

**MANUAL**



# AlphaLISA™ SureFire®

## Ultra™

### KRAS Total

|                     |                        |                        |                        |
|---------------------|------------------------|------------------------|------------------------|
| <b>Part number:</b> | <b>ALSU-TKRAS-A500</b> | <b>ALSU-TKRAS-A10K</b> | <b>ALSU-TKRAS-A50K</b> |
| Assay points:       | 500                    | 10,000                 | 50,000                 |

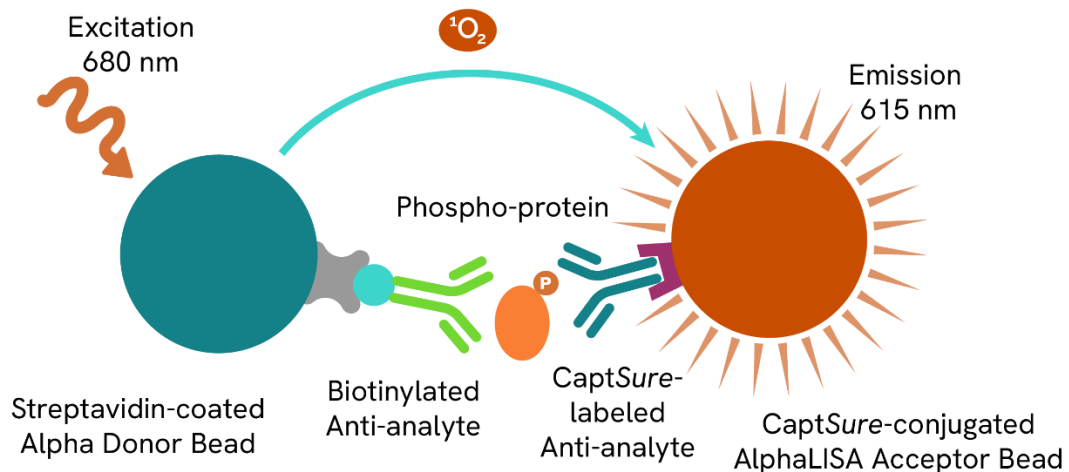
For assay-specific information, relating to Kit Specificity, Control Lysates and Representative Data, please refer to the Technical Data Sheet of the kit, available from [www.revvity.com](http://www.revvity.com).

**Note: For kit handling and disposal information see page 3 & 4 of this manual**

## ASSAY PRINCIPLE

The AlphaLISA™ *SureFire*® *Ultra*™ assay kits enable the rapid and sensitive detection of phosphorylated or total cellular proteins. The kit utilizes bead-based Alpha Technology. The Acceptor Bead is coated with a proprietary CaptSure™ agent to specifically immobilize the assay specific antibody which is labeled with a CaptSure™ tag. The Donor Bead is coated with streptavidin to capture the biotinylated antibody. In the presence of a target protein, the two antibodies bring the Donor and Acceptor Beads in close proximity. This enables the generation of an Alpha signal upon illumination of Donor Beads by an Alpha-enabled plate reader such as the EnVision™, EnVision Nexus™, Enspire™ and EnSight™ Multimode Plate Readers. The amount of light emission is directly proportional to the amount of target protein present in the sample.

*SureFire* assays have a simple and homogeneous no-wash assay format which make them highly amenable to automation and far less laborious than other techniques such as Western blotting or conventional ELISA. The workflow attributes and high performance characteristics (i.e. sensitivity, dynamic range and reproducibility) of our extensive *SureFire* assay offering are ideally suited to both high throughput screening in pharma drug discovery programs as well as lower throughput academic research applications.



## MAIN FEATURES

The AlphaLISA™ SureFire® Ultra™ assays are used to measure a phosphorylated or total protein in cellular extracts. The assays are an ideal system for the screening of modulators of receptor activation (e.g agonists and antagonists) as well as agents acting intracellularly, such as small molecule inhibitors of upstream events and also for targeted protein degradation approaches such as PROTAC. The assays measure changes in levels of cellular proteins in endogenous or engineered systems and can be applied to all types of cellular extracts, including cultured primary/non-primary cell lines and tissue lysates. The kits are very sensitive and formulated to provide improved signal:noise assay windows and to perform without interference in the presence of extraneous antibodies.

