Application note

Highly sensitive ELISAs in 90-minutes: SimpleStep ELISA® kits and the SPECTROstar® *Nano*

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- Single-wash protocol reduces assay time but maintains high sensitivity, specificity, and reproducibility
- Using the SPECTROstar *Nano* and MARS data reduction software simplifies assay detection and analysis

Introduction

Since its introduction in the early 1970's the enzyme-linked immunosorbent assay (ELISA) has become one of the most reliable and relied upon biochemical assays[1].Detecting the presence (and quantity) of an analyte has proved an invaluable tool as a diagnostic in medicine or in basic research spanning nearly every biological discipline.

The main drawbacks of the ELISA approach have always been the relatively low throughput and the tedium of multiple wash steps that normally take 3–5 h to perform. Abcam's SimpleStep ELISA® technology addresses these issues by streamlining the process to a semi homogeneous format that results in a simple 90-minute, single wash protocol.

Here, we describe the detection of this innovative ELISA by the SPECTROstar *Nano*. When combined with MARS data analysis you will get your great results in the fastest, easiest way possible.

Exemplary data are shown for three important targets:

Human Pro-Collagen I alpha: The protein is a component of type I collagen that is found in bones and cartilage. Hence, it is implicated in diseases characterized by fragile bones such as osteogenesis imperfecta or osteoporosis.

Human IL6: Interleukin 6 (IL6) is an inflammatory cytokine that is capable of inducing maturation of B-cells and fever. Therefore, it is often used as an inflammation marker.

GFP: Green fluorescent protein (GFP) is characterized by its ability to fluoresce in the green spectrum when excited by blue light. It is a popular protein, when coupled to a protein of interest.

Assay principle

The sandwich ELISA uses an antibody pair that bind to distinct parts of the analyte. One antibody coats the bottom of the well and serves to capture the analyte. Following capture, and requisite washing, the detector antibody is added. Finally, after additional washing, the detection can be performed.

Figure 1 shows how SimpleStep ELISA® technology enables the sandwich ELISA to be performed semi homogeneously by the addition of an immobilization antibody.

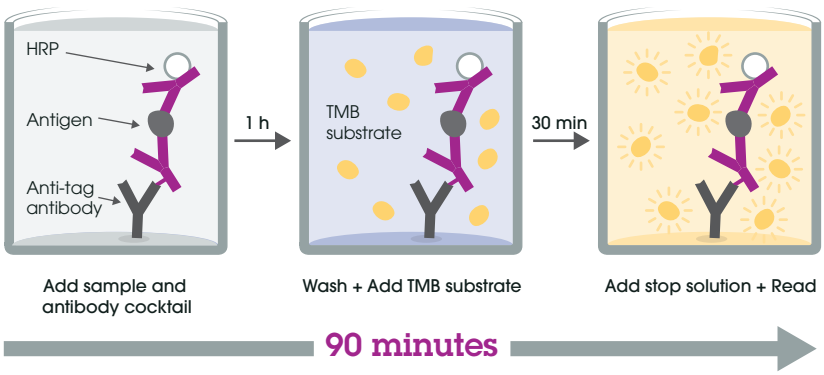

Figure 1. SimpleStep ELISA® kits assay principle. Samples are added along with the capture and detector antibody mix. The sandwich complex of antigen, capture and detector antibody is formed in solution. The complex bind to the immobilization antibody via an affinity tag on the capture antibody. After a single wash, color development can be performed followed by absorbance measurements.

Materials and methods

- SPECTROstar *Nano*
- SimpleStep ELISA® kits
  - Human Pro-Collagen I alpha 1 ELISA kit (ab210966)
  - Human IL-6 ELISA kit (ab178013)
  - GFP ELISA kit (ab171581)

Experimental procedure

For all kits, samples were handled in accordance with instructions[2]. For all tests, standard curves were prepared based on an 8-point dilution series using duplicate samples. Pro-Collagen 1 alpha and IL-6 used a 2-fold dilution series with starting concentrations of 2000 and 500 pg/mL respectively. GFP used a 3-fold dilution series with a starting concentration of 2000 pg/mL. Assay reproducibility was determined using biological replicates, which included 2 different dilutions of human serum for Pro-Collagen I alpha, 2 dilutions of stimulated peripheral mononuclear blood cells (PMBC) supernatant for IL-6, and 143B cells spiked with 500 and 250 pg/mL of GFP.

