Introducing AlphaLISA *SureFire*® Biotin Free: A Versatile Immunoassay Platform for Biotin-Rich Samples.

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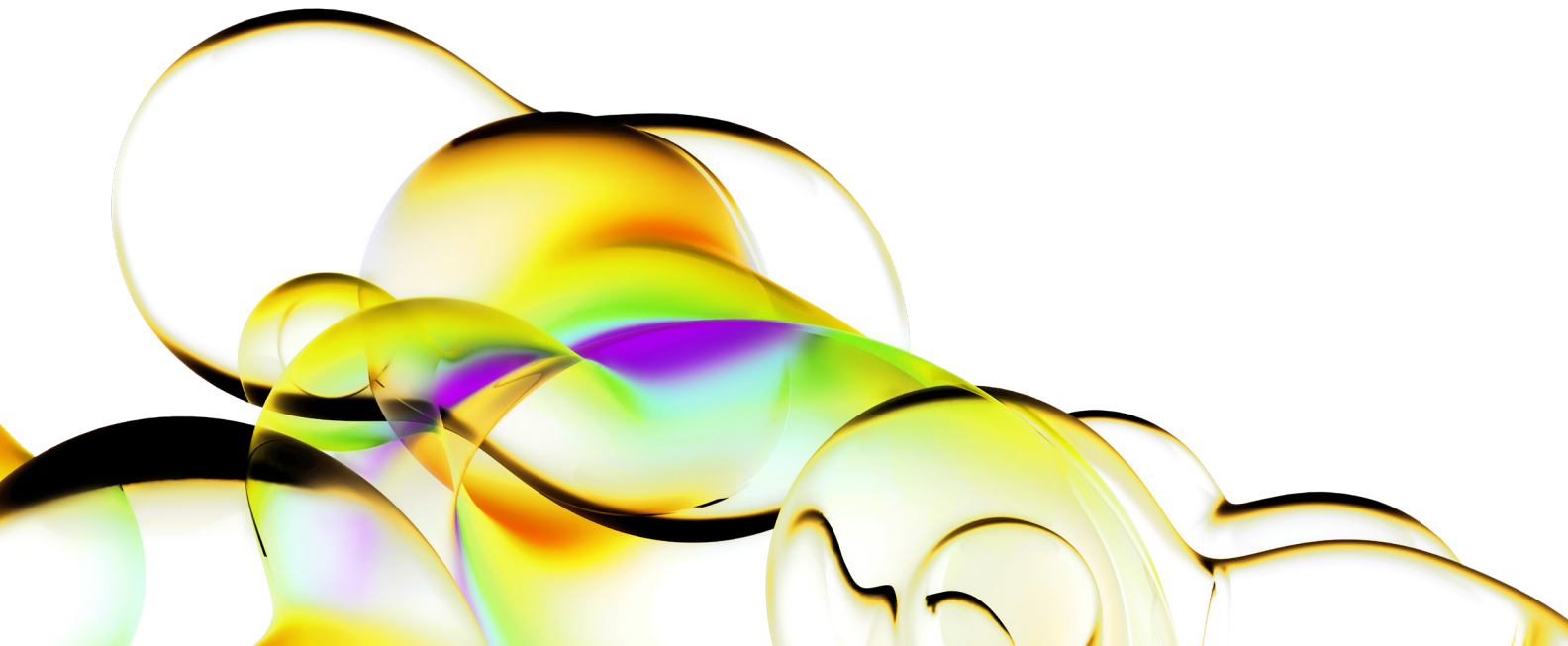
Introduction

The evolution of AlphaLISA™ *SureFire*® technology continues with the introduction of AlphaLISA *SureFire*® Biotin Free (ASBF). This innovative immunoassay platform integrates TGR's patented Capt*Sure*® antibody immobilization system with Revvity's renowned AlphaLISA technology, enabling no-wash, homogeneous detection of cell-based proteins (Figure 1). Importantly, ASBF eliminates the need to consider free biotin in cell culture media, making it perfectly suited for use with media like RPMI 1640 without compromising performance. Additionally, ASBF has the potential to be effectively utilized in other biological samples rich in biotin, such as fluids and tissues including blood, serum, plasma, liver, and brain. This versatility allows for broader application in various research and drug discovery settings, enhancing the analysis of biotin-containing matrices across different sample types. The *Amplified Luminescence Proximity Homogeneous Assay* technology, commonly known as 'Alpha,' has been closely associated with TGR's *SureFire*® technology for nearly two decades (Figure 2).