## KIT-SPECIFICITY / CONTROL LYSATE / REPRESENTATIVE DATA INFORMATION

The assay specific Technical Data Sheet and Certificate of Analysis (COA) are available on the website. Search for Lot Specific COA's from [www.revivity.com](http://www.revivity.com)

## KIT CONTENTS

|  | Kit size             |               |                          |
|--|----------------------|---------------|--------------------------|
|  | 500 points           | 10,000 points | 50,000 points            |
| Lysis Buffer (5X)  | 1 x 12 mL            | 4 x 60 mL     | 3 x 400 mL               |
| Activation Buffer  | 1 x 0.8 mL           | 1 x 10 mL     | 1 x 50 mL                |
| <b>10X Reaction Buffer 1 - ALSU</b>  | 1 x 0.15 mL          | 1 x 2.8 mL    | 1 x 14 mL                |
| <b>10X Reaction Buffer 2 - ALSU</b>  | 1 x 0.15 mL          | 1 x 2.8 mL    | 1 x 14 mL                |
| Dilution Buffer  | 2 x 3 mL             | 2 x 60 mL     | 1 x 500 mL<br>1 x 100 mL |
| AlphaLISA™ CaptSure™ Acceptor Beads<br>(2 mg/mL in PBS plus 0.03% Proclin-300) | 1 x 0.06 mL          | 1 x 1.1 mL    | 1 x 5.5 mL               |
| Alpha Streptavidin Donor Beads<br>(2 mg/mL in PBS plus 0.03% Proclin-300)      | 1 x 0.06 mL          | 1 x 1.1 mL    | 1 x 5.5 mL               |
| Positive Control Lysate  | 1 X Lyophilized tube |               |                          |

**The above volumes supplied are in excess to the actual volume required to perform assay.**

## STORAGE AND HANDLING CONDITIONS

Expiry date indicated on kit box.

|                     |                                      |  |
|---------------------|--------------------------------------|--|
| <b>Upon receipt</b> |                                      | Remove <u>10X Reaction Buffers 1 and 2</u> from kit and <b>store at -20°C</b><br>(formulation contains glycerol and will not freeze)   |
| <b>Opened kit</b>   | Lysis Buffer (5X)<br>Dilution Buffer | Store at 4°C   |
|                     | Activation Buffer                    | Precipitates at 4°C. To re-dissolve, warm to 37°C and mix before each use. Alternatively, once re-dissolved can be stored at room temperature with no loss in activity.                                      |
|                     | Acceptor/Donor Beads                 | Store at 4°C, in the dark zip lock bag or box provided. Vortex vigorously for 5-10 seconds, then give a quick spin (e.g 500 rpm for 1 second) to ensure bead suspension is at the bottom of the tube/bottle. |
|                     | Positive Control Lysate              | Store at -20°C.  |

## MATERIALS REQUIRED BUT NOT PROVIDED

| Item   | Suggested source | Part #  | Size    |
|--|------------------|---------|---------|
| Optiplate-384, White Opaque assay plate <sup>(1)</sup>                     | Revvity Inc.     | 6007290 | 50/box  |
| AlphaPlate-384, Light Gray Opaque assay plate <sup>(2)</sup>               | Revvity Inc.     | 6005350 | 50/box  |
| CulturPlate-384, White Opaque, Sterile, TC-Treated <sup>(3)</sup>          | Revvity Inc.     | 6007680 | 50/box  |
| ViewPlate-384, White with clear bottom, Sterile, TC-Treated <sup>(4)</sup> | Revvity Inc.     | 6007480 | 40/box  |
| White adhesive seal for the bottom of microplates <sup>(5)</sup> .         | Revvity Inc.     | 6005199 | 1X55    |
| Spectraplate-96, Clear, sterile TC-treated plate <sup>(6)</sup>            | Revvity Inc.     | 6005650 | 50/box  |
| TopSeal-A 384, clear adhesive sealing film                                 | Revvity Inc.     | 6050185 | 100/box |
| Envision™ or Ensign™ Alpha-reader  | Revvity Inc.     | -       | -       |

- (1) Plates used for the immunoassay or for the one-plate protocol (from cell seeding to immunoassay) using suspension cells; (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) using adherent cells; (4) Same as (3) but with the possibility to check cells by microscopy, in this case a white adhesive seal should be stuck to the bottom of the plate before plate reading; (5) This seal can be used to turn the clear bottom of microplates opaque; (6) 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.revvity.com](http://www.revvity.com).

