

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

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- Determining the expression of biomarkers is essential for optimizing immuno-oncology therapies
- SimpleStep ELISA® kits from Abcam provide a detection tool for hundreds of cancer-relevant markers
- SPECTROstar® Nano simplifies data collection, with results quickly achieved using MARS data analysis

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 strategies¹ as well as 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 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 pathway¹. In addition, both IL-8² and IFN-γ³ expression have been shown to be modulated in response to PD-1/PD-L1-targeting treatments.

Assay Principle

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

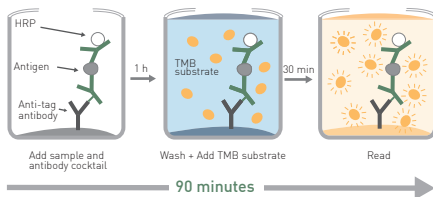


Fig. 1: SimpleStep ELISA® 90-minute workflow

Materials & Methods

- 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)

The 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 expression⁴.

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:

Instrument Settings

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	



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 R² 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.

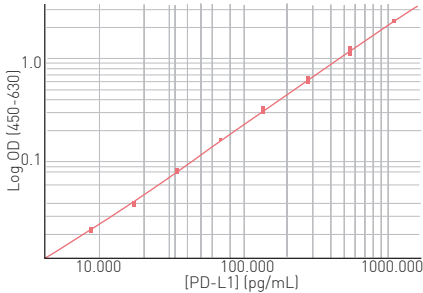


Fig. 2: Human PD-L1 SimpleStep ELISA® standard curve R²=0.9998

MDD (pg/mL)	%CV of blanks (n=16)	%CV of bio-replicates		
		50% Human Serum (n=8)	50% Human Serum + 500 pg/mL PD-L1 (n=8)	50% Stim. PBMC Culture Sup. (n=8)
<4.5	4.6	4.3	3.1	2.7

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 R² 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.

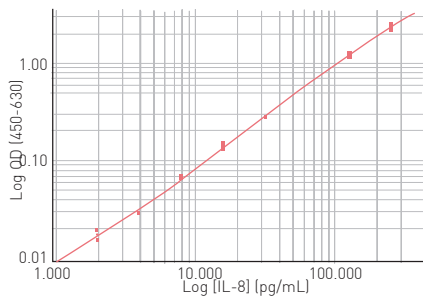


Fig. 3: Human IL-8 SimpleStep ELISA® standard curve. R²=0.9993

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).

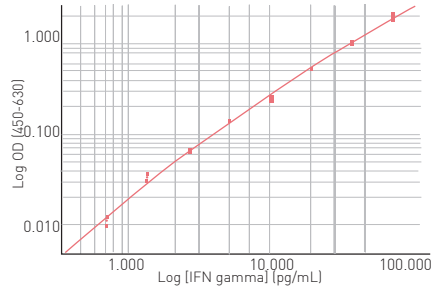


Fig. 4: Human IFN-γ SimpleStep ELISA® standard curve R² = 0.997

MDD (pg/mL)	%CV of blanks (n=15)	%CV of bio-replicates	
		Stim. PBMC Culture Sup. 25% (n=8)	Stim. PBMC Culture Sup. 12.5% (n=8)
5.5	3.4	1.9	1.4

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 immunology biomarkers to better understand cancer immune evasion strategies and allow for the development of individual, optimized treatment regimens for patients.

References

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