

# MANUAL



## Alpha SureFire® Ultra™ Multiplex

### Terbium SureFire® Ultra™ Assay Kit

For multiplexing with AlphaLISA™ SureFire® Ultra™ (ALSU)  
assay kits

Part number:	TBSU-XXXX-X500	TBSU-XXXX-X10K	TBSU-XXXX-X50K
Assay points:	500	10,000	50,000

This is a generic manual for all kits.

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 pages 4-6 of this manual

## ASSAY PRINCIPLE

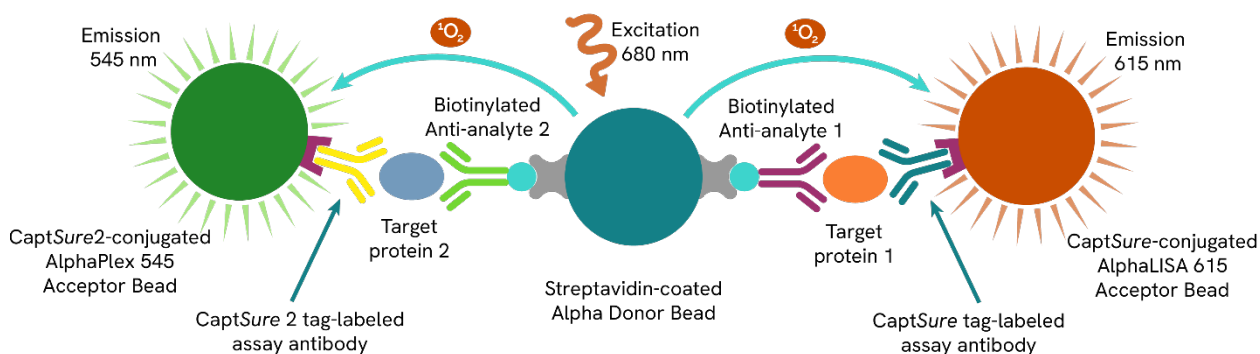
The Alpha SureFire® Ultra™ Multiplex kits allow the rapid, sensitive, and quantitative detection of two phosphoprotein targets in each well of an assay plate. This Multiplex measurement is achieved by the use of two types of Alpha Acceptor beads that emit at distinct wavelengths (Terbium, 545nm and Europium, 615nm).

The two distinct Alpha Acceptor beads report their binding to distinct antigens through their association with specific assay antibodies, as indicated below.

### **Terbium SureFire Ultra Assay kit for multiplexing with an AlphaLISA SureFire Ultra assay kit:**

The Terbium SureFire Ultra (TBSU) assay kits contain reagents to be combined with those of an AlphaLISA SureFire Ultra (ALSU) assay kit, to allow multiplexed measurement of two different phosphoprotein targets. Antibodies in each TBSU or ALSU kit measure a single target. Combining two such kits allows the highly flexible selection of two phosphoprotein targets to be measured.

The terbium “Alpha 545 Acceptor Beads” in the TBSU kit are conjugated with a “CaptSure2” agent, which binds a specific CaptSure2 tag on one of the assay antibodies. In combination with the biotinylated antibody provided, the 545 nm signal generated will report levels of the phosphoprotein of interest (Target 2). The TBSU kit is to be used in conjunction with the reagents in an ALSU kit, such that the ALSU kit will report that target of interest by Eu emission at 615nm (Target 1). This is shown diagrammatically below. The protocol details how to combine these kits to allow multiplexed measurement of the desired targets.



## MAIN FEATURES

The Terbium *SureFire*® *Ultra*™ Assay (TBSU) kit is used to measure the phosphorylation of a target of interest plus another target of choice from the AlphaLISA *SureFire Ultra* (ALSU) list of kits. The TBSU kit must be used in conjunction with an ALSU kit, and **cannot** be used alone to measure a single target.

Multiplexing is achieved through the use of two Alpha Acceptor beads. The first (545nm Tb) in this TBSU kit will report on the levels of the designated target of interest. The second (615nm Eu) Acceptor bead from another AlphaLISA *SureFire Ultra* kit reports on the protein designed to be measured using that kit.

The Donor beads in this kit are identical to those in a standard AlphaLISA *SureFire Ultra* kit.

Control lysates are provided in each kit to allow testing of the signal generated in the two channels (545 and 615 nm).