## KIT CONTENT INFORMATION

**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are generic for all kits and available from [www.revvy.com](http://www.revvy.com)

### **Lysis Buffer (5X)**

Lysis Buffer (5X) is a proprietary mixture of buffers, detergents and generic phosphatase inhibitors (Orthovanadate, Pyrophosphate and sodium fluoride), optimized for lysis of a broad range of cells without the excessive release of nuclear DNA. It does not contain protease inhibitors. Additives can be supplemented to the lysis buffer as required for particular cell types and may include excipients such as protease inhibitors or extra detergents. These will need to be tested on a case-by-case basis.

Buffers contain Triton X-100, otherwise known as p-tert-octylphenol ethoxylate, which must be disposed of as Controlled Waste in accordance with Local Regulations.

**Lysis Buffer color may vary from clear-yellow-green. The visual appearance has no impact on performance.**

### **10X Reaction Buffer 1 and 10X Reaction Buffer 2**

Dilute 10-fold in Dilution Buffer before use.

### **Alpha Streptavidin Donor Beads**

Alpha Streptavidin Donor Beads are light-sensitive. 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. The Donor Beads must NOT be used under red/orange light as can be found in photographic work darkrooms because red light (680 nm) excites the beads. All other assay reagents can be used under normal light conditions.

### **Positive Control lysate**

The Positive Control lysates are prepared from various cell types, which have been cultured and prepared to optimize the activation of the intracellular pathway of interest. The lysate is intended for use as an assay positive control only and should not be used for the absolute quantification of a particular protein or phosphorylated target. The lysate can be further diluted with Lysis Buffer (1X) and used to give an indication of the expected signal range for a given assay.

See the COA for the recommended dilution in the linear range of the assay.

## BUFFER PREPARATION AND SUBSEQUENT STORAGE CONDITIONS

Prepare volumes as needed and discard excess

|   |   |
|---|---|
| <p><b>1X Lysis Buffer</b></p>   | <p>Dilute 5X Lysis Buffer <b>5-fold</b> in deionized water.</p> <p><u>For example: For 10 mL of 1X Lysis Buffer:</u><br/>Add 2 mL of 5X Lysis Buffer to 8 mL deionized water.</p>   |
| <p><b>1X Reaction Buffer 1</b><br/>+Dilution Buffer<br/><b>1X Reaction Buffer 2</b><br/>+Dilution Buffer</p>                | <p>Dilute 10X Reaction Buffer 1 and 2 <b>10-fold</b> in Dilution Buffer.</p> <p><u>For example: For 150 mL of 1X Reaction Buffer 1 and 2:</u><br/>Add 15 µL of 10X Reaction Buffer 1 to 135 µL of Dilution Buffer.<br/>Add 15 µL of 10X Reaction Buffer 2 to 135 µL of Dilution Buffer.</p>   |
| <p><b>Acceptor Mix</b><br/>1X Reaction Buffer 1<br/>+ 1X Reaction Buffer 2<br/>+ Activation Buffer<br/>+ Acceptor Beads</p> | <p>Combine 1X Reaction Buffer 1 and 1X Reaction Buffer 2 in <b>equal parts</b>.<br/>Dilute Activation Buffer <b>25-fold</b> in final volume.<br/>Dilute Acceptor Beads <b>50-fold</b> in final volume.</p> <p><u>For example: For 300 µL of Acceptor Mix:</u><br/>Combine 141 µL of 1X Reaction Buffer 1 and 141 µL of 1X Reaction Buffer 2.<br/>Then add 12 µL of Activation Buffer.<br/>Then add 6 µL of Acceptor Beads.</p> <p>The Acceptor Mix should be made up and used within 30 minutes for best results.</p> |
| <p><b>Donor Mix</b><br/>Dilution Buffer<br/>+ Donor Beads</p>   | <p>Dilute Donor Beads <b>50-fold</b> in Dilution Buffer.</p> <p><u>For example: For 300 µL of Donor Mix:</u><br/>Add 6 µL Donor Beads to 294 µL of Dilution Buffer.</p> <p>The Donor Mix should be made up and used within 30 minutes for best results. <b>Prepare and use under low light conditions</b></p>   |
| <p><b>Positive Control Lysate</b></p>   | <p>Reconstitute with 250µL deionized water.<br/>Reconstituted lysate can be stored effectively at -80°C in single use aliquots but <u>should be tested on a case by case basis</u>. Dilute as required with 1X Lysis Buffer.</p>  |

# AlphaLISA™ SureFire® Ultra™ ASSAY PROTOCOL

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

### Cell Seeding

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

### Cell Treatment

2. Remove culture media and replace with 45  $\mu$ L of antagonists/inhibitors prepared 1X in serum-free media (22.5  $\mu$ L for 384-well plates).