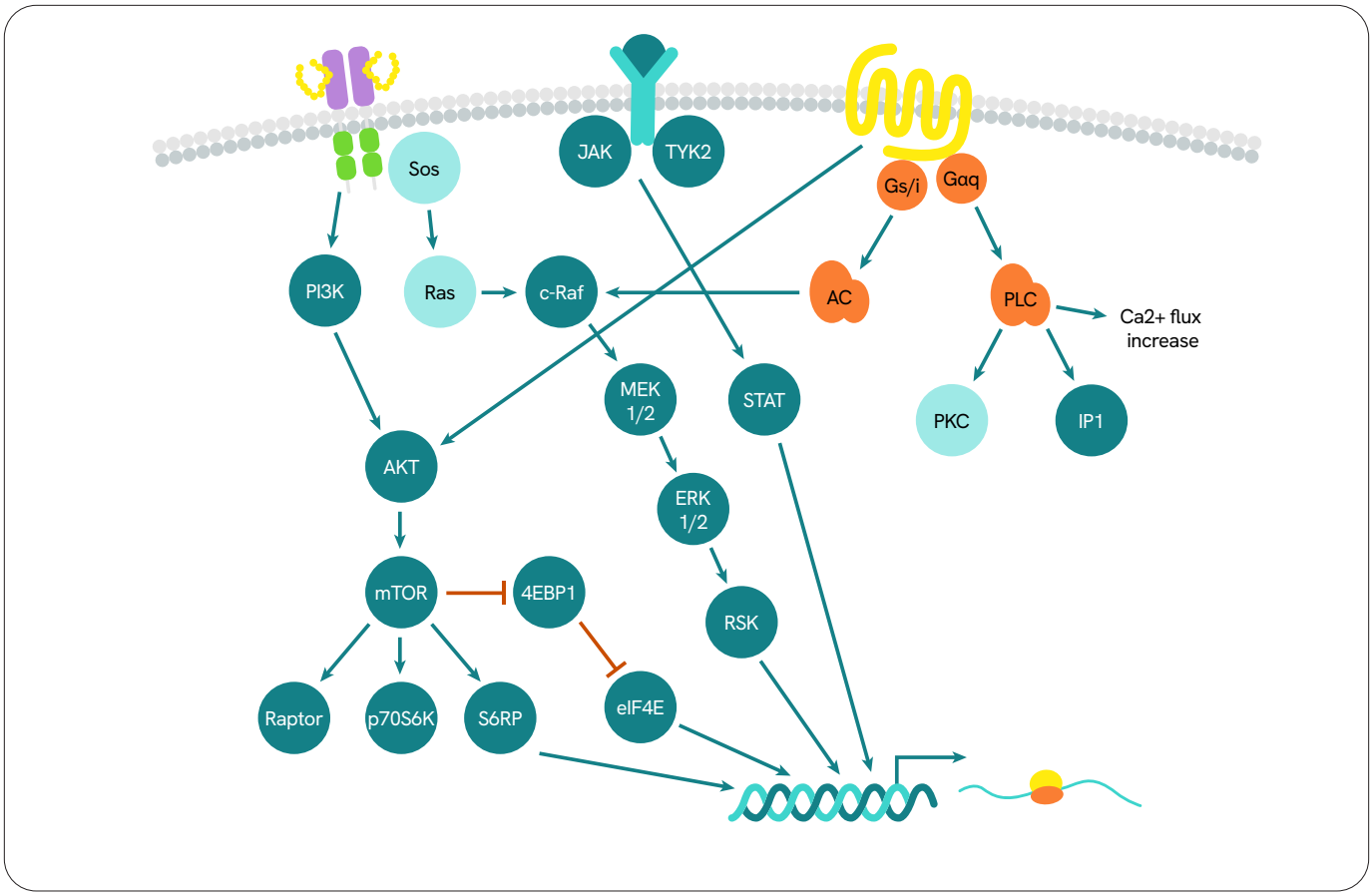


Figure 1: AlphaLISA *SureFire*® is an innovative immunoassay technology platform that addresses complex cell signalling biology.

- Phosphoproteins are attractive drug targets in high throughput screening systems and AlphaLISA *SureFire*® technology is predominantly known for its ability to measure phosphorylation.
- AlphaLISA *SureFire*® is also highly suited to targeted protein degradation approaches with the emergence of PROTAC technology in recent years.

