

APPLICATION NOTE

Quantitate interleukin-8 concentrations on the SpectraMax ABS Plus Microplate Reader with the SimpleStep ELISA kit

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Introduction

THP-1, a human monocytic cell line derived from an acute monocytic leukemia patient, is often used to study macrophage function in vitro. THP-1 cells are normally cultured in suspension, but when treated with phorbol 12-myristate 13-acetate (PMA), they differentiate into macrophages and become adherent. They also begin to release cytokines, including interleukin-8 (IL-8), which is secreted by a variety of cell types and plays a role in regulation of the acute inflammatory response.

Enzyme-linked immunosorbent assays (ELISAs) are commonly used to quantitate analytes like IL-8 in a 96-well microplate format. A typical ELISA can take over four hours to perform, but with Abcam's SimpleStep ELISA® kit, one can process an ELISA in just 90 minutes, decreasing the time spent by nearly two thirds, with no compromise in sensitivity and reproducibility (Figure 1). Here, we describe how to run the Human IL-8 SimpleStep ELISA and detect the results on the SpectraMax® ABS Plus Microplate Reader using a convenient preconfigured protocol in SoftMax® Pro Software.

Benefits

- Reduce ELISA experimentation time from 260 min to 90 min with no compromise in sensitivity and reproducibility using Abcam's SimpleStep ELISA kit
- Read an entire ELISA plate in as little as 5 seconds with the SpectraMax ABS Plus Microplate Reader
- Automatically calculate sample concentrations and plot results with SoftMax Pro Software

Materials

- Human IL-8 SimpleStep ELISA kit (Abcam cat. #ab214030)
- THP-1 human peripheral blood monocytes (ATCC cat. #TIB-202)
- THP-1 culture medium
 - RPMI-1640 Medium (ATCC cat. #30-2001)
 - Benchmark Fetal Bovine Serum (Gemini cat. #100-106)
 - 2-Mercaptoethanol (Sigma cat. #M6250-100mL)
 - Penicillin-Streptomycin (10,000 U/mL) (ThermoFisher cat. #15140122)
- SpectraMax ABS Plus Microplate Reader (Molecular Devices cat. #ABS Plus)

Methods

THP-1 Cell Preparation

20,000 undifferentiated THP-1 suspension cells were seeded into each well of a 96-well microplate. Afterwards, THP-1 cells were treated with 2.5 ng/μL PMA, or with DMSO (negative control), diluted in culture media. Following treatment, the cells were placed in an incubator at 37°C for 48 hours. Cell supernatants were then collected and stored at -80°C until the ELISA was performed.

ELISA

On the day of the ELISA, sample supernatants were thawed, spun down at 2,000 x g for 10 minutes in a micro-centrifuge to remove debris, and diluted 1:5, 1:10, and 1:50 in the provided Sample Diluent. The Human IL-8 SimpleStep ELISA reagents and standards were prepared according to the kit’s Protocol Booklet. Samples and standards were added to the provided microplate, Antibody Cocktail was added to the wells, and the plate was sealed and incubated at room temperature for one hour on a plate shaker. Wells of the plate were then washed three times with Wash Buffer PT. TMB Substrate was added to each well, and the plate was incubated for 15 minutes in the dark on a plate shaker. Stop Solution was added to each well, and absorbance (OD) was read at 450 nm on the SpectraMax ABS Plus Microplate Reader using a preconfigured protocol in SoftMax Pro Software (see Figure 1 for workflow).