**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% protease-free BSA).

3. Return cells to incubator at 37°C for 5 min to 2 hours.

**Note:** 1 hour is often sufficient for signal transduction inhibitors and 5 - 20 minutes for receptor antagonists. For short incubation times, the plate can stay at room temperature.

4. Stimulate the cells by the addition of 5  $\mu$ L of 10X agonist prepared in serum-free media (2.5  $\mu$ L for 384-well plates). *If not testing antagonists, directly add 50  $\mu$ L of 1X agonists prepared in serum-free media (25  $\mu$ L for 384-well plates).*

**Note:** Optimal agonist stimulation time is often between 5 and 20 minutes.

### Lysate Preparation

5. To lyse cells, remove medium from wells and add freshly prepared 1X Lysis Buffer (50-100  $\mu$ L for a 96 well plate, 20-25  $\mu$ L for a 384 well plate). Agitate on a plate shaker (~350 rpm) for 10 minutes at room temperature.

6. Take 10  $\mu$ L of the lysate and transfer to a 384-well Optiplate™ for assay. Add 10  $\mu$ L of Control lysates/1X Lysis Buffer to separate wells. *We recommend testing a serial dilution of Control lysate in 1X Lysis Buffer. See the COA for recommended dilution in the linear range of assay.*

### SureFire Ultra Assay

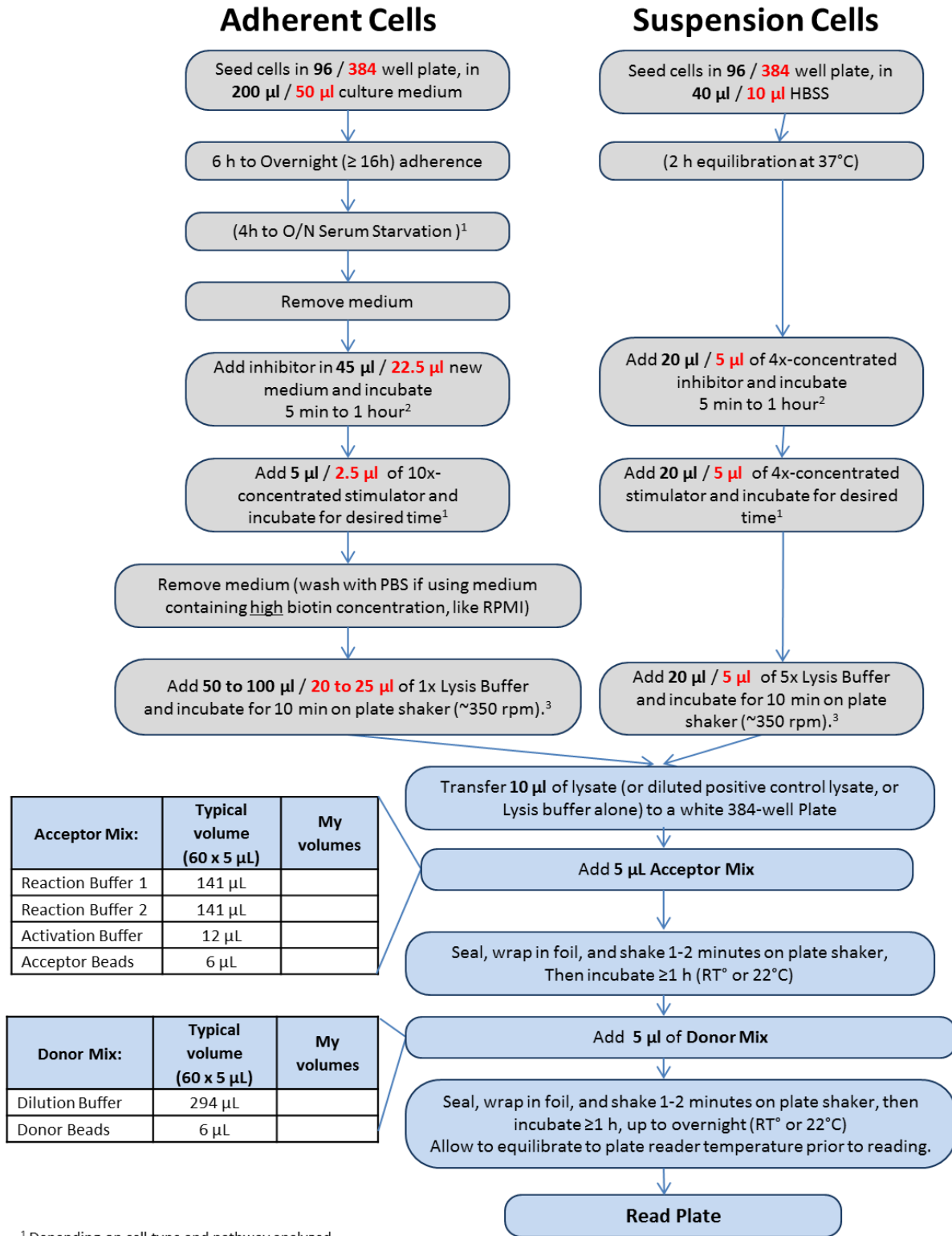
7. Add 5  $\mu$ L of Acceptor Mix to wells. Seal plate with Topseal-A adhesive film, and cover plate with foil. Incubate for 1 hour at room temperature.

