available from BMG. Each data point 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.