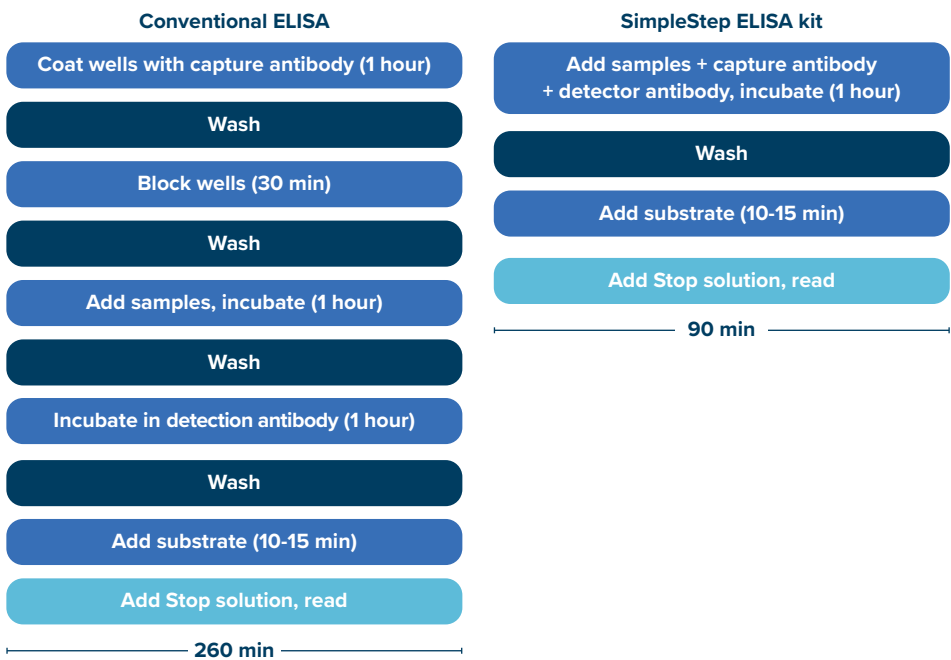


Figure 1. Comparison between SimpleStep ELISA kit and conventional ELISA workflows. Abcam’s SimpleStep ELISA kit provides results in 90 minutes with its streamlined protocol. Unlike conventional ELISAs, the SimpleStep ELISA kit only requires one incubation and one wash step, reducing assay time by nearly two thirds.

Results

A linear IL-8 standard curve with $r^2 > 0.99$ was generated in the 'HRP and TMB' protocol in SoftMax Pro Software (Figure 2). From the standard curve, the software interpolated IL-8 concentrations of samples derived from DMSO-treated negative control cells and PMA-treated cells. Samples with insufficient dilution were flagged in the

preconfigured protocol's group table as out of range ('R') of the standard curve (Figure 3). The concentration of IL-8 from PMA-treated cells was calculated to be 3400 pg/mL, 55-fold higher than the 63 pg/mL found in DMSO-treated negative controls (Figure 4).

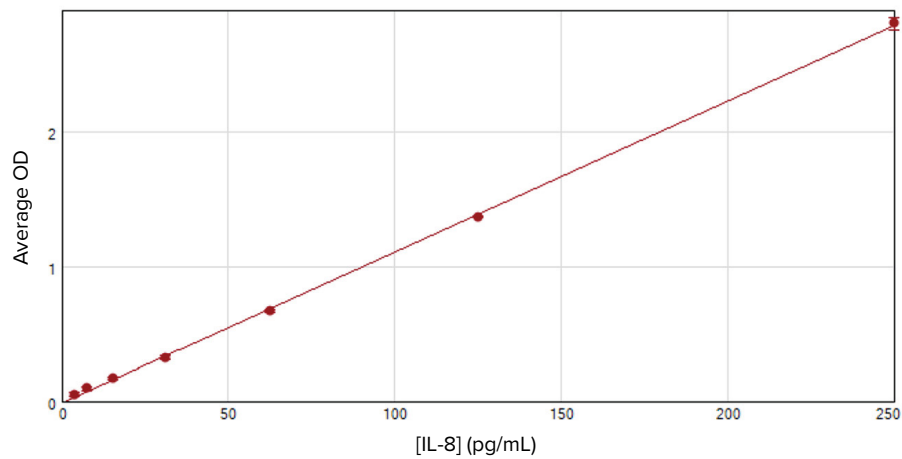


Figure 2. ELISA standard curve. The IL-8 standards were diluted in Sample Diluent and assayed using the Human IL-8 SimpleStep ELISA kit. A linear curve fit was applied to the standards in SoftMax Pro Software, and the generated standard curve demonstrated excellent linearity ($r^2 > 0.99$).