The Alpha *SureFire*® technology evolution

The original AlphaScreen™ *SureFire*® (ASSF) platform was launched in 2006, and it was built on Streptavidin Coated Donor Beads and Protein A Coated Acceptor Beads (Figure 2). Approximately 80 cell-based assays, predominantly phosphoproteins, were developed on the ASSF platform with a primary focus on high throughput screening and drug discovery research. Although ASSF was a highly successful platform, it was superseded in 2014 with the arrival of AlphaLISA *SureFire*® *Ultra*™ (ALSU), which catered better for the evolving needs of pharma/biotech customers. ALSU was a significant improvement on ASSF and was characterised by a change in both the coating and type of Acceptor Bead employed. The narrow emission properties of Europium (i.e. 615 nm) in the AlphaLISA Acceptor Bead made it less susceptible to interference by either artificial or natural compounds (e.g. such as hemoglobin) that absorb light between 500-600 nm. In addition, the substitution of Protein A with a proprietary Capt*Sure* coating advanced the ALSU platform significantly, making it compatible with the increasingly popular antibody-based biologics. In addition, the elimination of Protein A and the incorporation of Capt*Sure* IP also transformed ALSU into a far more powerful and universal platform due to its compatibility with all antibody isotype/species as possible immunoassay sandwich pairs. Continued R&D investment in response to customer demand has led to the generation of an expansive offering (> 400) of cell-based ALSU assays.

Further innovation led to the launch of Multiplex *SureFire*® *Ultra* (MPSU) in 2016, enabling the simultaneous measurement of both phospho and total target proteins in the same well. This platform was built using the same Streptavidin Donor Bead and Capt*Sure*-coated Europium Acceptor Beads (615 nm emission) from ALSU combined with a 'Capt*Sure* 2'-coated Terbium Acceptor Bead (545 nm emission).

Introducing our latest innovation - AlphaLISA *SureFire*® Biotin Free

Our innovative AlphaLISA *SureFire*® Biotin Free platform is characterized by the incorporation of a different coating on the Alpha Donor Bead. We have introduced another proprietary 'Capt*Sure* 3' coating on the Alpha Donor Bead to replace the streptavidin coating that has long been associated with legacy Alpha technology (Figure 3). The Capt*Sure* 3 coating is a rabbit monoclonal antibody that displays very high affinity and specificity against a small synthetic 'Capt*Sure* 3 peptide' that is not found in nature. This Capt*Sure* 3 peptide is then covalently conjugated to a target antibody of interest such that it can bind directly to the Capt*Sure* 3-coated Alpha Donor Bead and participate in a typical Alpha *SureFire*® immunoassay format.
The Capt*Sure* 3-coated Alpha Donor Bead is paired with our existing Capt*Sure*-coated AlphaLISA Acceptor Bead in all ASBF immunoassays to make it a completely streptavidin/biotin free system.

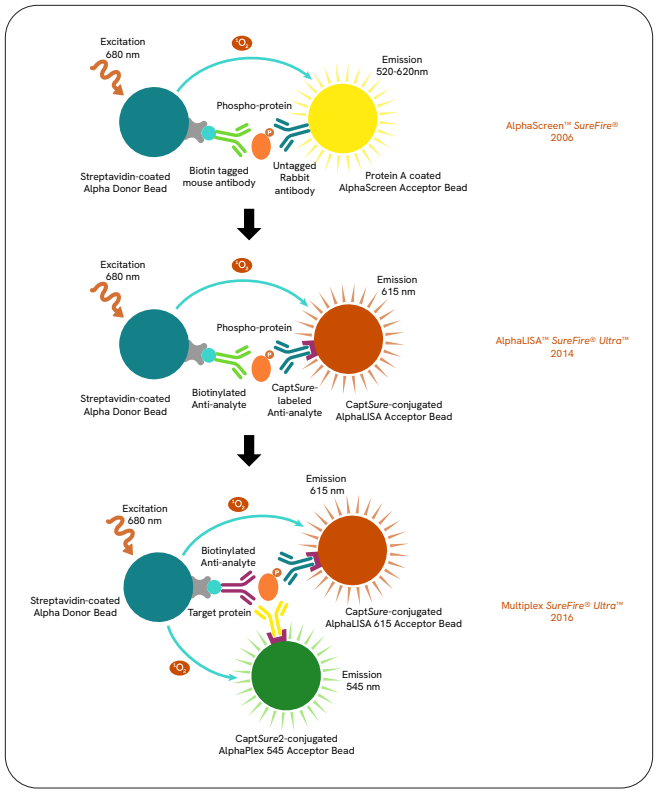


Figure 2: The evolution of AlphaLISA *SureFire*® technology.

