NEW Alpha Technique - Washer Free, Highly Sensitive Immunoassays

Celerion is proficient in a number of highly selective and sensitive immunoassay platforms to support pharmacokinetic and anti-drug-antibody analytics. Today, we want to introduce our newest achievement, the ALPHA (Amplified Luminescent Proximity Homogeneous Assay) technique, which combines the robustness of standard ELISAs with the broad dynamic range of ECL assays. Strong benefits of this technique are the rapid assay development and the lacking necessity for washers, which tend to bring variabilities into common immunoassay formats. There are two ALPHA techniques currently available, both of which Celerion`s brand new Tecan Infinite® M1000 Pro reader can perform:

a) ALPHAScreen, originally developed by PerkinElmer, is based on the principle of “Donor” and “Acceptor” beads displaying functional groups for biochemical conjugation of different, potentially interacting molecules in the sample. In case of interaction between already bound molecules, the beads are brought into proximity, leading to a cascade of chemical reactions. Upon laser excitation, this results in an amplified signal. A photosensitizer in the “Donor” bead converts ambient oxygen to a more excited singlet state. The singlet state oxygen molecules diffuse across to react with a chemiluminescer in the “Acceptor” bead that further activates fluorophores contained within the same bead. The fluorophores subsequently emit light at 520– 620 nm, which are detected by the Infinite® M1000 Pro.

b) ALPHALISA is a homogeneous, bead-based alternative to conventional ELISA assays, being either developed as sandwich or competitive assay. “Donor” and “Acceptor” beads get coated with specific antibody raised against different epitopes of the molecule of interest. During incubation with the “Donor” beads, the analyte binds to the antibody, before the “Acceptor” beads are added. Again, high energy excitation of photosensitizer molecules within the ALPHALISA donor beads at 680 nm converts ambient oxygen to singlet state oxygen, which is able to react with the chemistry in the acceptor beads if these, due to binding, are in close proximity. A strong output signal at 615 nm indicates specific binding between the molecules attached to the two bead types. The

fluorophores embedded in the ALPHALISA acceptor beads produce a narrower bandwidth signal than the acceptor beads used for classical ALPHAScreen assays, which makes this technology robust against signal interference at wavelengths of <600 nm, leading to increased sensitivity and robustness. The no-wash nature of this assay makes it fast and easy to use, and the use of dedicated ALPHALISA optics permits the analysis of target molecules in blood and serum by drastically reducing the effect of hemoglobin within a sample.

Further Reading: A Practical Guide to working with AlphaScreen, https://www.urmc.rochester.edu/MediaLibraries/URMCMedia/hts/documents/AlphaScreenPracticalGuide.pdf