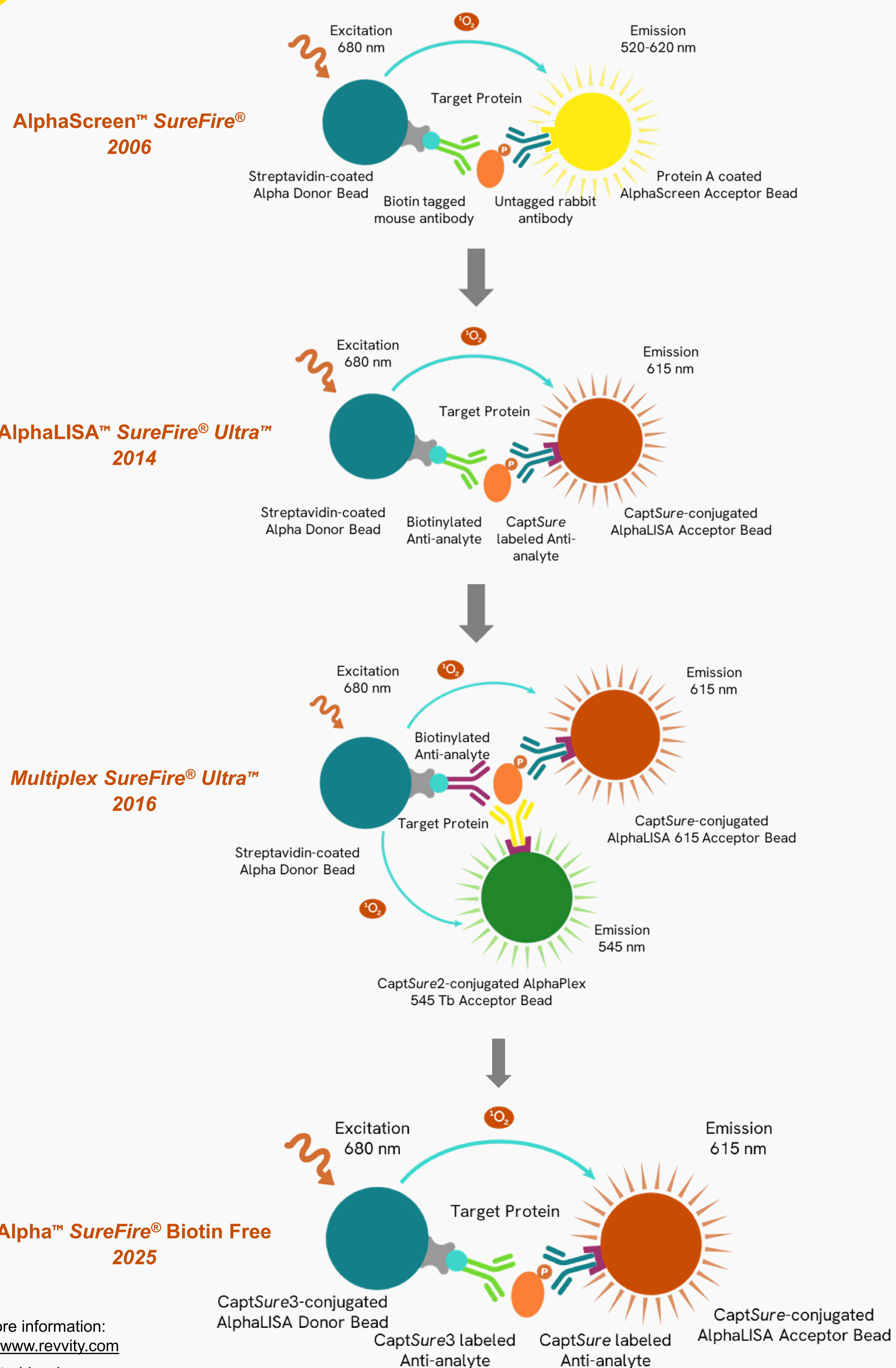


AlphaLISA™ SureFire® Biotin Free: Next-Generation Technology for Cell-Based Assays

