PK Assay Development

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METHODS

MSD’s electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates.

Electrochemiluminescence Technology

• Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
• The stimulation mechanism (electrolysis) is decoupled from the response (light signal), minimizing matrix interference.
• Only labels bound near the electrode surface are excited, enabling non-washed assays.
• Labels are stable, non-radioactive, and directly conjugated to biological molecules.
• Emission at ≤620 nm eliminates problems with color quenching.
• Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
• Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
• Surface coatings can be customized.

RESULTS

Uniformity Quality Control Results

Uniformity Measurements

Uniformity measurements for MSD GOLD Streptavidin and Small Spot Streptavidin-96-well plates are made by running whole plates with a constant amount of biotin-tagged IgG (BTI) at all 2 probes of IgG per well. The mean signal and coefficient of variation (CV) is calculated for each plate (interplate %CV) and across plates (interplate %CV). Mean inter-plate CV must be less than 6% with no plate having an interplate-CV greater than 12%

The mean interplate %CV from 487 MSD GOLD Streptavidin lots tested between 11/2014 and 11/2018 are shown in figure 1a. The individual plate results (14,917 plates) from each lot are shown in figure 1b.

Uniformity Quality Control Results (continued)

Inter-Lot Reproducibility

To verify the inter-lot reproducibility of MSD GOLD Streptavidin-96-well plates, the BTI is measured at 0.3, 0.2, 0.1, 0.025, and 0.028 pmole per well. These values correspond to typical capture antibody concentrations used in immunoassay and PK settings (25 µL of 1 µg/mL of an antibody is 0.1667 pmole of sodium IgG). A minimum of three plates per concentration are tested and the stability is defined as within 15% of the established target. A reference plate lot is run with each new test lot as a control to verify proper execution of the test. The results from a production run from 10/2014 to 11/2018 are shown in figures 2a-2e.

Real Time Stability at 2-8°C - BTI Titration Study

Stability Measurements

A real-time stability study was performed on the 96-well MSD GOLD Streptavidin plate format. The study was conducted over a 57-month period by using a plate layout that consisted of multiple BTI concentrations tested within a single plate, with a total of 3 plates being used for each time point. The graphs below show the mean signals (+/−16 replicates) collected for two different concentrations at each time point with the error bars representing +/−1 standard deviation of all replicates. The dotted line represents a +/-15% window around the mean signal measured across all the time points.

Adalimumab PK Assay Development

Primary Screening

Unbiased screening of all anti-Adalimumab antibodies was performed on MSD GOLD Small Spot Streptavidin plates by testing four-well combinations of antibodies labeled with either biotin or MSD SULFO-TAG™. The heat map shown in figure 3a is a visual representation of the signal intensities produced by each antibody combination. Intensity is represented on a red color scale where red represents a single antibody signal in the 50th percentile and red represents pairs with little to no signal. From this study, 11 antibody pairs were selected based on high signal and low background (data not shown) for continued screening.

Secondary Screening

The 11 antibody pairs selected during primary screening were evaluated further by testing full biotin or MSD SULFO-TAG™-labeled antibodies. The top 10 antibody pairs are highlighted in figure 3a. These 3 antibody pairs were then tested for dilution linearity, which was measured by plotting Adalimumab into four-well formats and then serially diluting 1:2, 1:10, 20, and 40 fold. Data for 2 out of 3 final pairs is shown in figure 3b.

CONCLUSIONS

We demonstrated the lot-to-lot reproducibility of the MSD GOLD Streptavidin and MSD GOLD Streptavidin Small Spot plate by presenting uniformity results across >350 lots and >40 lots, respectively. In addition, the robustness of the MSD GOLD Streptavidin-96-well plate format, when stored at 2-8°C, was highlighted by the consistent signal generation captured in the real-time stability study conducted over a 4-year time period. For application work, we demonstrated the utility of the MSD GOLD Streptavidin plate as an effective assay development tool that could be used during the antibody selection stage of assay design.

Adalimumab is a monoclonal antibody that is used to treat various types of arthritis, including rheumatoid arthritis and ankylosing spondylitis. It works by blocking the activity of the cytokine tumor necrosis factor-alpha (TNF-α), which plays a role in the development of arthritis and inflammatory bowel disease. Adalimumab is typically given by injection and is available in the United States under the brand name Humira. It is a humanized monoclonal antibody that binds to TNF-α and neutralizes its activity. Adalimumab has been shown to be effective in treating rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, and inflammatory bowel disease.