2.5ng/uL_PMA										
Sample	Well	DilutionFactor	R	OD	Result	Adj Concentration	MeanResult	Std.Dev.	CV%	
01	C4	5.000	R	3.864	346.196	17309.794	17309.794	0.000	0.000	
	C5		R	3.864	346.196	17309.794				
	C6		R	3.864	346.196	17309.794				
02	D4	10.000	R	3.766	337.485	16874.253	16334.926	536.960	3.287	
	D5		R	3.644	326.603	16330.161				
	D6		R	3.526	316.007	15800.364				
03	E4	50.000		0.788	71.380	3569.021	3440.220	149.428	4.344	
	E5			0.764	69.263	3463.151				
	E6			0.700	63.563	3178.151				
	F4			0.794	71.934	3596.717				
	F5			0.756	68.584	3429.201				
	F6			0.751	68.102	3405.079				

Figure 3. Group table displaying calculated sample concentrations. The IL-8 concentrations of PMA-treated cells were automatically interpolated from the standard curve by the preconfigured SoftMax Pro protocol and displayed in a group table. Any samples showing an 'R' in the 'R' column had a calculated concentration that is outside the range of the standard curve, in this case indicating the need to further dilute the sample.

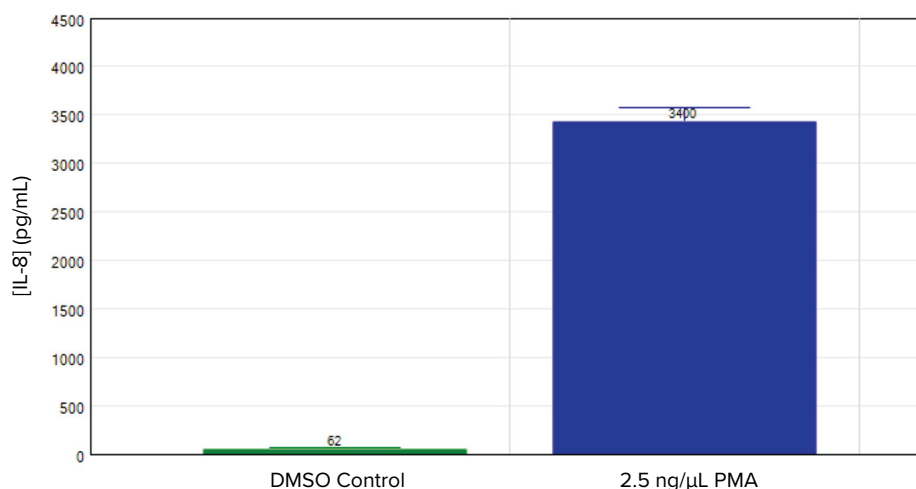


Figure 4. IL-8 concentrations measured in undifferentiated (DMSO control) and differentiated (2.5 ng/μL PMA) THP-1 cells. 2.5 ng/μL PMA-treated THP-1 cells produced 3400 pg/mL of IL-8 compared to the 62 pg/mL IL-8 produced by undifferentiated THP-1 cells, a 55-fold increase.

Conclusion

The SpectraMax ABS Plus Microplate Reader provides rapid and sensitive IL-8 detection with Abcam's Human IL-8 SimpleStep ELISA kit. The SimpleStep ELISA's proprietary antibody binding technology enables quantitation of IL-8 in THP-1 cell supernatant in as little as 90 minutes, only about a third of the time required to perform a conventional ELISA, with no compromise in sensitivity and reproducibility.

The reader's 8-channel detector can read an entire 96-well ELISA plate in as little as 5 seconds. A preconfigured protocol in SoftMax Pro Software automatically plots a standard curve, calculates sample concentrations, and can plot results for negative controls vs. treated cells, further reducing the time needed to obtain publication-quality results.

SimpleStep ELISA® is a registered trademark of Abcam plc.



SpectraMax ABS Plus Microplate Reader

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