Abstract

Proteins, cytokines, and chemokines play a significant role in both basic research and clinical diagnostics. High throughput assay platforms are therefore necessary in order to assess the levels of analytes in complex samples. The U-PLEX multiplexing platform is an efficient method for this application. This platform can be used with primary antibodies that have been labeled with biotin or streptavidin, and secondary detection antibodies labeled with either enzyme or MSD SULFO-TAG™ label. The U-PLEX protocol offers the advantage of running all assays in a single platform.

Methods

Calibration Curves and Limits of Detection

To assess linearity, normal human serum and EDTA plasma samples (both obtained from a commercial source) and cell culture supernatants were spiked with recombinant calibrator and diluted 2, 4, 8, and 16 fold before testing. The average percent recovery shown in Table 1 was calculated as follows:

\[
\text{Percent Recovery} = \frac{\text{Average Conc. of Recombinant Calibrator}}{\text{Average Conc. of Recombinant Calibrator in Supernatant}} \times 100
\]

Spike & Recovery

The native analytes are detectable in normal serum and EDTA plasma as well as in serum and EDTA plasma that were spiked with culture supernatants obtained from stimulated PBMCs.

Dilution Linearity

The U-PLEX platform can be used with primary antibodies that have been labeled with biotin or streptavidin, and secondary detection antibodies labeled with either enzyme or MSD SULFO-TAG™ label. The U-PLEX protocol offers the advantage of running all assays in a single platform.

Specificity

The specificity of each of the markers was evaluated by testing for cross-reactivity for each capture–detector pair with all 40 analytes included in U-PLEX Biomarker Group 1 (human). The following table indicates the analytes tested for specificity.

<table>
<thead>
<tr>
<th>Markers</th>
<th>Spike &amp; Recovery</th>
<th>Dilution Linearity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>≤ 20% CV</td>
<td>&gt; 0.95 r² Value</td>
<td>≥ 20%</td>
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</tbody>
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