Development of a Flexible, Personalized Multiplexing Platform

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Abstract

Coupling multiple assays allows multiplexing, facilitating complex assays to be repeated, or advanced technologies to improve workflow and reduce costs of analysis. The U-PLEX assay platform enables users to consistently create high quality multiplexed assays, using proprietary linkers and antibodies. This U-PLEX assay platform demonstrates high level specificity and reproducibility for all linkers tested across multiple runs. The U-PLEX assay platform allows users to develop multiplexed immunoassays, using high quality commercial streptavidin and recombinant antibodies. Cross-reactivity of streptavidin antibodies was determined using an antibody competition assay. Linkers demonstrated high specificity for their corresponding capture materials with 0.02% non-specificity. Specificity of the linkers was tested in two ways: 1) a calibration curve demonstration and 2) a cross-reactivity demonstration. Results were obtained using high quality antibodies, and confirm the high level specificity of the U-PLEX assay platform.

Methods

Microplates were manufactured using Meso Scale Discovery’s proprietary electrode technology, using the TARC® platform which has been shown to detach and release signal data. The TARC® platform is a 2D, 24-well microplate with a metal electrode surface that can be excited by a laser. The TARC® platform allows for the creation of high quality microarrays that can be used for high throughput analysis of cell lines and cell populations. The TARC® platform can be used to create high quality microarrays that can be used for high throughput analysis of cell lines and cell populations.

Specificity of Linkers

A high level of specificity was observed for all linkers tested. Cross-reactivity was determined using a calibration curve demonstration, where a protein was immobilized to the plate through the U-PLEX linkers, and the amount of antibody remaining on the surface was determined using SULFO-TAG conjugated Protein A/G. The inter-lot %CV was less than 18% for all linkers.

Reproducibility

Intra-plate %CVs were below 5%, and inter-plate %CVs were below 8%. The mean signal and CV were calculated for each plate (intra-plate %CV) and across plates (inter-plate %CV, 6 plates from each production run). The results were obtained using high quality antibodies, and confirm the high level specificity of the U-PLEX assay platform.

Conclusions

Labeled antibodies can be produced using the U-PLEX assay platform, allowing for the creation of high quality microarrays that can be used for high throughput analysis of cell lines and cell populations. The U-PLEX assay platform demonstrates high level specificity and reproducibility for all linkers tested across multiple runs. The U-PLEX assay platform allows users to develop multiplexed immunoassays, using high quality commercial streptavidin and recombinant antibodies.