8. Add 5  $\mu$ L 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.

9. Read plate on an Alpha Technology-compatible plate reader, using standard AlphaLISA settings.

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



<sup>1</sup> Depending on cell type and pathway analyzed.

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

<sup>3</sup> 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™ ASSAY PROTOCOL

### B. 1 Plate Assay - protocol for suspension 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  $\mu$ L of cells/well into a 384-well white opaque culture plate (e.g. Revvity Cat # 6007680).

**Note:** As engaging less cells per well can result in increased signal to background ratios, it is important to optimize this factor.

#### Cell Treatment

2. If using test agents/inhibitors, add 2  $\mu$ L/well of 3X 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% protease-free BSA).

3. Return cells to incubator at 37°C for 5 min to 2 hours.

**Note:** 1 hour is often sufficient for signal transduction inhibitors and 5-20 minutes for receptor antagonists. For short incubation times, the plate can stay at room temperature.

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

**Note:** Optimal agonist stimulation time is often between 5 - 20 minutes.

#### Lysate Preparation

5. To lyse the cells add 2  $\mu$ L/well of 5X Lysis Buffer. Add 10  $\mu$ L of Control lysate / 1X Lysis Buffer to separate wells. *We recommend testing a serial dilution of Control lysate in 1X Lysis Buffer. See the COA for recommended dilution in the linear range of assay.*