This kit has been formulated to provide optimal signal:background (i.e. S:B) assay windows, and to perform without interference in the presence of extraneous antibodies.

Unless otherwise indicated, the antibodies in each TBSU kit are identical to the antibodies to the same target in the corresponding ALSU kit, except for the CaptSure or CaptSure2 tag.

The assay utilizes the bead-based Alpha Technology, and requires an Alpha Technology-compatible plate reader capable of reading dual emission wavelengths. See [www.revvity.com](http://www.revvity.com) for more information about the AlphaPlex technology and download the “AlphaPlex Quick Start Guide” and the “AlphaPlex Assay Development Guide” to find guidance about filters and mirrors selection, instrument protocol and channels crosstalk correction. It is to be noted that, as the analytes recognized by both assays (i.e. the phosphorylated protein and the total protein) cannot be dissociated, it is not possible to omit one or the other analyte for the establishment of the channels crosstalk correction, but one or the other type of acceptor beads needs to be omitted instead. i.e. all the assay components but the Alpha 615 beads must be assembled to establish the crosstalk of the Alpha 545 beads into the 615 nm channel, and all the assay components but the Alpha 545 beads must be assembled to establish the crosstalk of the Alpha 615 beads into the 545 nm channel.

## 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.revvity.com](http://www.revvity.com).

## KIT CONTENTS

	Kit size		
	500 points	10,000 points	50,000 points
Reaction Buffer 3 – TBSU ( <i>Biotinylated anti-target antibody</i> )	1 x 0.15 mL	1 x 2.8 mL	1 x 14 mL
Reaction Buffer 4 – TBSU ( <i>CaptSure2-tagged anti-target antibody</i> )	1 x 0.15 mL	1 x 2.8 mL	1 x 14 mL
Alpha 545 CaptSure2 Acceptor Beads (2mg/mL in PBS plus 0.05% Proclin-300)	1 x 0.06 mL	1 x 1.1 mL	1 x 5.5 mL
Alpha Streptavidin Donor Beads (2mg/mL in PBS plus 0.05% Proclin-300)	1 x 0.06 mL	1 x 1.1 mL	1 x 5.5 mL
Positive Control Lysate	1 lyophilized tube		

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

**IMPORTANT:** The components of this kit will be used in conjunction with the components of another standard AlphaLISA SureFire Ultra (ALSU) kit to provide multiplexing. On page 8 we detail how to set up the assay solutions from both kits.

## STORAGE AND HANDLING CONDITIONS

Expiry date indicated on kit box.

<b>Unopened kit</b>		Store at 4°C. DO NOT freeze the kit. The Reaction Buffer contains antibodies and freeze/thaw cycles can lead to a loss of activity.
<b>Opened kit</b>	Reaction Buffer 3 - <i>Ultra</i> Reaction buffer 4 - <i>Ultra</i>	Store at 4°C.
	Acceptor/Donor Beads	Store at 4°C, in the dark zip lock bag or box provided
	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 with adequate AlphaPlex filters (see table below)	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).

**Table:** AlphaPlex Optics for EnVision Multilabel Reader – for complete information about how to set an AlphaPlex reading, please refer to the AlphaPlex Guides available at [www.revvity.com](http://www.revvity.com).

	Description	Part #	Barcode	Recommendations
Mirrors	AlphaScreen	2101-4010	444	For Tb and Eu single and sequential reading ; not for Sm
	AlphaPlex Single Tb-Eu-Sm	2102-5910	605	Preferred mirror for all sequential AlphaPlex applications
	AlphaPlex Dual Tb-Eu	2102-5900	653	For <b>simultaneous</b> duplexing of Tb with Eu
Filters	AlphaScreen	2100-5710	244	Suitable for AlphaPlex single plexing, not for multiplexing
	Resorufine/ Amplex Red	2100-5570	124	Suitable for Tb single plexing and Tb/Eu duplexing.
	Europium	2100-5090	203	Preferred filter for all Eu applications and multiplexing
	AlphaPlex Tb	2100-5930	701	Preferred filter for all Tb applications and multiplexing

## 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) - Ultra (from AlphaLISA SureFire Ultra kit)**

Lysis Buffer (5X) - *Ultra* 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.

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

Lysis Buffer B (5X) - *Ultra* and Lysis Buffer C (5X) - *Ultra* are assay specific and should not be interchanged.

