AlphaLISA® SureFire® Ultra™

p-p38 MAPK (Thr180/Tyr182) Assay Kit

Manual

<table>
<thead>
<tr>
<th>Assay Points</th>
<th>Catalog #</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>ALSU-PP38-A500</td>
</tr>
<tr>
<td>10 000</td>
<td>ALSU-PP38-A10K</td>
</tr>
<tr>
<td>50 000</td>
<td>ALSU-PP38-A50K</td>
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</tbody>
</table>

For Research Use Only. Not for use in Diagnostic Procedures

For a full, electronic, version of this manual, please go to: www.perkinelmer.com/pp38MAPK
**Assay Principle**

The AlphaLISA® SureFire® Ultra™ assay kits allow the rapid, sensitive, and quantitative detection of phosphoproteins from cells. The kits utilize Alpha beads that are each coated to specifically capture one of the assay antibodies. The Donor bead is coated with streptavidin to capture the biotinylated antibody. The Acceptor bead is coated with a proprietary CaptSure™ agent that immobilizes the other assay antibody which is labeled with a CaptSure™ tag. As such, this assay system performs well in the presence of extraneous antibodies, such as antibody biotherapeutics, and can be used to screen such reagents. In the presence of phosphorylated protein, the two antibodies bring the Donor and Acceptor beads close to each other, enabling the generation of an Alpha signal upon illumination of Donor beads by the Alpha-enabled plate reader, such as the EnVision® Multilabel Plate Reader or Enspire® and EnSight™ Multimode Plate Readers. The amount of light emission is directly proportional to the amount of phosphoprotein present in the sample.

The AlphaLISA® SureFire® Ultra™ assay kits are also optimized for enhanced signal-to-noise windows, while using shorter incubation times and larger volumes for pipetting compared to the AlphaScreen® SureFire® kits. This assay eliminates the need for laborious techniques, such as Western blotting or conventional ELISA. It is a homogeneous assay, in that no sample washing steps are required, which allows for minimal handling, short assay times, better well-to-well reproducibility (lower CV%), and robotic operation if desired. The assay utilizes the bead-based Alpha Technology, and requires an Alpha Technology-compatible plate reader.

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General Information on the AlphaLISA® SureFire® Ultra™ p-p38 MAPK (Thr180/Tyr182) assay

The AlphaLISA® SureFire® Ultra™ p-p38 MAPK assay is used to measure the phosphorylation of endogenous p38 MAP kinase at Thr180/Tyr182, in cellular lysates. The assay is an ideal system for the screening of both modulators of receptor activation (e.g. agonists and antagonists) as well as agents acting intracellularly, such as small molecule inhibitors of upstream events. The assay will measure p38 MAPK activation by either recombinant or endogenous receptors, and can be applied to primary cells.

This kit has been formulated to provide improved signal:noise assay windows, and to perform without interference in the presence of extraneous antibodies.

Kit-Specificity information

This assay kit contains antibodies which recognize the phospho-Thr180/Tyr182 epitope, and a distal epitope, on p38 MAPK. The protein detected by this kit corresponds to GenBank Accession NP_001306. Also known as p38; SAPK2A; p38alpha; MAPK14.

These antibodies recognize p38 MAPK of human, mouse, rat origin. Other species should be tested on a case-by-case basis.

Kit Contents (store at 4°C)

<table>
<thead>
<tr>
<th>Kit Size</th>
<th>500 points</th>
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<tr>
<td>Lysis Buffer (5X) - Ultra</td>
<td>1 x 12 mL</td>
<td>4 x 60 mL</td>
<td>3 x 400 mL</td>
</tr>
<tr>
<td>Activation Buffer - Ultra</td>
<td>1 x 0.8 mL</td>
<td>1 x 10 mL</td>
<td>1 x 50 mL</td>
</tr>
<tr>
<td>Reaction Buffer 1 - Ultra</td>
<td>1 x 1.2 mL</td>
<td>1 x 23.5 mL</td>
<td>1 x 117.5 mL</td>
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<tr>
<td>Reaction Buffer 2 - Ultra</td>
<td>1 x 1.2 mL</td>
<td>1 x 23.5 mL</td>
<td>1 x 117.5 mL</td>
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<tr>
<td>Dilution Buffer - Ultra</td>
<td>1 x 2.5 mL</td>
<td>1 x 49 mL</td>
<td>1 x 245 mL</td>
</tr>
<tr>
<td>AlphaLISA® CaptSure™ Acceptor Beads (2mg/mL in PBS plus 0.05% Proclin-300)</td>
<td>1 x 50 µL</td>
<td>1 x 1.0 mL</td>
<td>1 x 5 mL</td>
</tr>
<tr>
<td>AlphaScreen® Streptavidin Donor Beads (2mg/mL in PBS plus 0.05% Proclin-300)</td>
<td>1 x 50 µL</td>
<td>1 x 1.0 mL</td>
<td>1 x 5 mL</td>
</tr>
<tr>
<td>Positive Control Lysate</td>
<td>1 tube to be re-dissolved in 250 µL H2O</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Storage Conditions Upon Receipt

The kit should be placed at 4°C upon receipt. DO NOT freeze the kit buffers or beads – the Reaction buffer contains antibodies and freeze/thaw cycles can lead to a loss of activity.