#### SureFire Ultra Assay

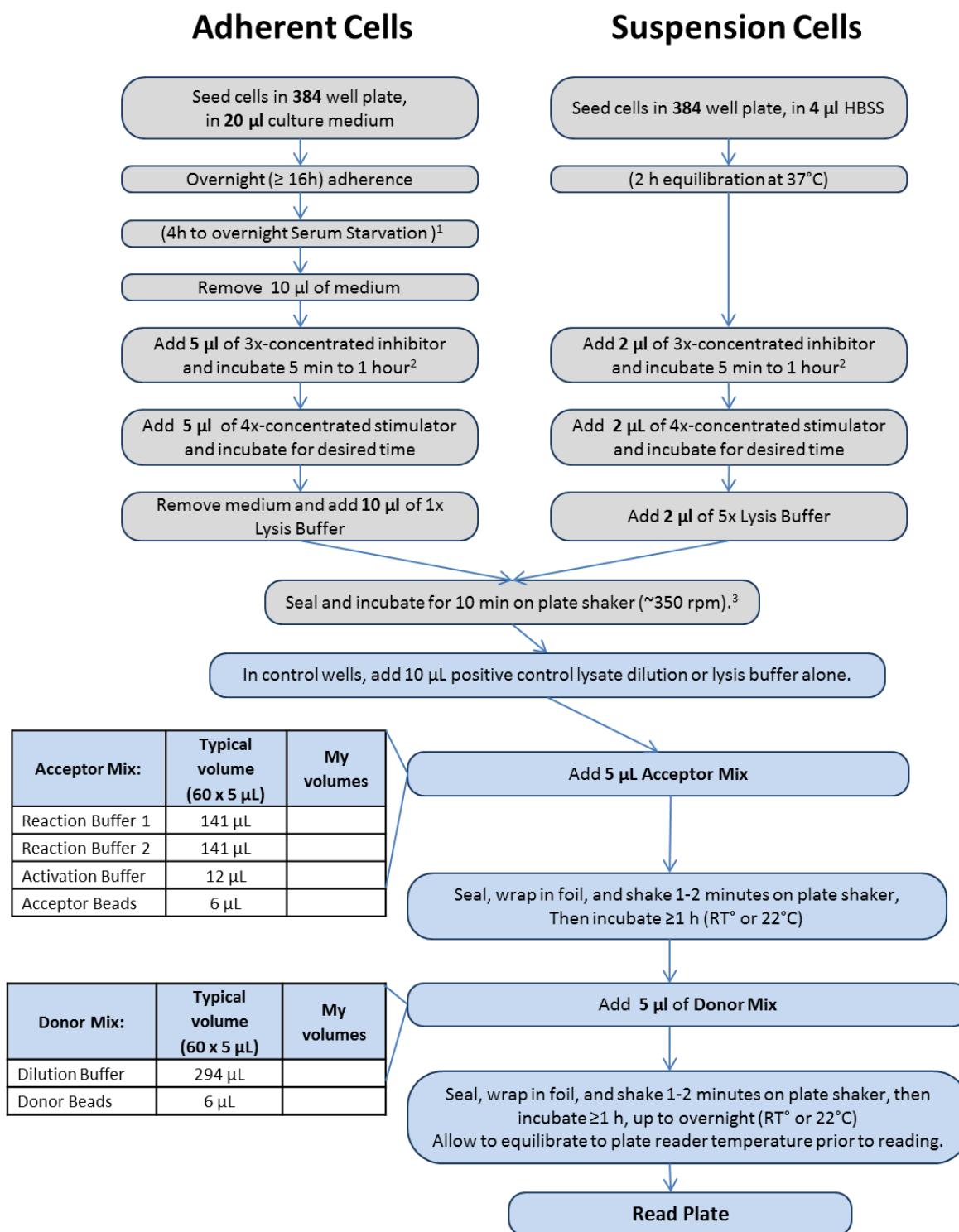
6. Add 5  $\mu$ L of Acceptor Mix to wells. Seal plate with Topseal-A adhesive film, and cover plate with foil. Incubate for 1 hour at room temperature.

7. Add 5  $\mu$ L 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



<sup>1</sup> Depending on cell type and pathway analyzed.

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

<sup>3</sup> May stop and freeze lysates at -20°C if desired. If doing this, re-shake after thawing to ensure homogeneity of lysate before pipetting.

## SUPPLEMENTARY BUFFERS AND BEADS

If using the standard protocol, sufficient amount 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 part numbers:

| Item  | Suggested source | Part numbers     | Size   |
|---|------------------|------------------|--------|
| Lysis Buffer (5X)                             | Revvity Inc.     | ALSU-LB-10mL     | 10 mL  |
|   | Revvity Inc.     | ALSU-LB-100mL    | 100 mL |
|   | Revvity Inc.     | ALSU-LBC-100mL   | 100 mL |
| Activation                                    | Revvity Inc.     | ALSU-AB-10mL     | 10 mL  |
|   | Revvity Inc.     | ALSU-AB-100mL    | 100 mL |
|   | Revvity Inc.     | ALSU-ABC-100mL   | 100 mL |
| Dilution Buffer                               | Revvity Inc.     | ALSU-DB-10mL     | 10 mL  |
|   | Revvity Inc.     | ALSU-DB-100mL    | 100 mL |
| Alpha Streptavidin Donor Beads -2 mg/mL       | Revvity Inc.     | ALSU-ASDB-0.06mL | 60 µL  |
|   | Revvity Inc.     | ALSU-ASDB-1.2mL  | 1.2 mL |
|   | Revvity Inc.     | ALSU-ASDB-6mL    | 6 mL   |
| AlphaLISA™ CaptSure™ Acceptor Beads - 2 mg/mL | Revvity Inc.     | ALSU-ACAB-0.06mL | 60 µL  |
|   | Revvity Inc.     | ALSU-ACAB-1.2mL  | 1.2 mL |
|   | Revvity Inc.     | ALSU-ACAB-6mL    | 6 mL   |



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