All Lysis 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

### **Activation Buffer - Ultra**

Activation Buffer B - *Ultra* and Activation Buffer C - *Ultra* are assay specific and should not be interchanged.

### **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 should 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 Certificate of Analysis for the recommended dilution in the linear range of the assay.

## BUFFER PREPARATION AND SUBSEQUENT STORAGE CONDITIONS

1. Prepare sufficient **Acceptor Mix** from the ALSU AlphaLISA *SureFire Ultra* kit as you would for a standard single target assay, according to that kit manual.
2. The Terbium *SureFire Ultra* Assay kit reagents will be added to the pre-prepared ALSU Acceptor mix from step 1 to complete the Multiplex Acceptor Mix.
3. We recommend that you combine the Donor beads from both ALSU and TBSU kits into a single tube prior to use. Donor Mix will then be made up as indicated below.

<p><b>1X Lysis Buffer</b></p> <p>Note: Lysis Buffer from ALSU kit</p>	<p>Dilute Lysis buffer (5X) - Ultra in deionised water to a final concentration of 1X</p> <p>For example: for 10 mL of 1X Lysis Buffer, add: 2 mL of 5X Lysis Buffer - <i>Ultra</i> to 8 mL deionised water.</p> <p>Discard unused 1X buffer.</p>
<p><b>Multiplex Acceptor Mix</b></p> <p>Prepare Acceptor Mix as normal from ALSU <i>SureFire Ultra</i> kit.</p> <p>To this :</p> <p>Add TBSU Reaction Buffer 3 (1:20) Add TBSU Reaction buffer 4 (1:20)</p> <p>Vortex</p> <p>Add Alpha 545 CaptSure2 Acceptor beads (1:50)</p> <p>Vortex</p>	<p><b>Important: Combine the reagents in the order indicated below</b></p> <p>For example: for 60 samples, require a minimum 300 µL of Multiplex Acceptor Mix:</p> <p>Make 300 µL of ALSU kit Acceptor Mix, according to kits protocol. To this ALSU Acceptor Mix add 15µL of Reaction buffer 3 and 15µL of Reaction buffer 4. Mix combined by vortex. Then add 6µL of Alpha 545 CaptSure 2 Acceptor Bead 545. Mx again by vortex</p> <p>The Multiplex Acceptor mix should be made up and used within 30min for best results. Excess mix should be discarded.</p>
<p><b>Donor Mix</b></p> <p>Alpha Streptavidin Donor beads (1:25)</p> <p>Note: Dilution buffer from ALSU kit</p>	<p>Dilute Donor beads <b>25-fold</b> in Dilution buffer</p> <p>For example: for 60 samples, require a minimum 300 µL of Donor Mix: Add 12 µL Donor Beads to 288 µL of Dilution Buffer from ALSU kit.</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> Excess Donor Mix should be discarded.</p>
<p><b>Positive control lysate</b></p>	<p>Reconstitute with 250µL deionised water. Reconstituted lysate can generally be stored effectively at -20°C in single use aliquots but should be tested on a case by case basis. Dilute as required with 1X Lysis Buffer.</p>



## ASSAY PROTOCOL for dual target measurement using combined Terbium SureFire® Ultra™ kit + AlphaLISA™ SureFire® Ultra™ kit

### 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 - *Ultra* (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 of Control lysates 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.*

#### Alpha SureFire Ultra Multiplex Assay

5. Add 5 µL of Multiplex Acceptor Mix to wells. Seal plate with Topseal-A adhesive film. 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.