AlphaScreen Donor Beads need to be stored at 4°C in the dark, and should be returned to the kit box after use.

The Activation Buffer precipitates at 4°C. To re-dissolve, warm to 37°C and mix before each use. Alternatively, Activation buffer can be stored at room temperature with no loss in activity. All other components to be returned to 4°C after each use.

The Positive control lysate tube should be placed at -20°C or -80°C for long term storage.

This product is stable for at least 9 months from the manufacturing date if used and stored under recommended conditions.

Materials Required But Not Provided

<table>
<thead>
<tr>
<th>Item</th>
<th>Suggested source</th>
<th>Catalog #</th>
<th>Size</th>
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<tr>
<td>Optiplate™-384, White Opaque assay plate</td>
<td>PerkinElmer Inc.</td>
<td>6007290</td>
<td>50/box</td>
</tr>
<tr>
<td>AlphaPlate-384, Light gray assay plate</td>
<td>PerkinElmer Inc.</td>
<td>6005350</td>
<td>50/box</td>
</tr>
<tr>
<td>CulturPlate-384, white, sterile, with lid, 384-well</td>
<td>PerkinElmer Inc.</td>
<td>6007680</td>
<td>50/box</td>
</tr>
<tr>
<td>ViewPlate-96 F, TC, black frame with clear bottom, sterile, with lid, 96-well</td>
<td>PerkinElmer Inc.</td>
<td>6005182</td>
<td>50/box</td>
</tr>
<tr>
<td>TopSeal-A 384, clear adhesive sealing film</td>
<td>PerkinElmer Inc.</td>
<td>6050185</td>
<td>100/box</td>
</tr>
<tr>
<td>Envision®, Enspire® or Ensign™ Alpha-reader</td>
<td>PerkinElmer Inc.</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(1) Plates used for the immunoassay; (2) Same as (1) but optimal if cross-talk needs to be reduced; (3) plates for assays run in a 1-plate protocol (from cell seeding to immunoassay); (4) plates used to seed and stimulate cells before Lysis and transfer of lysate in an immunoassay plate.

For more assay plates options, please go to [www.perkinelmer.com/microplates](http://www.perkinelmer.com/microplates)

Precautions

*Only the AlphaScreen® Donor beads are light-sensitive. All the other assay reagents can be used under normal light conditions. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco, or the equivalent) can be applied to light fixtures.
## Buffer Preparation and Subsequent Storage Conditions

<table>
<thead>
<tr>
<th>Buffer Type</th>
<th>Preparation and Storage Conditions</th>
</tr>
</thead>
</table>
| **1X Lysis Buffer**                 | Dilute 5X Lysis buffer in MilliQ water to a final concentrations of 1X  
For example: for 10 mL of 1X Lysis Buffer, add: 2 mL of 5X Lysis Buffer to 8 mL MilliQ water. Discard unused 1X buffer.                                                                         |
| **Acceptor Mix**                    | Dilute Activation Buffer **25-fold** in combined Reaction Buffer 1 and Reaction buffer 2  
Dilute Acceptor beads **50-fold** in combined Reaction Buffers  
For example: for 300 µL of Acceptor Mix: Combine 141µL of Reaction Buffer 1 and 141µL of Reaction buffer 2, and to this add 12µL Activation Buffer and 6µL Acceptor Beads  
The Acceptor mix should be made up and used immediately when required for best results. Excess mix should be discarded. |
| **Donor Mix***                      | Dilute Donor beads **50-fold** in Dilution buffer  
For example: for 300 µL of Donor Mix, add: 6 µL Donor Beads to 294 µL of Dilution Buffer  
The Donor mix should be made up and used immediately when required for best results. Excess mix should be discarded.                                                                  |
| **Positive control lysate**         | Stable while lyophilized at -20°C to expiry date. After reconstitution in 250µL of water, lysate should be frozen at -20°C in single use aliquots and used within 1 month.                                                                                       |

* Prepare and use under low-light conditions.  
Note: the buffers (lysis, activation, reaction, dilution) in the AlphaLISA SureFire Ultra kits have a different formulation compared to the buffers from the AlphaScreen SureFire kits, and buffers from the two types of kits should not be interchanged.
AlphaLISA® SureFire® Ultra™ p-p38 MAPK (Thr180/Tyr182) Assay Protocols

A. 2-Plate Assay - assay protocol for adherent cells

Cell Seeding
1. Seed cells (200 μL of cells for 96 well plates, 50 μL for 384 well plates) in tissue culture plates. Incubate at 37°C overnight in serum-containing media.