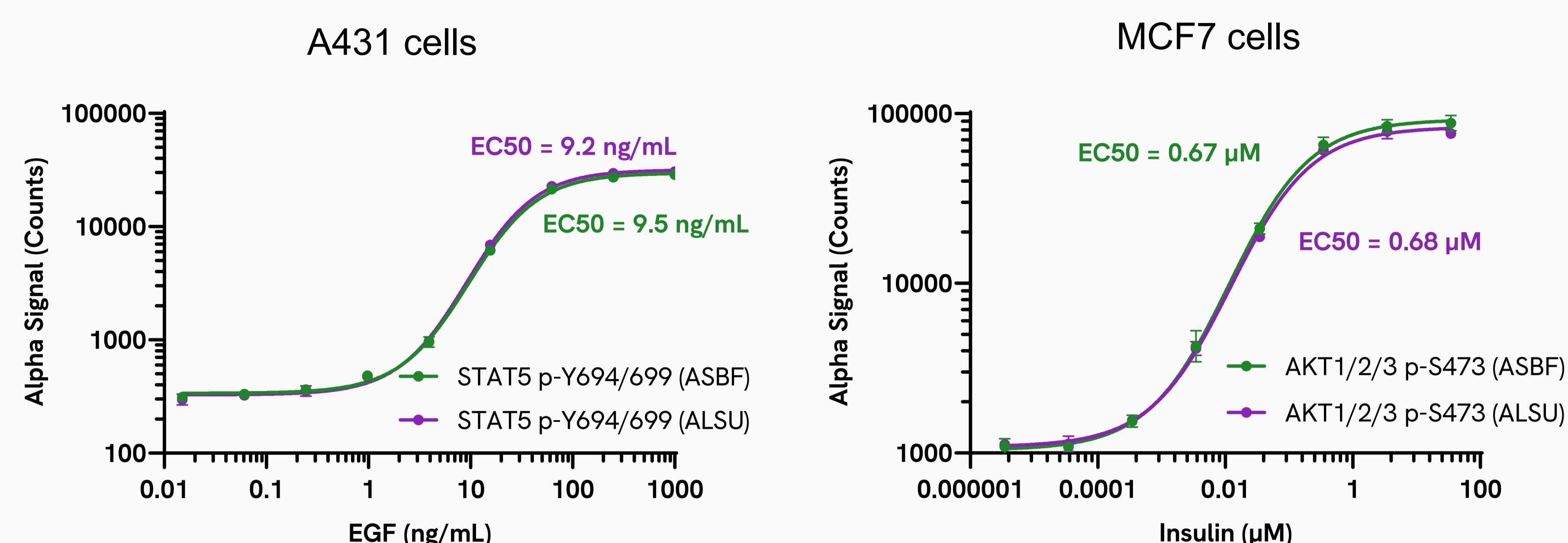
1 Overview

The evolution of AlphaLISA™ SureFire® technology continues with the introduction of AlphaLISA SureFire® Biotin Free (ASBF). This innovative immunoassay platform integrates TGR's patented CaptSure® antibody immobilization system with Revvity's renowned AlphaLISA technology, enabling no-wash, homogeneous detection of cell-based proteins. 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 ASBF data presented here establish a new benchmark in no-wash immunoassay technology, showcasing TGR's commitment to innovation and its support for basic and drug discovery research across the academic, biotech and pharma sectors worldwide.