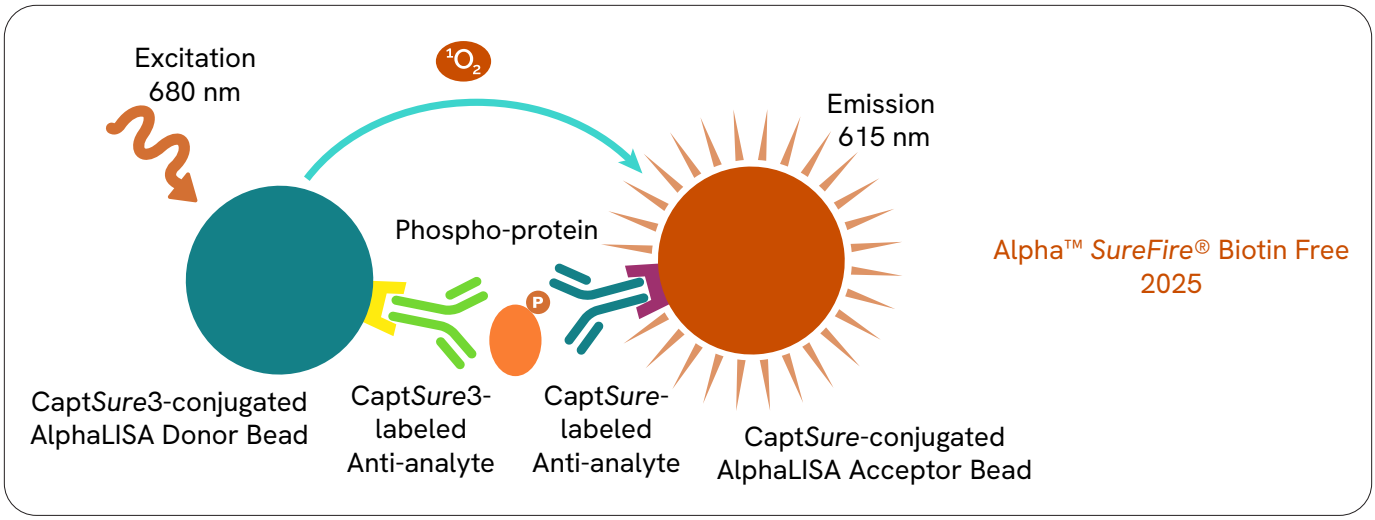


Figure 3: AlphaLISA *SureFire*® Biotin Free (ASBF) immunoassay format.

The case for a streptavidin/biotin-free AlphaLISA *SureFire*® assay platform

Free biotin is commonly found in many cell culture media like RPMI 1640 at almost micromolar concentrations. It is commonly used when culturing suspension cells like THP-1, Jurkat, U937 and Ramos cells. The high sensitivity of the ALSU platform allows the use of biotinylated assay antibodies in the 0.3-1.5 nM concentration range which is approximately 1000-fold less than the levels of free biotin found in RPMI 1640 medium. The ALSU platform has successfully found ways to overcome the use of biotin-containing medium by either washing cells or substituting media with a HBSS (i.e. Hank's Buffered Salt Solution) formulation when treating cells prior to lysis and detection.

With the launch of ASBF, the 'free biotin' workflow consideration is no longer relevant, and it is perfectly suited to handling media like RPMI 1640 while not compromising the high performance that is expected of AlphaLISA *SureFire*® assay technology.

ASBF seamlessly integrates with an 'all-in-one well' assay format and is highly compatible with the high-throughput screening and automation systems used in global pharma and biotech industries.

ASBF and ALSU assays: equivalent performance with outstanding sensitivity and dynamic range

Although the femtomolar affinity of streptavidin/biotin is well established as being one of the strongest known biological interactions, the Capt*Sure* IP that underpins ASBF is cleverly designed to maintain the exceptional immunoassay performance consistently demonstrated with legacy ALSU technology. When benchmarking ALSU and ASBF STAT5 (Y694/699) and AKT1/2/3 (S473) performance in A431 and MCF7 cells cultivated in DMEM and MEM media respectively, superimposable curves were generated showing the induction of phosphorylation in response to EGF and insulin treatments respectively (Figure 4A and 4B). The EC_{50} for EGF-mediated STAT5 induction was 9.2-9.5 ng/mL and 0.67-0.68 µM for insulin-mediated AKT phosphorylation. The large signal windows (i.e. 100-fold cellular response) and modest cell number (i.e. 4,000 cells/datapoint) requirement are typical characteristics of the AlphaLISA *SureFire*® technology, and these attributes have also been captured with the next generation ASBF platform.