Cell Treatment
2. Remove culture media, and stimulate the cells with 50 μL agonists prepared in serum-free media (25 μL for 384-well plates). (If testing antagonists, prior to stimulation remove culture medium and replace with 50 μL serum-free media containing antagonists (25 μL for 384-well plates)). Return cells to 37°C incubator for desired time. 1 hour is often sufficient for signal transduction inhibitors, and 5-20 minutes for receptor agonists.

Note: Peptidic agonists and antagonists can often stick to plastic surfaces. To minimize this effect, dilute in serum-free media containing a suitable carrier protein (e.g. 0.1% BSA)

Lysate Preparation
3. To lyse cells, remove medium from wells, and add freshly prepared 1X Lysis Buffer (50-100 μL for a 96 well plate, 25 μL for a 384 well plate). Agitate on a plate shaker (~350 rpm) for 10 minutes at room temperature.

4. Take 10 μL of the lysate and transfer to a 384-well Optiplate™ for assay. (Add 10 μL Control lysates to separate wells if required).

SureFire Ultra Assay
5. Add 5 μL of Acceptor Mix to wells. Seal plate with Topseal-A adhesive film, and cover plate with foil. Incubate for 1 hour at room temperature.

6. Add 5 μL of Donor Mix to wells under subdued light. Seal plate with Topseal-A adhesive film, and cover plate with foil. Incubate for 1 hour at room temperature in the dark.

Note: Longer incubation may give greater sensitivity. Plates can be incubated overnight if required.

7. Read plate on an Alpha Technology-compatible plate reader, using standard AlphaLISA settings.
AlphaLISA® SureFire® Ultra™: 2-plates / 2-incubation assay flowchart

### Adherent Cells

- **Seed cells in 96 / 384 well plate, in 200 μl / 50 μl culture medium**
- **6 h to Overnight (≥16 h) adherence**
- **(4h to O/N Serum Starvation)³**
- **Remove medium**
- **Add inhibitor in 45 μl / 22.5 μl new medium and incubate 5 min to 1 hour²**
- **Add 5 μl / 2.5 μl of 10x concentrated stimulator and incubate for desired time¹**
- **Remove medium (wash with PBS if using medium containing high biotin concentration, like RPMI)**
- **Add 50 to 100 μl / 20 to 25 μl of 1x Lysis Buffer and incubate for 10 min on plate shaker (≈350 rpm).³**
- **Transfer 10 μl of lysate (or diluted positive control lysate, or Lysis buffer alone) to a white 384-well Plate**
- **Add 5 μl Acceptor Mix**
- **Seal, wrap in foil, and shake 1-2 minutes on plate shaker, Then incubate ≥1 h (RT² or 22°C)**
- **Add 5 μl of Donor Mix**
- **Seal, wrap in foil, and shake 1-2 minutes on plate shaker, then incubate ≥1 h, up to overnight (RT² or 22°C) Allow to equilibrate to plate reader temperature prior to reading.**

### Suspension Cells

- **Seed cells in 96 / 384 well plate, in 40 μl / 10 μl HBSS**
- **(2 h equilibration at 37°C)**
- **Add 20 μl / 5 μl of 4x-concentrated inhibitor and incubate 5 min to 1 hour²**
- **Add 20 μl / 5 μl of 4x-concentrated stimulator and incubate for desired time¹**
- **Add 20 μl / 5 μl of 5x Lysis Buffer and incubate for 10 min on plate shaker (≈350 rpm).³**

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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 lysate before pipetting.
**AlphaLISA® SureFire® Ultra™ p-p38 MAPK (Thr180/Tyr182)**

**1 Plate Assay** - assay protocol for non-adherent cells, and for high-throughput applications.

**Cell Seeding**
1. Harvest cells by centrifugation, and re-suspend cells in HBSS at a suitable cell density. We recommend 10^7 cells/mL as a starting point. Seed 4 μL of cells/well into a 384-well white opaque culture plate (e.g. PerkinElmer Cat # 6007680).