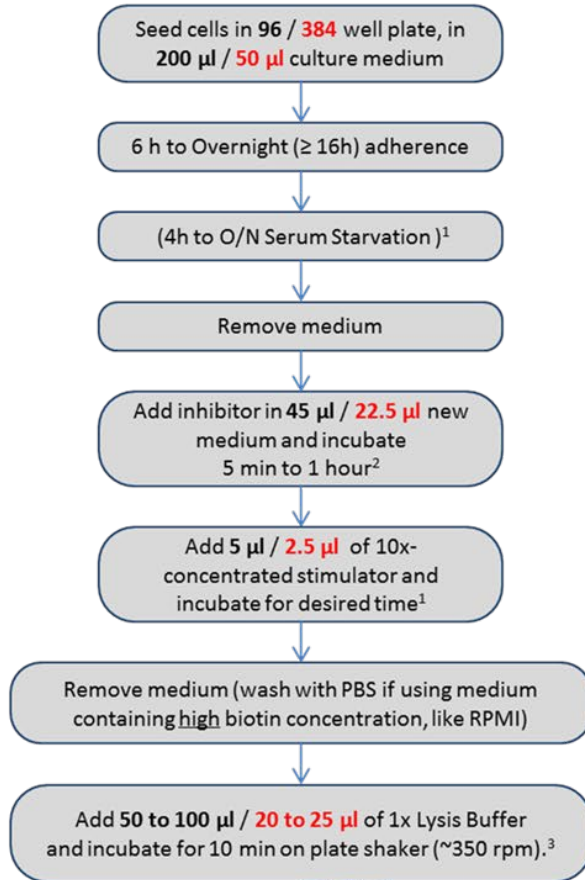
**Note:** Longer incubation may give greater sensitivity. Plates can be incubated overnight if required.

7. Read plate on an AlphaPlex Technology-compatible plate reader, using standard AlphaPlex settings (see above).

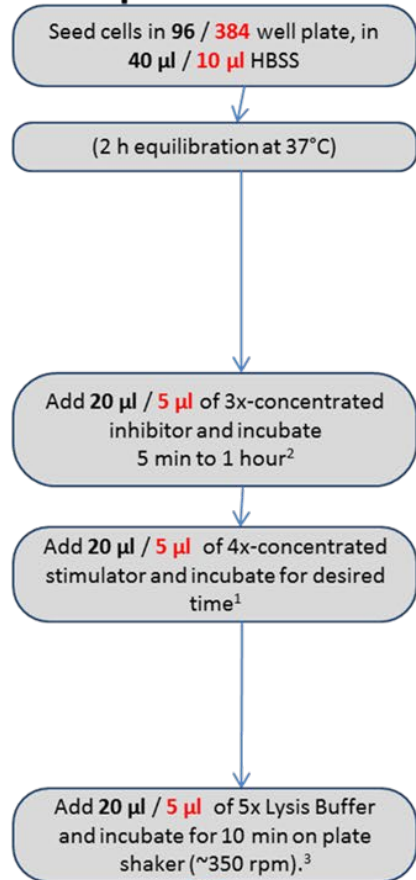
# Alpha SureFire® Ultra™ Multiplex: 2-plates / 2-incubation assay flowchart

## Dual Targets

### Adherent Cells

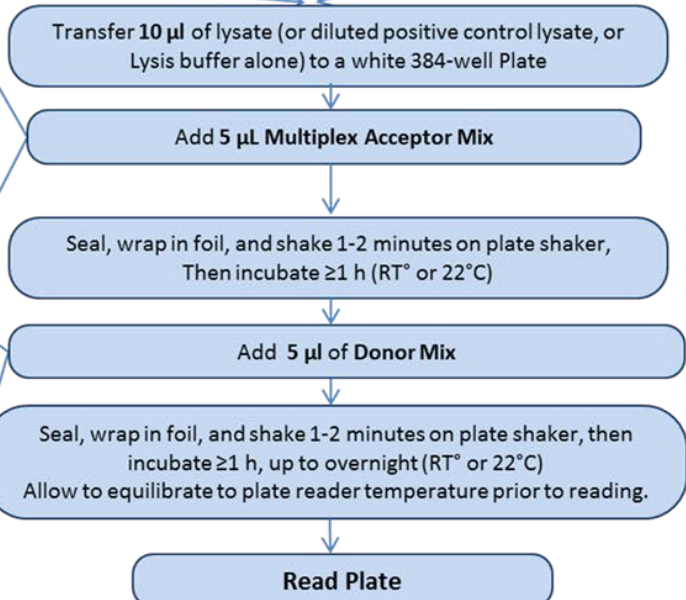


### Suspension Cells



Multiplex Acceptor Mix:	Typical volumes (60 x 5 µL)	My volumes
ALSU Acceptor Mix	300	
TBSU Reaction Buffer 3	15 µL	
TBSU Reaction Buffer 4	15 µL	
CaptSure2 Alpha 545 Acceptor Beads	6 µL	

Donor Mix:	Typical volume (60 x 5 µL)	My volumes
Dilution Buffer	288 µL	
Donor Beads	12 µL	



<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.