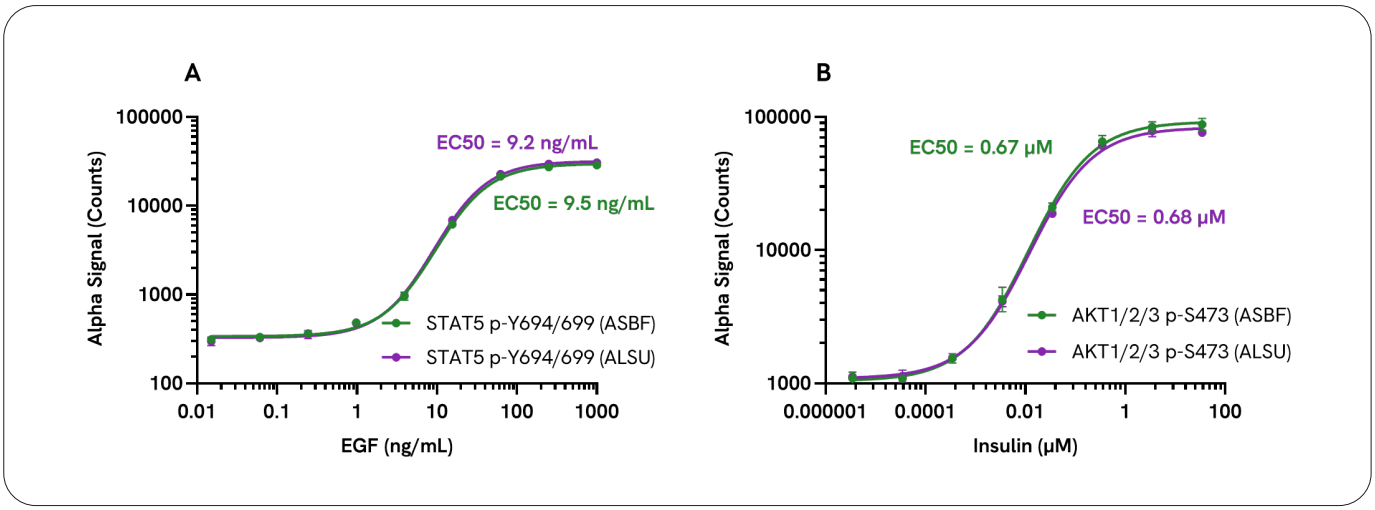


Figure 4: Sensitivity comparison between ASBF and ALSU assays. A431 **(A)** and MCF7 **(B)** cells were seeded at 40,000 cells/well in a 96-well plate and incubated overnight in DMEM and MEM, respectively. Cells were serum starved for 2 hours and then treated with increasing concentrations of EGF for 15 minutes **(A)** or insulin for 30 minutes **(B)**. Cells were lysed with 100 µL of Lysis Buffer. For the detection step, 10 µL of lysate (approximately 4,000 cells/datapoint) was used in respective assays (ASBF and ALSU) to measure STAT5 **(A)** and AKT **(B)** phosphorylation.

Exceptional performance of ASBF assays in biotin-containing media

ASBF has been designed with the incorporation of Capt*Sure* IP on both the Alpha Donor and Acceptor Beads for compatibility with biotin-containing matrices. ASBF platform validation work has demonstrated the utility of the ASBF platform in both 1-plate and 2-plate cell-based assay formats in presence of RPMI 1640 media (Figure 5). Impressive Phospho (Y641) STAT6 signal windows (> 200-fold) were seen for both a 1-plate and 2-plate assay format when screening lysates from THP-1 cells treated with a dose range of IL-4. As expected, in both formats, total STAT6 levels remained constant during the 20-minute exposure period to IL-4 (Figure 5A). Similarly, excellent ASBF assay performance was demonstrated when measuring Phospho (S473) AKT1/2/3 in THP-1 cells treated with insulin. A 16-fold and 22-fold induction of AKT1/2/3 phosphorylation was observed in the 1-plate and 2-plate assay formats respectively, while Total AKT1/2/3 levels remained unchanged as expected in both assay formats across the insulin dose-range (Figure 5B).

