AlphaScreen® SureFire®: 1-plate/2-steps assay flowchart

**Adherent Cells**

Seed cells in **384** well plate, in **20 μl** culture medium

1. Overnight (≥ 16h) adherence
2. (4h to overnight Serum Starvation)¹
3. Remove 10 μl of medium
4. Add 5 μl of 4x-concentrated inhibitor and incubate 5 min to 1 hour²
5. Add 5 μl of 4x-concentrated stimulator and incubate for desired time
6. Remove medium and add 4 μl of 1x Lysis Buffer

Incubate for 10 min on plate shaker (~350 rpm).³

In control wells, add positive or negative control lysates.

**Suspension Cells**

Seed cells in **384** well plate, in **4 μl** HBSS

1. (2 h equilibration at 37°C)
2. Add 2 μl of 4x-concentrated inhibitor and incubate 5 min to 1 hour²
3. Add 2 μl of 4x-concentrated stimulator and incubate for desired time
4. Add 2 μl of 5x Lysis Buffer

**Acceptor Mix:**

<table>
<thead>
<tr>
<th>Typical volume</th>
<th>My volumes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reaction Buffer</strong></td>
<td>392 μL</td>
</tr>
<tr>
<td><strong>Activation Buffer</strong></td>
<td>98 μL</td>
</tr>
<tr>
<td><strong>Acceptor Beads</strong></td>
<td>10 μL</td>
</tr>
</tbody>
</table>

**Donor Mix:**

<table>
<thead>
<tr>
<th>Typical volume</th>
<th>My volumes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dilution Buffer</strong></td>
<td>190 μL</td>
</tr>
<tr>
<td><strong>Donor Beads</strong></td>
<td>10 μL</td>
</tr>
</tbody>
</table>

³ Important note: the volumes shown here are the ones for most common kits. For a few kits (e.g. c-Jun, coflin, GSK3β, H3, MEK-1 and VEGFR2), the dilution factors are different. In this case please refer to the manual of the kits for volumes to be used.

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.