## ASSAY PROTOCOL for dual target measurement using combined Terbium SureFire® Ultra™ kit + AlphaLISA™ SureFire® Ultra™ kit

### B. 1 Plate Assay - 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.
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% BSA).

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

#### Cell Treatment

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.)

#### Lysate Preparation

5. To lyse the cells, add 2  $\mu$ L/well of 5X Lysis Buffer. Add 10  $\mu$ L of Control lysates 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.*

#### Alpha SureFire Ultra Multiplex Assay

6. Add 5  $\mu$ L of Acceptor Mix to wells. Seal plate with Topseal-A adhesive film. 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 in the dark.

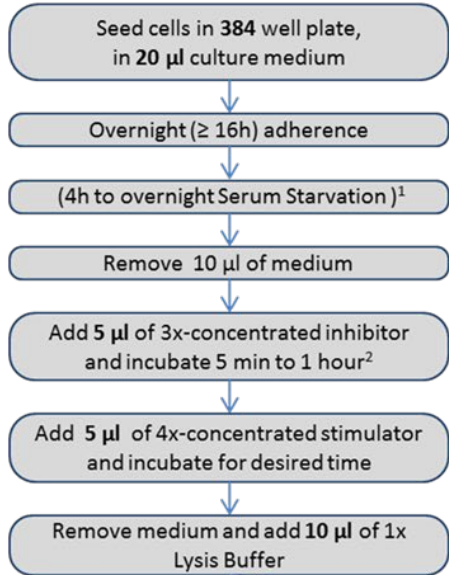
**Note:** Longer incubation may give greater sensitivity. Plates can be incubated overnight if required.

8. Read plate on an AlphaPlex Technology-compatible plate reader, using standard AlphaPlex settings (see above).

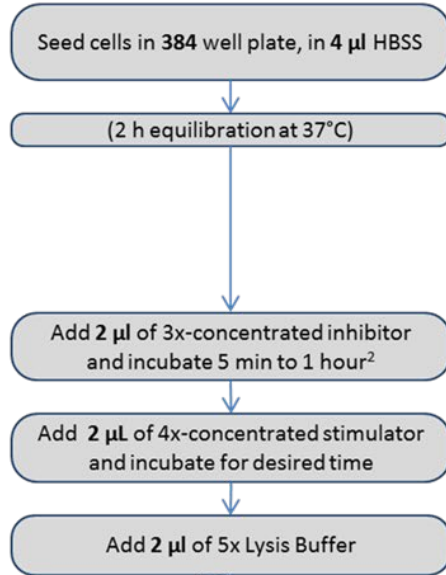
# Alpha SureFire® Ultra™ Multiplex: 1-plate / 2-incubation assay flowchart

## Dual Targets

### Adherent Cells



### Suspension Cells



Seal and incubate for 10 min on plate shaker (~350 rpm).<sup>3</sup>

In control wells, add 10 µL positive control lysate dilution or lysis buffer alone.

Multiplex Acceptor Mix:	Typical volumes (60 x 5 µL)	My volumes
ALSU Acceptor Mix	300	
TBSU Reaction Buffer 3	15 µL	
TBSU Reaction Buffer 4	15 µL	
CaptSure2 Alpha 545 Acceptor Beads	6 µL	

Donor Mix:	Typical volume (60 x 5 µL)	My volumes
Dilution Buffer	288 µL	
Donor Beads	12 µL	

Add 5 µL Multiplex Acceptor Mix

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

Read Plate

<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.