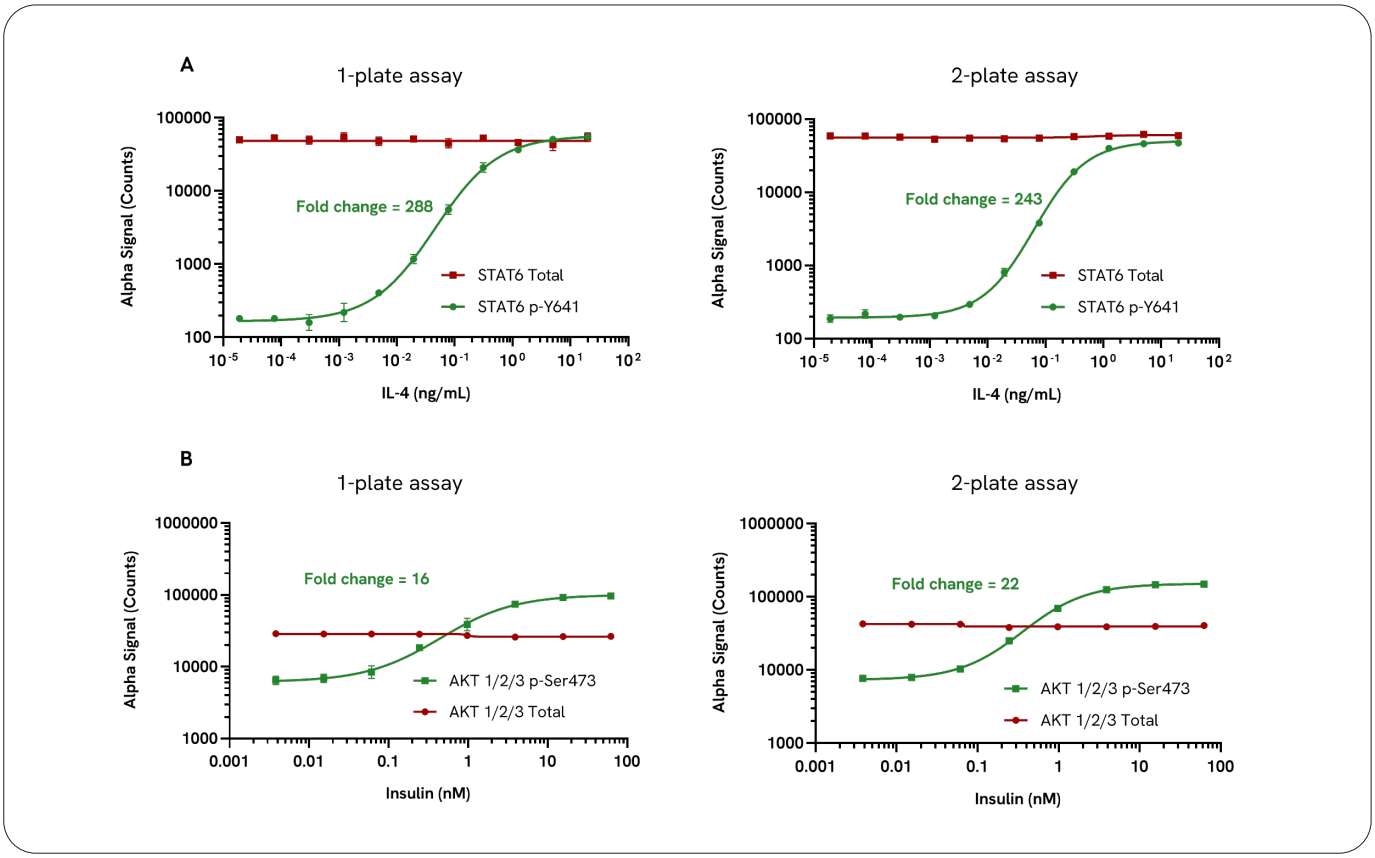


Figure 5: ASBF performance in biotin containing media. THP-1 cells were harvested and seeded in a 384-well Optiplate (1-plate assay) or in a 96-well culture plate (2-plate assay) in RPMI media. Cells were treated with IL-4 for 20 minutes **(A)** or serum starved for 2 hours and then treated with insulin for 5 minutes (**B**). Cells were lysed with the addition of 5X Lysis Buffer. Lysates were used in respective ASBF assays to measure Phospho (Y641) and Total STAT6 **(A)** or Phospho (S473) and Total AKT1/2/3 **(B)**. For the 1-plate assay experiment, cell seeding, treatment, lysis, and assay were done in a single 384-well Optiplate. Approximately 8,000 cells/datapoint. For the 2-plate assay, cell seeding, treatment, and lysis were done in a 96-well culture plate. Lysates were then transferred into a 384-well Optiplate for the detection step. Approximately 16,000 cells/datapoint. Both protocols were performed as described in the AlphaLISA *SureFire*® Biotin Free manual. Schematic representation of a 1-plate and 2-plate assay workflow is depicted in Figures 6 and 7.

