Development of a homogeneous AlphaLISA ubiquitination assay using ubiquitin binding matrices as universal components for the detection of ubiquitinated proteins

The Ubiquitin Proteasome Pathway (UPP) regulates the degradation of proteins within the cell, controlling a variety of cellular processes. UPP pathway proteins can potentially be modulated by small molecules, making them promising targets for therapeutic intervention. Among these potential targets, E3 ligases have been implicated in cancer, inflammation and metabolic disease.

In this study, researchers set out to develop a homogenous assay for autoubiquitination of MDM2, an E3 ubiquitin ligase, using AlphaLISA® technology. Other assay technologies have yet to yield promising hits in ubiquitination screening campaigns. Theoretically, this may be due in part to their reliance on tagged ubiquitin or tagged proteins that hinder the physiological mechanism of ubiquitin transfer. AlphaLISA technology enabled the use of native ubiquitin and Tandem-repeated Ubiquitin-Binding Entities (TUBEs) in a functional ubiquitination assay, providing a key advantage over other technologies. AlphaLISA also provided the sensitivity, simplicity, and performance required for high-throughput screening. This study shows the development of an AlphaLISA ubiquitination assay that can be used in high-throughput screening for the discovery of novel drugs targeting ubiquitination pathways.

Figure 1: E3 autoubiquitination. Detection of autoubiquitination occurs when the pre-bound glutathione donor bead and GST-TUBE reagent bind to the polyubiquitin chain of the E3, and the specific antibody (α-Mdm2) pre-bound to the protein A AlphaLISA® acceptor bead binds to Mdm2. The proximity of the donor to the acceptor bead under laser excitation of 680 nm sets off a cascading chemical reaction from which a singlet oxygen molecule excites the acceptor bead, emitting light that can be detected at 615 nm.

Figure 2: Assay Development. The graphs measure MDM2 autoubiquitination activity. B) Ubch5b (E2) was titrated from 1000 nM in 2 fold dilution steps to 16.5 nM for each concentration of ubiquitin (titrated in the same range). C) Mdm2 (E3) was titrated from 20 nM in 2 fold dilutions to 0.031 nM. D) Signal to background increases dose dependently with Mdm2 concentration.

Figure 3: Ubiquitin chain formations. Summary of the activity and chain formation requirements between Mdm2 and Traf6 with respect to ubiquitin derivatives.