Plates were read on a SPECTROstar *Nano* with the following settings.

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 and discussion

BMG LABTECH microplate readers are equipped with a spectrometer for absorbance detection. Collection of spectral data can be done in the same time as reading a single wavelength. This allows easy evaluation of data quality (Figure 2). MARS data analysis templates allow automatic wavelength selection including peak, off -peak and reference wavelengths, for additional calculations that are also performed automatically by the system.

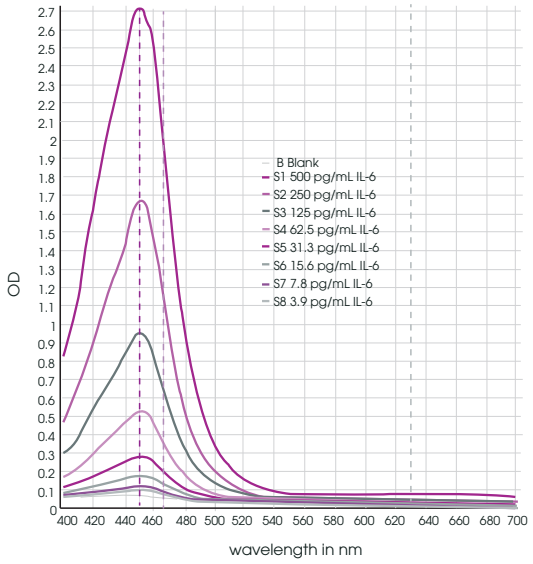

Figure 2. Representative spectral data. Spectral data collected on samples containing the indicated concentrations of IL-6. Selection of peak (450 nm) off-peak (466 nm) and reference (630 nm) wavelengths are indicated by dashed lines.

Figure 3 demonstrates how data from the SimpleStep ELISA® kit conforms to the suggested 4-parameter fit. An R2 of at least 0.9995 was seen for all three kits used.

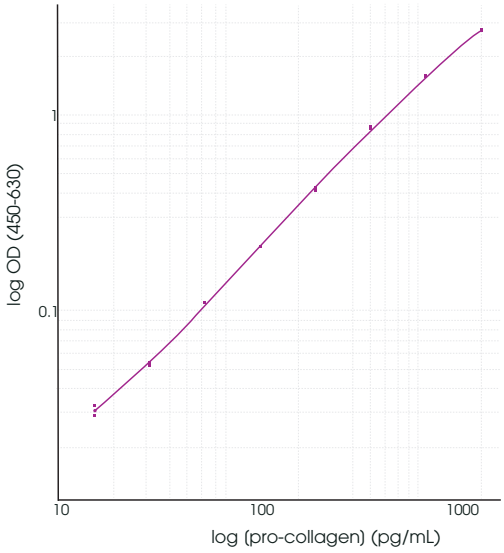

Fig. 3: Representative 4-parameter fit curve from curve from Human Pro-Collagen I alpha 1 SimpleStep ELISA® An 8-point standard curve for pro-collagen SimpleStep ELISA® (Human Pro-Collagen I alpha 1 ELISA kit (ab210966)) exhibits (R^2 =0.9998).

Table 1 shows the results of sensitivity and reproducibility metrics calculated for all 3 kits. These results indicate that SimpleStep ELISA® kits do not sacrifice performance while improving ease-of-use taking only one-third of the time to complete than a typical ELISA.

Table 1: SimpleStep ELISA® Sensitivity and Assay Precision MDD (minimum detectable dose) = recalculated concentration of $Avg_{Blank}+2*SD_{Blank}$. Blank (n=15), bio-replicates (n = 8).

	MDD (pg/mL)	%CV of Blanks	%CV of bio-replicates	
			Dil. 1	Dil. 2
Pro-collagen	<8.1	4.7	1.3	3.3
GFP	1.16	4.4	3.8	7.6
IL-6	<1.99	8.4	3.8	3.4

Conclusion

SimpleStep ELISA® kits offer excellent sensitivity and performance while saving significant time and effort. Reading microplates with the SPECTROstar *Nano* provides notable data quality while offering the convenience of linking data analysis to MARS templates, for immediate and accurate results.

References

1. Lequin, R.M. Enzyme Immunoassay (EIA)/Enzyme-Linked Immunosorbent Assay (ELISA). Clin. Chem.(2005) **51**: 2415-2418.

2. SimpleStep ELISA® kits