Immuno-oncology biomarker measurement with high sensitivity and speed using SimpleStep ELISA® kits and SPECTROstar® Nano

Introduction

Cancer therapies based on harnessing patients’ immune responses have shown impressive results in the clinic. Despite these successes, there is still a significant unmet need, as a large proportion of patients either do not respond or can become resistant to current drugs. The development of more efficacious treatments requires better understanding of cancer immune evasion strategies and the identification of predictive biomarkers for clinical outcomes or pharmacodynamic markers for target engagement. Altogether, this has the potential to tailor therapies to the individual and optimize treatment regimens.

Here we describe the successful implementation of the SimpleStep ELISA® kits for detection of 3 relevant biomarkers: Programmed Death Ligand 1 (PD-L1), Interleukin-8 (IL-8), and Interferon Gamma (IFN-γ).

Treatments targeting the Programmed Death 1 (PD-1) / PD-L1 pathway have seen successes due to the role of this pathway as an immune checkpoint. Beyond the immune regulator role, the expression level of PD-L1 in tumors, and in some instances, immune cells, has been correlated to the clinical outcome of blocking this pathway. In addition, both IL-8 and IFN-γ expression have been shown to be modulated in response to PD-1/PD-L1-targeting treatments.

Materials & Methods

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- SPECTROstar® Nano
- SimpleStep ELISA® kits
- Human PD-L1 (ab214565)
- Human IL-8 (ab214030)
- Human IFN-γ (ab236895)

Experimental Procedure

Samples were all handled according to kit instructions including an 8-point standard curve, in duplicate, with a 2-fold dilution series. Starting concentrations for PD-L1, IL-8, and IFN-γ were 1100, 250, and 800 pg/mL respectively. Replicates of blanks and appropriate biological replicates for each kit were employed to assess reproducibility of the assays.

- Human PD-L1 (ab214565)
  - Bio-replicates used were human serum, human serum spiked with PD-L1, and the supernatant from a peripheral blood mononuclear cell (PMBC) culture that had undergone a mitogenic stimulation regimen with phytohemagglutinin-M (PHA-M) to promote cytokine expression.
- Human IL-8 (ab214030)
  - Two different dilutions of supernatant from a PHA-M stimulated PMBC culture were used as bio-replicates.
- Human IFN-γ (ab236895)
  - Bio-replicates are dilutions of PHA-M stimulated PMBC culture supernatant.

Data analysis (4-parameter curve fit, R2 calculation, minimum detectable dose (MDD) calculation, determination of %CV) was performed in MARS using a data analysis template following absorbance detection using the SPECTROstar® Nano. The following settings were used for detection:

- Optic settings
  - Absorbance spectrum, Endpoint test
  - Wavelength range (step width): 400-700 (2) nm
- General settings
  - Number of flashes: 45
  - Settling time: 0.2 s

Assay Principle

The SimpleStep ELISA® improves upon the established ELISA platform, providing a semi-homogeneous approach that saves both time and effort (Figure 1).
Results & Discussion

All SimpleStep ELISA kits demonstrated high data quality and reproducible results. Figure 2 shows outstanding correlation for the data from the Human PD-L1 SimpleStep ELISA® kit in the 4-parameter fit, based on the $R^2$ value. MDD showed high sensitivity similar to the value reported in the kit literature and all bio-replicates and blanks exhibited low variability with %CV of less than 5.

![Figure 2: Human PD-L1 SimpleStep ELISA® standard curve](image)

**Table 1:**

<table>
<thead>
<tr>
<th>MDD (pg/mL)</th>
<th>%CV of blanks (n=16)</th>
<th>%CV of bio-replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50% Human Serum (n=8)</td>
</tr>
<tr>
<td>&lt;4.5</td>
<td>4.6</td>
<td>4.3</td>
</tr>
</tbody>
</table>

The results from the Human IL-8 SimpleStep ELISA kit are shown in Figure 3. The standard curve correlates with a 4-parameter fit based on $R^2$ and the MDD agreed with the kit literature. Both bio-replicates showed very low variability with a %CV of less than 4 while the blanks %CV were less than 5.

![Figure 3: Human IL-8 SimpleStep ELISA® standard curve](image)

Figure 4 shows the results for the Human IFN-γ SimpleStep ELISA® kit. The standard curve correlates to a 4-parameter fit and MDD agreed with kit literature. The blanks and bio-replicates for this assay showed very low variability (%CV < 4).

![Figure 4: Human IFN-γ SimpleStep ELISA® standard curve](image)

**Table 2:**

<table>
<thead>
<tr>
<th>MDD (pg/mL)</th>
<th>%CV of blanks (n=15)</th>
<th>%CV of bio-replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Stim. PBMC Culture Sup. 25% (n=8)</td>
</tr>
<tr>
<td>5.5</td>
<td>3.4</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Conclusion

This study has demonstrated the ability of SimpleStep ELISA® kits, when used in conjunction with SPECTROstar® Nano and MARS data analysis, to deliver the fast and easy detection of predictive and pharmacodynamic immuno-oncology biomarkers to better understand cancer immune evasion strategies and allow for the development of individual, optimized treatment regimens for patients.

References