2. If using test agents/inhibitors, add 2 μL/well of 4X inhibitors prepared in HBSS.

**Note:** Peptidic agonists and antagonists can often stick to plastic surfaces. To minimize this effect, dilute in HBSS containing a suitable carrier protein (e.g. 0.1% IgG free BSA - Jackson Immunoresearch Cat #001-000-161).

3. Return cells to incubator at 37°C for 1-2 hours.

**Cell Treatment**
4. Stimulate cells with agonists by addition of 2 μL/well of 4X agonist stock in HBSS containing 0.1% BSA. The final volume in the wells should be 8 μL. *(if no antagonists were used in step 2, stimulate the cells with 4 μL/well of 2X agonist, to give a final volume in the wells of 8 μL.)*

**Lysate Preparation**
5. To lyse the cells, add 2 μL/well of 5X Lysis Buffer.

*(Add 10 μL control lysates to separate wells if required)*

**SureFire Ultra Assay**
6. Add 5 μL of Acceptor Mix to wells. Seal plate with Topseal-A adhesive film, and cover plate with foil. Incubate for 1 hour at room temperature.

7. Add 5 μL of Donor Mix to wells under subdued light. Seal plate with Topseal-A adhesive film, and cover plate with foil. Incubate for 1 hour at room temperature.

**Note:** Longer incubation may give greater sensitivity. Plates can be incubated overnight if required.

8. Read plate on an Alpha Technology-compatible plate reader, using standard AlphaLISA settings.
AlphaLISA® SureFire® Ultra™: 1-plate / 2-incubation assay flowchart

**Adherent Cells**
- Seed cells in 384 well plate, in 20 µl culture medium
- Overnight (≥16h) adherence
- (4h to overnight Serum Starvation)¹
- Remove 10 µl of medium
- Add 5 µl of 3x-concentrated inhibitor and incubate 5 min to 1 hour²
- Add 5 µl of 4x-concentrated stimulator and incubate for desired time
- Remove medium and add 10 µl of 1x Lysis Buffer
- Seal and incubate for 10 min on plate shaker (∼350 rpm).³
- In control wells, add 10 µl positive control lysate dilution or lysis buffer alone.

**Suspension Cells**
- Seed cells in 384 well plate, in 4 µl HBSS
- (2 h equilibration at 37°C)
- Add 2 µl of 3x-concentrated inhibitor and incubate 5 min to 1 hour²
- Add 2 µl of 4x-concentrated stimulator and incubate for desired time
- Add 2 µl of 5x Lysis Buffer
- Seal, wrap in foil, and shake 1-2 minutes on plate shaker, Then incubate ≥1 h (RT° or 22°C)
- Seal, wrap in foil, and shake 1-2 minutes on plate shaker, then incubate ≥1 h, up to overnight (RT° or 22°C) Allow to equilibrate to plate reader temperature prior to reading.
- Read Plate

<table>
<thead>
<tr>
<th>Acceptor Mix:</th>
<th>Typical volume (60 x 5 µl)</th>
<th>My volumes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Buffer 1</td>
<td>141 µl</td>
<td></td>
</tr>
<tr>
<td>Reaction Buffer 2</td>
<td>141 µl</td>
<td></td>
</tr>
<tr>
<td>Activation Buffer</td>
<td>12 µl</td>
<td></td>
</tr>
<tr>
<td>Acceptor Beads</td>
<td>6 µl</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Donor Mix:</th>
<th>Typical volume (60 x 5 µl)</th>
<th>My volumes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution Buffer</td>
<td>294 µl</td>
<td></td>
</tr>
<tr>
<td>Donor Beads</td>
<td>6 µl</td>
<td></td>
</tr>
</tbody>
</table>

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 lysate before pipetting.
Control Lysate Information
Positive Control Lysate: Prepared from HeLa cells, cultured to confluence in T175 flasks in 10% FBS containing medium for 3 days, then treated with anisomycin (2 µg/mL) for 15 min and lysed in 8 mL of 1X SureFire Ultra Lysis buffer.

Representative Data

Data obtained with 2-incubation protocol.