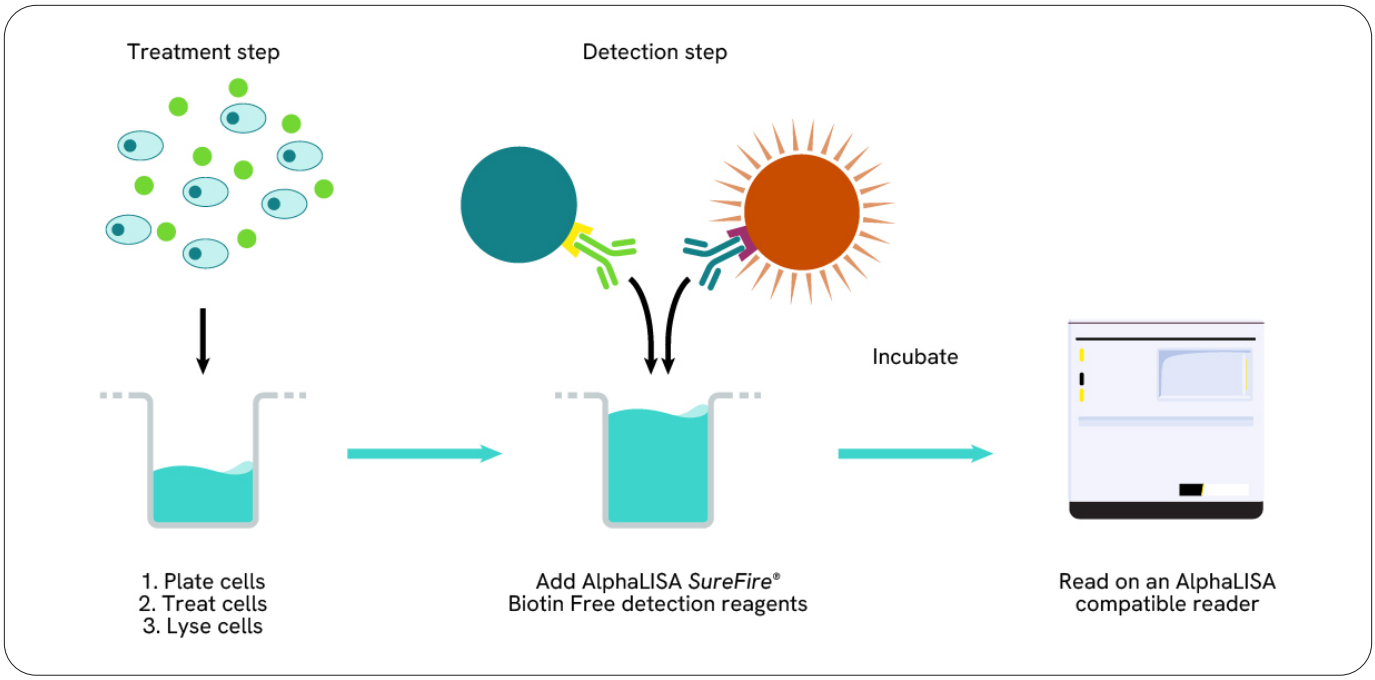


Figure 6: The 1-plate assay protocol involves cell culturing, treatment, lysis, and detection of the target protein in a single well of a 384-well Optiplate. This high throughput screening designed protocol enables miniaturization while maintaining robust AlphaLISA *SureFire*® quality.