## SUPPLEMENTRY BUFFERS AND BEADS

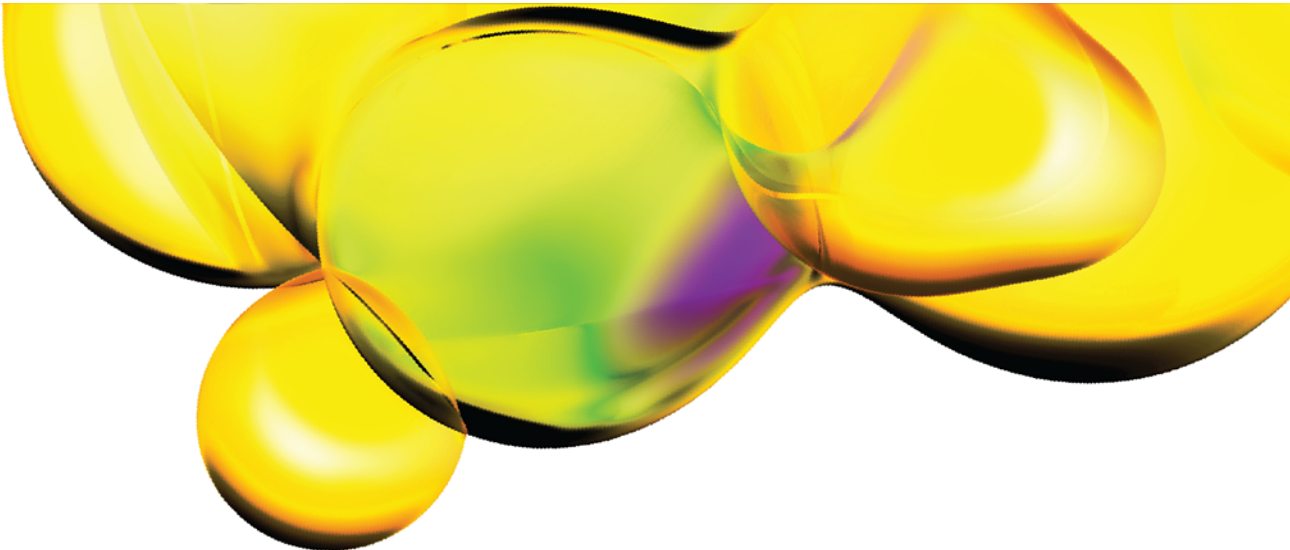
If using the standard protocol, sufficient amounts of buffers and beads are provided in the kit. However, in case the standard protocol would be modified, more buffers or beads may be needed. In this case, you can order additional buffers and beads using the following catalog numbers:

Item	Suggested source	Catalog #	Size
Lysis Buffer (5X) - <i>Ultra</i>	Revvity Inc.	ALSU-LB-10mL	10mL
	Revvity Inc.	ALSU-LB-100mL	100mL
Lysis Buffer B (5X) - <i>Ultra</i>	Revvity Inc.	ALSU-LBB-10mL	10 mL
	Revvity Inc.	ALSU-LBB-100mL	100 mL
Lysis Buffer C (5X) - <i>Ultra</i>	Revvity Inc.	ALSU-LBC-10mL	10 mL
	Revvity Inc.	ALSU-LBC-100mL	100 mL
Activation Buffer - <i>Ultra</i>	Revvity Inc.	ALSU-AB-10mL	10mL
	Revvity Inc.	ALSU-AB-100mL	100mL
Activation Buffer B - <i>Ultra</i>	Revvity Inc.	ALSU-ABB-10mL	10 mL
	Revvity Inc.	ALSU-ABB-100mL	100 mL
Activation Buffer C - <i>Ultra</i>	Revvity Inc.	ALSU-ABC-10mL	10 mL
	Revvity Inc.	ALSU-ABC-100mL	100 mL
Dilution Buffer - <i>Ultra</i>	Revvity Inc.	ALSU-DB-10mL	10mL
	Revvity Inc.	ALSU-DB-100mL	100mL
AlphaLISA™ CaptSure™ Acceptor Beads -2mg/ml	Revvity Inc.	ALSU-ACAB-0.06mL	60µL
	Revvity Inc.	ALSU-ACAB-1.2mL	1.2mL
	Revvity Inc.	ALSU-ACAB-6mL	6mL
Alpha Streptavidin Donor Beads -2mg/mL	Revvity Inc.	ALSU-ASDB-0.06mL	60µL
	Revvity Inc.	ALSU-ASDB-1.2mL	1.2mL
	Revvity Inc.	ALSU-ASDB-6mL	6mL
Alpha 545 (Tb) CaptSure2 Acceptor Beads -2mg/mL	Revvity Inc.	MPSU-CS2B-0.06mL	60µL
	Revvity Inc.	MPSU-CS2B -1.2mL	1.2mL
	Revvity Inc.	MPSU-CS2B -6mL	6mL