Supplementary Buffers and Beads
If using the standard protocol, sufficient amounts of buffers and beads are provided in the kit. However in case the standard protocol would be modified, more buffers or beads may be needed. In this case, you can order additional buffers and beads using the following catalog numbers:

<table>
<thead>
<tr>
<th>Item</th>
<th>Suggested source</th>
<th>Catalog #</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
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<td>PerkinElmer Inc.</td>
<td>ALSU-LB-10mL</td>
<td>10mL</td>
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<td></td>
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<td>ALSU-LB-100mL</td>
<td>100mL</td>
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<tr>
<td>Activation Buffer - Ultra</td>
<td>PerkinElmer Inc.</td>
<td>ALSU-AB-10mL</td>
<td>10mL</td>
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<tr>
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<td>PerkinElmer Inc.</td>
<td>ALSU-AB-100mL</td>
<td>100mL</td>
</tr>
<tr>
<td>Dilution Buffer - Ultra</td>
<td>PerkinElmer Inc.</td>
<td>ALSU-DB-10mL</td>
<td>10mL</td>
</tr>
<tr>
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<td>PerkinElmer Inc.</td>
<td>ALSU-DB-100mL</td>
<td>100mL</td>
</tr>
<tr>
<td>AlphaScreen® Streptavidin Donor Beads - 2mg/mL</td>
<td>PerkinElmer Inc.</td>
<td>ALSU-ASDB-0.06mL</td>
<td>60µL</td>
</tr>
<tr>
<td></td>
<td>PerkinElmer Inc.</td>
<td>ALSU-ASDB-1.2mL</td>
<td>1.2mL</td>
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<tr>
<td></td>
<td>PerkinElmer Inc.</td>
<td>ALSU-ASDB-6mL</td>
<td>6mL</td>
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<tr>
<td>AlphaLISA® CaptSure™ Acceptor Beads - 2mg/mL</td>
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<td>ALSU-ACAB-0.06mL</td>
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<td>PerkinElmer Inc.</td>
<td>ALSU-ACAB-1.2mL</td>
<td>1.2mL</td>
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<tr>
<td></td>
<td>PerkinElmer Inc.</td>
<td>ALSU-ACAB-6mL</td>
<td>6mL</td>
</tr>
</tbody>
</table>
**Note:**

This assay can also be carried out as a single 2 hour incubation after separate additions of Acceptor Mix then Donor Mix, as indicated below in the flow chart. This can be applied to either 1-plate or 2-plate assays. Shown below is the 2-plate protocol in this format. This assay format may result in a slightly reduced sensitivity.

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**AlphaLISA® SureFire® Ultra™: 2-plates / 1-incubation assay flowchart**

**Adherent Cells**
- Seed cells in 96 / 384 well plate, in 200 µl / 50 µl culture medium
- 6 h to Overnight (≥ 16h) adherence
- (4h to O/N Serum Starvation)¹
- Remove medium
- Add inhibitor in 45 µl / 22.5 µl new medium and incubate 5 min to 1 hour²
- Add 5 µl / 2.5 µl of 10x-concentrated stimulator and incubate for desired time³
- Remove medium (wash with PBS if using medium containing high biotin concentration, like RPMI)
- Add 50 to 100 µl / 20 to 25 µl of 1x Lysis Buffer and incubate for 10 min on plate shaker (~350 rpm)³

**Suspension Cells**
- Seed cells in 96 / 384 well plate, in 40 µl / 10 µl HBSS
- (2 h equilibration at 37°C)
- Add 20 µl / 5 µl of 4x-concentrated inhibitor and incubate 5 min to 1 hour²
- Add 20 µl / 5 µl of 4x-concentrated stimulator and incubate for desired time³
- Add 20 µl / 5 µl of 5x Lysis Buffer and incubate for 10 min on plate shaker (~350 rpm)³

---

**Transfer 10 µl of lysate (or diluted positive control lysate, or Lysis buffer alone) to a white 384-well Plate**

**My volumes**
- Add 5 µl of Acceptor Mix

**My volumes**
- Add 5 µl Donor Mix ⁴

Seal, wrap in foil, and shake 1-2 minutes on plate shaker, then incubate ≥2 h, up to overnight (RT or 22°C) Allow to equilibrate to plate reader temperature prior to reading.

**Read Plate**

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**Accept Mix:**
- **Typical volume** (60 x 5 µl)
- **My volumes**
  - Reaction Buffer 1: 141 µl
  - Reaction Buffer 2: 141 µl
  - Activation Buffer: 12 µl
  - Acceptor Beads: 6 µl

**Donor Mix:**
- **Typical volume** (60 x 5 µl)
- **My volumes**
  - Dilution Buffer: 294 µl
  - Donor Beads: 6 µl

---

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 lysate before pipetting.
⁴ We recommend adding Acceptor Mix and Donor Mix in 2 sequential steps to maximize assay performance.
Useful Links

For FAQ and troubleshooting, please go to:
www.perkinelmer.com/SureFireFAQ

For a complete list of AlphaLISA SureFire Ultra kits, please go to:
www.perkinelmer.com/SureFire
or
www.tgrbio.com

For technical support please go to:
www.perkinelmer.com/ASK

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