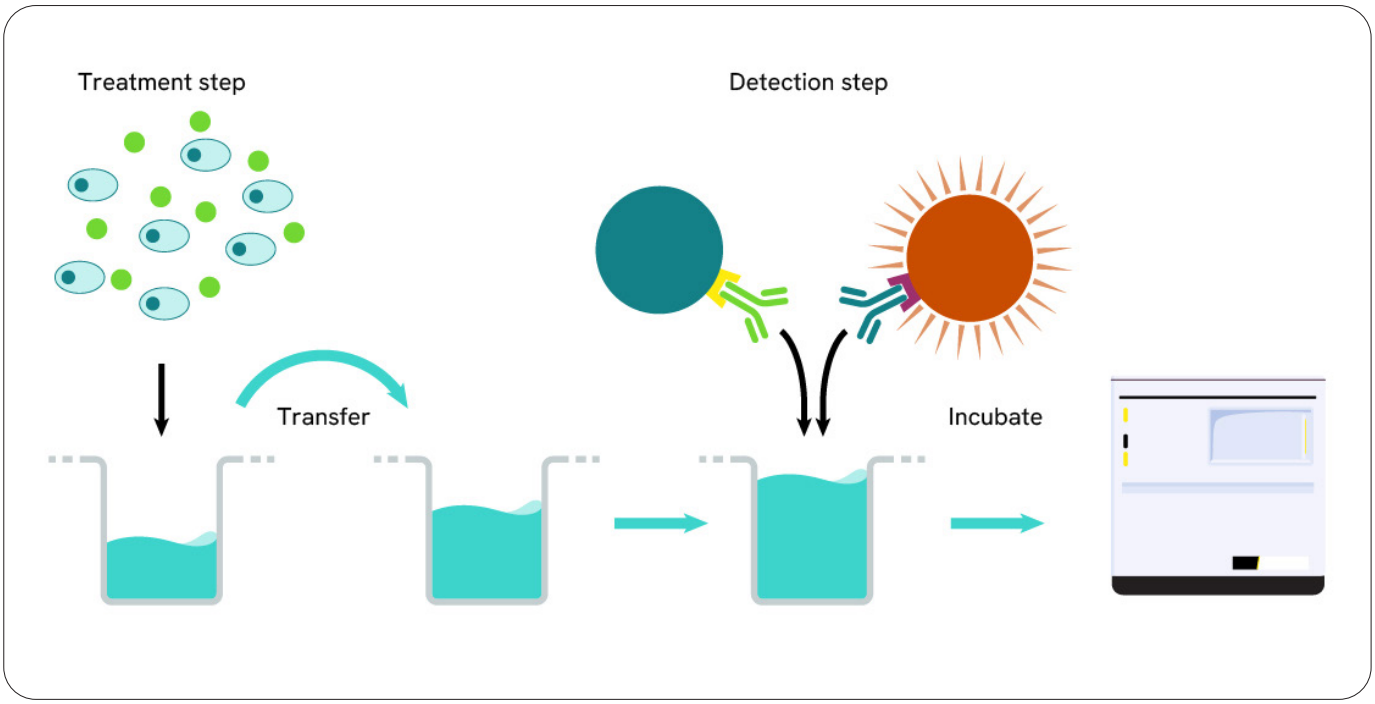


Figure 7: The 2-plate assay protocol involves culturing and treating the cells in a 96-well plate before lysis, then transferring lysates into a 384-well Optiplate plate before the addition of Alpha *SureFire*® Biotin Free detection reagents. This protocol enables the cells viability and confluence to be monitored. In addition, lysates from a single well can be used to measure multiple targets.

Conclusion

The ASBF platform eliminates the need to consider free biotin in cell culture media, such as RPMI 1640, while maintaining high performance. It integrates seamlessly with high-throughput screening and automation systems used in the pharma and biotech industries.

ASBF retains the exceptional performance of legacy ALSU technology, demonstrated by comparable results in cell-based assays for STAT5 and AKT1/2/3 phosphorylation.

Validation work has shown its effectiveness in both 1-plate and 2-plate assay formats, with impressive signal windows and consistent total protein levels.

The platform's design, incorporating Capt*Sure* IP on both Alpha Donor and Acceptor Beads, ensures compatibility with biotin-containing matrices and establishes a new benchmark in no-wash immunoassay technology.

This advancement underscores Revvity's commitment to innovation, aiming to best support basic and drug discovery research across the academic, biotech, and pharma sectors worldwide.

New AlphaLISA™ *SureFire*® Biotin Free detection assays	
Protein	**ASBF – 500 Assay Points***
AKT1/2/3, Phospho-S473	ASBF-PAKT-A500
AKT1/2/3, Total	ASBF-TAKT-A500
CDK1, Phospho-T14	ASBF-PCDK1-A500
CDK1, Total	ASBF-TCDK1-A500
ERK1/2, Phospho-T202/Y204	ASBF-PERK-A500
ERK1/2, Total	ASBF-TERK-A500
Rb, Phospho-S780	ASBF-PRB-A500
Rb, Phospho-S807/811	ASBF-PRB-B500
Rb, Phospho-T821	ASBF-PRB-C500
Rb, Phospho-T826	ASBF-PRB-D500
Rb, Total	ASBF-TRB-A500
STAT1, Phospho-Y701	ASBF-PST1-A500
STAT1, Total	ASBF-TST1-A500
STAT3, Phospho-Y705	ASBF-PST3-A500
STAT3, Total	ASBF-TST3-A500
STAT5, Phospho-Y694/699	ASBF-PST5-A500
STAT5, Total	ASBF-TST5-A500
STAT6, Phospho-Y641	ASBF-PST6-A500
STAT6, Total	ASBF-TST6-A500
SYK, Phospho-Y525/526	ASBF-PSYK-A500
SYK, Total	ASBF-TSYK-A500

*also available in other pack sizes.

Please note that the list of products in the table represents the kits launched in January 2025. This list is not exhaustive. For a complete and updated list of kits launched, please refer to the Alpha *SureFire* Ultra product list.

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