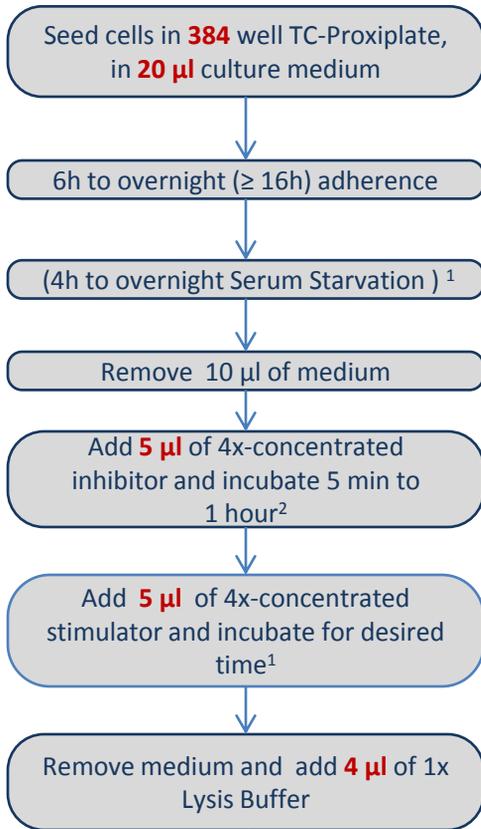
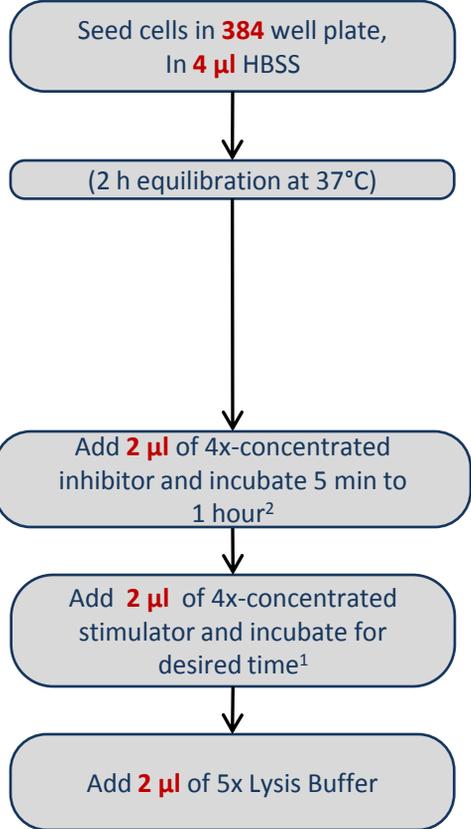


AlphaScreen® SureFire®: 1-plate/ 1-step assay flowchart (p-ERK1/2 and p-MEK1 kits only)

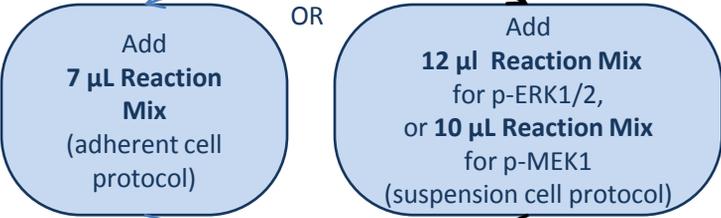
Adherent Cells



Suspension Cells



In control wells, add positive or negative control lysates. Incubate for 10 min on plate shaker (~350 rpm).³



Seal, wrap in foil, and (shake 1-2 min on plate shaker), then incubate ≥ 2 h (RT° or 22°C)

Read Plate

Reaction Mix:	Typical volume for p-ERK1/2 ⁴	Typical volume for p-MEK1 ⁴
Reaction Buffer	587 µL	636 µL
Activation Buffer	98 µL	49 µL
Acceptor Beads	10 µL	10 µL
Donor Beads	5 µL	5 µL

⁴ **Important note:** only the p-ERK and p-MEK1 assays can be run using a single step immunoassay. For other kits; a separate Acceptor Mix and Donor Mix procedure needs to be performed.

¹ Depending on cell type and pathway analyzed.

² Depending on type of inhibitor used: 5 min is generally enough for receptor antagonists; more time is needed to block intracellular targets.

³ May stop and freeze lysates at -20°C if desired. If doing this, re-shake after thawing to ensure homogeneity of solution.