2 The Alpha SureFire® technology evolution

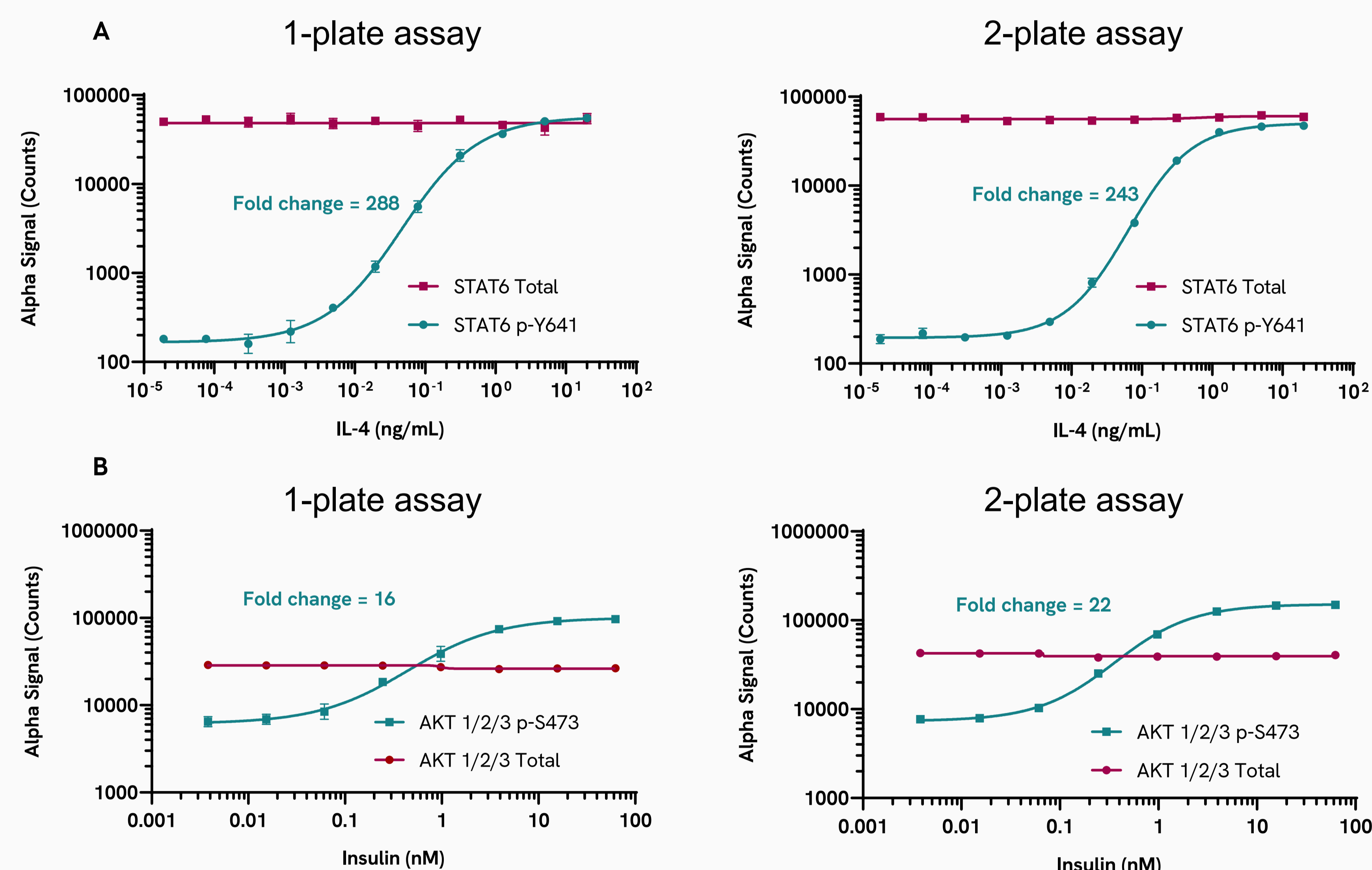


3 ASBF and ALSU assays: equivalent performance, sensitivity and dynamic range



- Superimposable curves were generated using ALSU and ASBF assays in response to EGF and insulin in both A431 and MCF7 cell lines, respectively.
- Large phosphorylation signal windows (100-fold cellular response) for STAT5 and AKT1/2/3 were obtained on both platforms using low cell numbers (4,000 cells/datapoint).

4 Exceptional performance of ASBF assays in biotin-containing media



- ASBF technology was evaluated in both 1-plate and 2-plate cell-based assay formats in the presence of RPMI media.
- THP-1 cells were treated with IL-4 (A) or Insulin (B) and lysed with the addition of 5X Lysis Buffer.
- Very good Phospho STAT6 (Y641) and AKT 1/2/3 (S473) signal windows were generated in 1-plate and 2-plate assay formats.

5 Conclusions

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