| Kinase: | SRCN1 | P2904 (10 µg) |
|---|---|--------------------------------|
| Antibody: | LanthaScreen™ Tb-PY20 Antibody | PV3552 (25 μg) PV3553 (1 mg) |
| Substrate: | Fluorescein-Poly GAT Fluorescein-Poly GT | PV3611 (1 mg) PV3610 (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 μ M ATP in a total volume of 10 μ L in a black Corning low-volume 384-well plate (Corning #3676). After a 60 minute incubation at room temperature, 5 μ L of TR-FRET dilution buffer containing EDTA was added followed by 5 μ L of TR-FRET dilution buffer containing Tb-labeled phosphospecific antibody. The final volume per well was 20 μ L, the final concentration of EDTA was 10 mM, and the final concentration of antibody was 2 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 four wells.

The data generated under these conditions are shown in the graphs 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.