## **List of Available TBSU kits**

Assay	Part Numbers		
	500 point	10,000 point	50,000 point
p-4E-BP1 (T37/46)	TBSU-P4EBP-A500	TBSU-P4EBP-A10K	TBSU-P4EBP-A50K
p-Akt1/2/3 (T308)	TBSU-PAKT-A500	TBSU-PAKT-A10K	TBSU-PAKT-A50K
p-Akt1/2/3 (S473)	TBSU-PAKT-B500	TBSU-PAKT-B10K	TBSU-PAKT-B50K
Total Cofilin	TBSU-TCOF-A500	TBSU-TCOF-A10K	TBSU-TCOF-A50K
p-CREB (S133)	TBSU-PCREB-A500	TBSU-PCREB-A10K	TBSU-PCREB-A50K
p-EGF Receptor (Y1068)	TBSU-PEGFR-A500	TBSU-PEGFR-A10K	TBSU-PEGFR-A50K
p-eIF2 $\alpha$ (S51)	TBSU-PEIF2-B500	TBSU-PEIF2-B10K	TBSU-PEIF2-B50K
p-eIF4E (S209)	TBSU-PEIF4-A500	TBSU-PEIF4-A10K	TBSU-PEIF4-A50K
p-ERK1/2 (T202/Y204)	TBSU-PERK-A500	TBSU-PERK-A10K	TBSU-PERK-A50K
p-GSK3 $\beta$ (S9)	TBSU-PGS3B-A500	TBSU-PGS3B-A10K	TBSU-PGS3B-A50K
p-IGF-1 Receptor $\beta$ (Y1135/1136)	TBSU-PIGFR-B500	TBSU-PIGFR-B10K	TBSU-PIGFR-B50K
p-IKK $\alpha$ (S176/180)	TBSU-PIKKA-A500	TBSU-PIKKA-A10K	TBSU-PIKKA-A50K
p-Insulin Receptor $\beta$ (Y1150/1151)	TBSU-PINR-A500	TBSU-PINR-A10K	TBSU-PINR-A50K
p-JNK1/2/3 (T183/Y185)	TBSU-PJNK-A500	TBSU-PJNK-A10K	TBSU-PJNK-A50K
p-MEK1 (S218/222)	TBSU-PMEK1-A500	TBSU-PMEK1-A10K	TBSU-PMEK1-A50K
p-mTOR (S2448)	TBSU-PMTOR-A500	TBSU-PMTOR-A10K	TBSU-PMTOR-A50K
p-NF- $\kappa$ B p65 (S536)	TBSU-PNFKB-A500	TBSU-PNFKB-A10K	TBSU-PNFKB-A50K
p-p38 MAPK (T180/Y182)	TBSU-PP38-B500	TBSU-PP38-B10K	TBSU-PP38-B50K
p-p70 S6K (T389)	TBSU-PP70-A500	TBSU-PP70-A10K	TBSU-PP70-A50K
p-Ribosomal Protein S6 (S240/244)	TBSU-PS6R-A500	TBSU-PS6R-A10K	TBSU-PS6R-A50K
p-SLP-76 (S376)	TBSU-PSLP-A500	TBSU-PSLP-A10K	TBSU-PSLP-A50K
p-SMAD1 (S463/465)	TBSU-PSM1-A500	TBSU-PSM1-A10K	TBSU-PSM1-A50K
p-SMAD3 (S423/425)	TBSU-PSM3-A500	TBSU-PSM3-A10K	TBSU-PSM3-A50K
p-STAT1 (S727)	TBSU-PST1-B500	TBSU-PST1-B10K	TBSU-PST1-B50K
p-STAT1 (Y701)	TBSU-PST1-A500	TBSU-PST1-A10K	TBSU-PST1-A50K
p-STAT3 (Y705)	TBSU-PST3-A500	TBSU-PST3-A10K	TBSU-PST3-A50K
p-STAT4 (Y693)	TBSU-PST4-A500	TBSU-PST4-A10K	TBSU-PST4-A50K
p-STAT5 (Y694/699)	TBSU-PST5-B500	TBSU-PST5-B10K	TBSU-PST5-B50K
p-STAT6 (Y641)	TBSU-PST6-A500	TBSU-PST6-A10K	TBSU-PST6-A50K
p-SYK (Y525/526)	TBSU-PSYK-A500	TBSU-PSYK-A10K	TBSU-PSYK-A50K
p-VEGF Receptor 2 (Y1175)	TBSU-PVGFR-A500	TBSU-PVGFR-A10K	TBSU-PVGFR-A50K





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